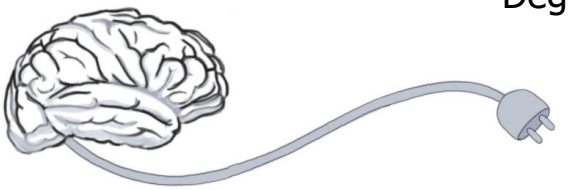
UNIVERSITAT DE BARCELONA

Facultat de Farmàcia
i Ciències de l'Alimentació

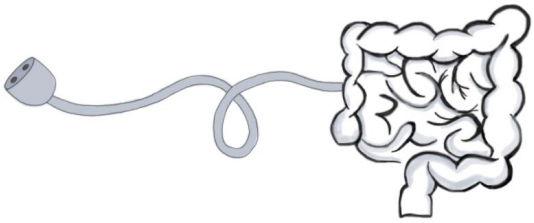
FINAL DEGREE PROJECT

Degree in Pharmacy



THE MICROBIOTA-GUT-BRAIN AXIS:

FROM DYSBIOSIS TO NEURODEGENERATIVE DISEASE



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Physiology and physiopathology

Microbiology

Pharmacology and therapeutics

Facultat de Farmàcia i Ciències de l'Alimentació

Universitat de Barcelona

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3 GOOD HEALTH AND WELL-BEING

5 GENDER EQUALITY

9 INDUSTRY, INNOVATION AND INFRASTRUCTURE

CONTENT

ABBREVIATIONS

The most repeated and relevant abbreviations for the understanding of this project are provided:

Aβ: amyloid beta

AD: Alzheimer's disease

ANS: autonomic nervous system

BBB: blood-brain barrier

BDNF: brain-derived neurotrophic factor

CNS: central nervous system

ECC(s): enterochromaffin cell(s)

EEC(s): enteroendocrine cell(s)

ENS: enteric nervous system

FMT: faecal microbiota transplantation

GBA: gut-brain axis

GF: germ-free

GI: gastrointestinal

GM: gut microbiota

HPA: hypothalamic-pituitary-adrenal

IB: intestinal barrier

IBD: inflammatory bowel disease

IBS: irritable bowel syndrome

LPS: lipopolysaccharide

MGBA: microbiota-gut-brain axis

ND: neurodegenerative

NDD(s): neurodegenerative disease/disorder(s)

PD: Parkinson's disease

SCFA: short-chain fatty acids

α-Syn: alpha-synuclein

τ: tau

TLR: Toll-like receptor

VN: vagus nerve

WHO: World Health Organization

1. ABSTRACT

The relation between the gut and the brain has been discussed for years, but the concept of gut microbiota (GM) has not been linked for so long. Having suggested its involvement in neurodegenerative disorders, this literature review aims to summarise the current knowledge on the possible bidirectional pathways that could explain it, while discussing the composition and alterations of the GM and the main pathological features that characterise neurodegeneration. It also presents the existing scientific evidence on the use of GM as a therapeutic target, accompanied by a systematic review-meta-analysis that evaluates the indication of one of the possible interventions: the faecal microbiota transplantation (FMT).

GM is made up of the trillions of microorganisms in the gut, mostly bacteria, which interact dynamically with the host, contributing to both health and disease. These, through neural, endocrine and immune pathways, based on mechanisms that require further research, can modify the gut-brain axis promoting neurodegenerative processes such as neuroinflammation, protein misfolding and loss of integrity of the intestinal and blood-brain barriers, thus facilitating the passage of components derived from a deregulated GM that has been characterised in Alzheimer's and Parkinson's patients.

Although more studies are needed, the promising results obtained in animal and more limited human trials, using GM modulating interventions such as oral bacteriotherapy or FMT, give hope for the cure and early detection of these increasingly prevalent diseases.

RESUM

-Eix microbiota-intestí-cervell: d'una disbiosi a una malaltia neurodegenerativa-

Ja fa anys que es parla de la relació entre l'intestí i el cervell, però no en fa tants que s'hi ha unit el concepte de microbiota intestinal (MI). Després d'haver-se suggerit la seva implicació en les alteracions neurodegeneratives, aquesta revisió bibliogràfica pretén resumir el coneixement actual sobre les possibles rutes bidireccionals que ho explicarien, aprofundint a més en la composició i alteracions de la MI i en els principals trets patològics que caracteritzen la neurodegeneració. També s'hi recull l'evidència científica existent sobre l'ús de la MI com a diana terapèutica, acompanyant-se d'una revisió sistemàtica-metaanàlisi que avalua la indicació d'una de les possibles intervencions: el trasplantament de microbiota fecal (TMF).

La MI la conformen els trilions de microorganismes que tenim a l'intestí, majoritàriament bacteris, que interactuen de manera dinàmica amb l'hoste, contribuint tant a la salut com a la malaltia. Aquests, mitjançant vies neurals, endocrines i immununològiques, basades en mecanismes que encara requereixen d'investigació, poden modificar l'eix intestí-cervell afavorint processos neurodegeneratius com són la neuroinflamació, el mal plegament de proteïnes i la pèrdua d'integritat de les barreres intestinal i hematoencefàlica, fet que facilita el pas de components derivats d'una MI desregulada que s'ha pogut caracteritzar en pacients d'Alzheimer i Parkinson.

Tot i ser necessaris més estudis, els resultats prometedors obtinguts en assaigs animals i d'altres més limitats en humans, fent ús d'intervencions moduladores de la MI com són la bacterioteràpia oral o el TMF, donen esperances per a la cura i detecció precoç d'aquestes malalties de prevalença creixent.

2. INTEGRATION OF THE DIFFERENT FIELDS

The main focus of this project is the microbiota-gut-brain axis. The aim, then, is to understand through which physiological pathways or mechanisms this bidirectional communication can occur in our organism and how its existence can lead to changes in the course of neurodegenerative diseases, whose main pathological features are also studied. It is for these reasons that this work is mainly framed in the field of **physiology and physiopathology**.

In addition, although to a lesser extent, this work is also related to two other teaching areas: **microbiology** and **pharmacology and therapeutics**. The former is best represented at the beginning of the project, when the definition, composition and functions of the gut microbiota are explained; it is essential to know the connections it maintains with the organism, more specifically with the brain. The latter takes place at a more advanced stage of the work, when it is intended to analyse the currently approved drugs (and their respective mechanisms of action) for neurodegenerative diseases, in order to determine whether other alternatives based on modifying the gut microbiota are necessary, as well as effective and safe.

3. IDENTIFICATION AND REFLECTION ON THE SUSTAINABLE DEVELOPMENT GOALS (SDGS)

The community on which this work can have an impact, and which motivates its realisation, is people suffering from neurodegenerative diseases, which greatly influence their quality of life as well as their life expectancy. In the world population, the evident constant growth in life span is causing a big increase in the prevalence of this type of disease, whose main risk factor is age. This, added to the lack of effective therapeutic strategies for these pathologies, leads to an increase in the diseased population, which entails a major health, social and economic burden.

In the face of this threat, this project is aimed at studying the connection between the brain and the gut microbiota, knowledge that is expanding due to the emergence of new sequencing technologies that still need to be improved (putting innovation above the economic challenge), in order to consider the possible analysis or modulation of microbiota as a tool for early diagnosis or as a new treatment approach, respectively. Not immediately, but in the long term, this research could be useful in clinical practice to prolong survival and increase the well-being of both patients and caregivers. Although studies are limited, promising results have been seen in animals and are beginning to be confirmed in preliminary studies in humans. In these, despite being diseases mostly prevalent in women, a greater participation of men has been detected, besides a major use of male animals; this may limit the ability to ensure women's health on equal terms with men.

For all these reasons, two of the SDGs addressed in this project are considered to fall within the scope of "**people**", being included in SDG 3 "Good health and well-being", specifically in target 3.4 "Reduce mortality from non-communicable diseases and promote mental health", and in SDG 5 "Gender equality", pointedly in target 5.1 "End discrimination against women and girls". Furthermore, given the biotechnological novelty that the use of microbiota as a therapeutic target would represent, another SDG considered, within the scope of "**prosperity**", would be number 9 "Industry, innovation and infrastructure", specifically in target 9.5 "Enhance research and upgrade industrial technologies". No indicator that explicitly reflects these aspects has been identified in any of the cases.

4. INTRODUCTION

More than 2000 years ago, the Greek physician Hippocrates, often lauded as the father of modern medicine, made the proclamation "**all disease begins in the gut**", which underlined the fundamental role of the gastrointestinal (GI) tract in maintaining homeostasis. Furthermore, along with other philosophers such as Plato and Aristotle, the intrinsic connection between the brain and the rest of the body was postulated.(1)

It was in the 19th century that an unfortunate injury to a Canadian fur trader, Alexis St. Martin, created a serendipitous opportunity to progress with the study of this idea: when he became angry or irritable, the rate of digestion was greatly affected, indicating that a gut-brain axis (GBA) existed. However, it was not until the early to mid-20th century that the first, still limited, scientifically recorded observations correlating changes in gut physiology with changes in emotion were made. Then, with the advent of brain imaging technology in the 1980s, the full appreciation of the **bidirectionality** of this axis emerged, linking emotional and cognitive centres of the brain with peripheral intestinal functions through neuro-immuno-endocrine mediators.(1,2)

Knowledge about the gut microbial communities that live in symbiosis or mutual support with humans had had, until now, little impact on medicine. This has changed radically in recent years with the **characterisation of the human microbiome** (a milestone in the history of biomedicine) and the development of recent epidemiological, physiological and -omics studies, complemented by cellular studies and animal experiments, aiming to get a deeper understanding on the genetic material that resides in our gut and on how it interferes with our health and disease.(2,3)

Therefore, it is in recent decades that the fields of microbiology and neuroscience have become increasingly intertwined and that the gut microbiota (GM), being the trillions of microorganisms that reside in the intestine, has emerged as a key regulator of the GBA, now better named **microbiota-gut-brain axis** (MGBA). This suggests that gut microbes, apart from various environmental factors which can affect their metabolism, may shape neuronal function, modulate neurotransmission and oxidative stress, cause neuroinflammation and affect behaviour, thereby contributing to the pathogenesis and progression of many neurodevelopmental, neuropsychiatric, and neurological conditions.(4) Moreover, alterations in GI function and GI symptoms have been reported to accompany an increasing number of central nervous system (CNS) disorders.

Furthermore, studies in widely used germ-free (GF) animals, lacking exposure to microorganisms from birth, demonstrated that the brain, as well as host physiology and behaviour, is affected in the absence of microbiota. It has been shown that fundamental central neuronal processes, such as myelination, neurogenesis and microglial activation, depend on the composition of the GM and vice versa. GF mice also demonstrated abnormal cognitive development and memory dysfunction, suggesting the possible involvement of gut microbial balance in **neurodegenerative diseases** (NDDs).(1,5)

NDDs, including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD) are among the leading causes of disability and death worldwide and represent a major threat to human health. These disorders start with cognitive decline and alterations of neurovegetative functions, and progress towards language deficit, memory loss, motor difficulties and ultimately death. Its prevalence is expected to increase at an alarming rate due to the steadily increasing life expectancy. Currently, nearly 50 million people worldwide suffer from them, and this number is expected to reach 152 million by 2050.(6,7)

The most common NDDs, AD (prevalence of 5.7 million people in the USA in 2018 and one in ten individuals aged ≥65 years; more prevalent among women, as with most dementias) and PD (prevalence of 2–3% of the global population aged >65 years in 2017; more prevalent among men), are clearly predominant in elderly individuals (as shown in **Fig. 1**). Since ageing constitutes the accumulation of damage at the cellular and molecular level in different organs (including the brain), which then translates into disease (8,9), and given the proved existence of an age-related deterioration of the GM (increased gut permeability) and of the homeostatic systems, **ageing** is considered the primary risk factor for most NDDs. Thus, in view of increasing life span (global ageing population is expected, according to World Health Organization -WHO-, to double by 2050 to an astounding 2 billion people), what appeared to be a mere physiological phenomenon is becoming a growing **socio-economic burden** worldwide.

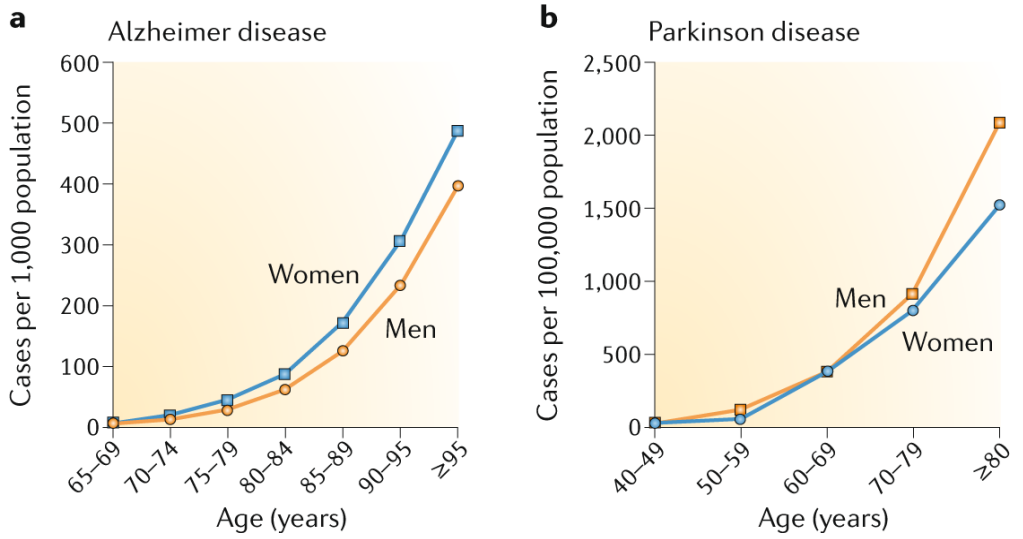

Fig. 1. Illustrative graphics of the prevalence of Alzheimer's disease per 1000 men and women by age in the USA (a) and the prevalence of Parkinson's disease per 100000 men and women by age globally (b).(9)

Added to all the problems that lie ahead and despite decades of clinical research regarding pathogenesis, diagnosis and treatment of these pathologies (mostly of unknown cause), they remain debilitating and fatal conditions, with **no effective therapeutic approaches** to prevent, delay or reverse them, and with only a few approved palliative drugs to alleviate the progressively worsening symptoms which, unfortunately, usually appear in very advanced stages of the degeneration.(10) New and more effective preventive and/or therapeutic alternatives to combat these devastating diseases are **urgently needed**.

That is why, given the critical involvement, a better understanding of the relation between GM and NDDs and the mechanisms underlying the MGBA may help in the development of promising therapeutic strategies to avoid, postpone, or make these common but poorly understood diseases less severe.(1,2,11) **GM modulation**, what appears to be a novel and safe approach with the aim to reverse established gut microbial imbalance (dysbiosis), will be considered and tested throughout this work.

5. OBJECTIVES

The main objective of this project is to deepen the current knowledge on the connection between the gut microbiota and the brain, in order to understand its role in neurological diseases, more specifically in neurodegenerative diseases. Additionally, in relation to this subject, a series of questions are posed with the **aim** of providing answers to them:

1. What is the gut microbiota and what is it made up of? Does it have any important function in the body? Which are the main factors that can alter it?

2. Through which pathways or mechanisms is the bidirectional connection between the gut microbiota and the brain established? How can these influence the hallmarks of neurodegeneration?

3. Is there an effective cure for neurodegenerative diseases? Could this axis be exploited as a diagnostic tool or therapeutic target for them? Is there scientific evidence on whether this works in practice?

6. MATERIALS AND METHODS

This project falls into the category of **bibliographic research**. Most of the information it contains has been obtained by consulting scientific literature, mainly review articles, in databases such as PubMed (Medline), SpringerLink, Web of Science, Scopus, ScienceDirect or SciELO, as well as the scientific journal Nature Reviews and the ClinicalTrials.gov database to search for and analyse clinical trials of interest. In all these sources, an advanced search was performed using specific terminology such as "gut microbiota", "gut brain axis", "neurodegenerative disease", "microbiota gut brain axis [AND] neurodegeneration", "microbiota gut brain axis [AND] Alzheimer disease", "faecal microbiota transplantation [AND] Parkinson disease", and so on. The topic addressed in this work has attracted a lot of interest, especially in the last few years. Therefore, in order to obtain up-to-date information, only references published from 2016 onwards have been selected.

Apart from this, it has been possible to **contact several experts** on the subject, from different fields. Some have provided information from their own publications, while others have shared other people's articles: Prof. Mónica de la Fuente (Professor of Physiology at the UCM; co-author of "Documento de consenso sobre la microbiota y el uso de probióticos/prebióticos en patologías neurológicas y psiquiátricas" as part of the board of the *Sociedad Española de Microbiota, Probióticos y Prebióticos*), Andreu Prados (scientific editor specialised in microbiota and probiotics; involved in the project "Gut Microbiota for Health"), Dr. Iria Grande (psychiatrist at Hospital Clínic; principal investigator in several projects on the gut-brain axis), Dr. Chaysavanh Manichanh (head of the microbiota laboratory in the physiology and digestive physiopathology research group at Vall d'Hebron Institut de Recerca) and Lisset de la Vega (PhD student at Institut d'Investigació Biomèdica de Girona; involved in microbiota-brain axis projects in animal models).

Finally, there has been the opportunity to complement the knowledge with an arranged visit to the *Alzheimer Center Barcelona* (ACE) by contacting Xavier Morato (Clinical Trials deputy director) and Amanda Cano (PhD in genetics and molecular biology), and also with an interview to an expert in the novel therapy of faecal microbiota transplantation, Andrea Aira (PhD student in gut microbiota at Fundació Clínic per a la Recerca Biomèdica). The extra information obtained from these last contacts can be found in the annexes of this project.

7. Results and Discussion

This section **7.1**-**7.5** presents the totality of the bibliographic research that makes up this project, discussing and responding in an orderly manner to the objectives that had been proposed.

7.1. Gut Microbiota

7.1.1. Definition and Composition

The human **microbiota** consists of a wide variety of microorganisms, more than a thousand different species of mainly bacteria (high proportion of anaerobes), but also viruses, archaea and eukaryotes (fungi, protozoa and even worms).(3) These microorganisms are known to establish complex trophic relationships with each other and with their human host, ranging from symbiosis to parasitism.(12) Thus, the microbiota interacts with the host in a dynamic way, contributing to both health and disease.(13) The human **microbiome**, which are the genes of the trillions of microbial cells contained in the human body, is much more diverse than that of human cells.(11) It represents a genetic repertoire that is more than one order of magnitude higher in genes than the human genome.(14)

These communities of microorganisms inhabit our skin, mouth, respiratory, urogenital and GI tracts(15), but reach their highest density, by far, in the **intestinal compartment** (especially in the colon, with 10^{10}-10^{14}/g), where they form the complex microbial population known as the gut microbiota (**GM**).(12) This community represents more than 75% of our microorganisms and lives in the digestive tracts not only of humans, but also of animals, including insects.(16)

It is known that there is great variability, from cavities where there is an absolute predominance of one or a few bacterial genera, such as the vagina, to others where there is great diversity, such as the large intestine, where representatives of all the major biological groups are found. Abundance also varies greatly: there are very inhospitable locations, such as the stomach, where colonisation is very low, while others, such as the colon, harbour quantities of microorganisms equal to the number of cells in our own organism.(3)

In the colon, microbial density and diversity are enormous; acidity has virtually disappeared, toxic components of bile have been reabsorbed through enterohepatic circulation and, above all, the intestinal contents are remanifested, which favours the accumulation of microorganisms.(3)

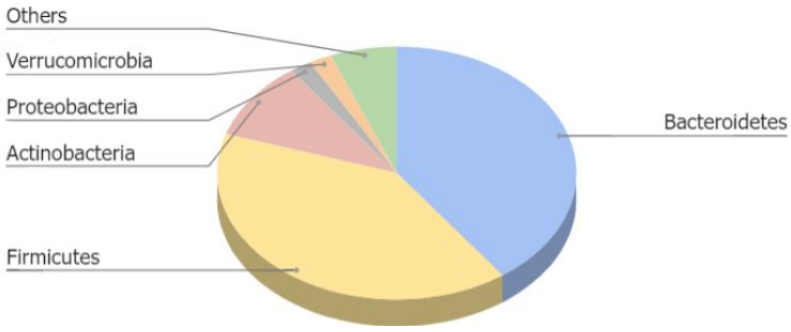

Fig. 2. Representation of the estimated abundances of the different bacterial phyla in human gut.(3)

Within the **bacterial phyla** inhabiting the large intestine (shown in **Fig. 2**), the most abundant are *Firmicutes* (mainly of the order *Clostridiales* as *Faecalibacterium prausnitzii*) and *Bacteroidetes* (with the genera *Bacteroides* and *Prevotella* as the main representatives)(11), which constitute approximately the 40% of the total in each case, followed by *Actinobacteria* (represented by the

genus *Bifidobacterium*), which would be around 10%, *Proteobacteria* (mainly enterobacteria) and *Verrucomicrobia* (*Akkermansia muciniphila*) with concentrations of less than 3%(3), making up to 90% of the total bacterial population in humans gut. Among the genera involved, *Bacteroides* is the most abundant; species of this genus alone comprise about 30% of the bacteria in the gut, suggesting that it is particularly significant in the functioning of the host organism.(16)

Until recently, this **colonisation** process was thought to begin at birth. However, this dogma of a sterile in utero environment has been challenged. A growing body of scientific evidence has provided indications of bacterial presence in the placenta, umbilical cord, and amniotic fluid in healthy full-term pregnancies.(12)

With regard to the interactions established between the microbiota and the colonised individuals, it is known that there is a strong tendency to maintain the microbiota present in each habitat within the same person (concept of homeostasis) and that the functions of the microbiota remain more or less constant in each of their locations in the organism (functional redundancy).(3)

The **diversity** of microbial communities in different habitats is governed by dispersal (a natural process causing an increase in diversity in local microbial communities), local diversification (based on rapid microbial adaptation via mutation or recombination), environmental selection (supporting the selection of microbial characteristics that allow survival and growth in the host) and ecological drift (responsible for the disappearance of low-abundance species).(12)

The relative **distribution** of gut bacteria and archaea is unique to each individual, both because of the diversity of strains and microbial growth rates and because of considerable inter-individual variation in environmental exposure and host genetics. However, although a **healthy GM** has not yet been defined, in general, a high taxa diversity, high microbial gene richness and stable microbiome functional cores characterise healthy gut microbial communities.(14)

7.1.2. FUNCTIONS

The information encoded by the mammalian genome is not sufficient to maintain health and protect against disease, which is why the microbiota is essential for all living organisms, establishing **predominantly** with humans a **mutualist symbiosis**, in which the host supplies a habitat to the microorganisms while they allow the adequate functions of the host.(11)

After millions of years of co-evolution, microorganisms provide humans with many **key functions** ranging from protection against invasion by opportunistic pathogens (through their exclusion by the biofilm formed or their destruction by production of several types of antimicrobial compounds) and development and function of the immune system, to collaboration in the digestion of dietary components, metabolism, nutrient biosynthesis, angiogenesis, bone growth and development, detoxification, epithelial cell proliferation and differentiation and neurological development in the early stages of life by influencing gut-brain communication.

In addition, the GM provides enzymes for the synthesis of vitamin K, B vitamins and amino acids. It also has the capacity to synthesise some neurochemicals that can affect the central nervous and peripheral enteric systems like gamma-aminobutyric acid (GABA) and is involved in the synthesis of bile acids and cholesterol. Another major function of this bacterial population is to obtain energy through the metabolism of non-digestible complex polysaccharides from food by anaerobic fermentation and the subsequent generation of short-chain fatty acids (**SCFA**) such as propionate, butyrate and acetate, described to improve gut health by regulating water absorption, mucous production and GI motility, and which furthermore provide essential nutrients and other compounds with promising anti-inflammatory and chemo-preventive properties. This energy

serves as their own supply, but also corresponds to 5-10% of the daily energy needs of a human; it is a significant energy source for intestinal epithelial cells and therefore strengthen the mucosal barrier. Lastly, the GI tract is also a site of production of hormones involved in energy homeostasis (e.g., insulin, glucagon, leptin and ghrelin) and growth.(3,11,13,14,16)

Thus, on the basis of all these capacities, the GM can be currently considered an additional **organ**, as it plays a significant role in maintaining homeostasis, normal gut physiology and health.(15) This "**holobiont**" concept (assemblage of a host and the many other species living in or around it) represents a paradigm shift in modern medicine and in our understanding of many aspects of biology and human evolution.(11)

7.1.3. Factors That Affect

The shaping and multiplication of the GM starts at birth, while the modification of its composition and maturation depends mainly on various genetic, nutritional and environmental factors (some examples are shown in **Fig. 3**), which will condition intestinal permeability, digestion, metabolism and immune responses.(16)

Hence, during all the life, many factors such as the diet, perinatal conditions, physical exercise, mood, emotions, circadian clock, environment and social contacts, antibiotic usage, family genetics and lifestyle, among others, determine each individual's GM and consequently their homeostatic systems, as well as the dialog between them. The almost infinite combinations of these factors are responsible for the **unique** microbial population harboured by the gut of each individual. For this reason, the health status and risk of morbidity in adulthood and in the ageing process is very different between individuals, even if they are from the same population.(11,12)

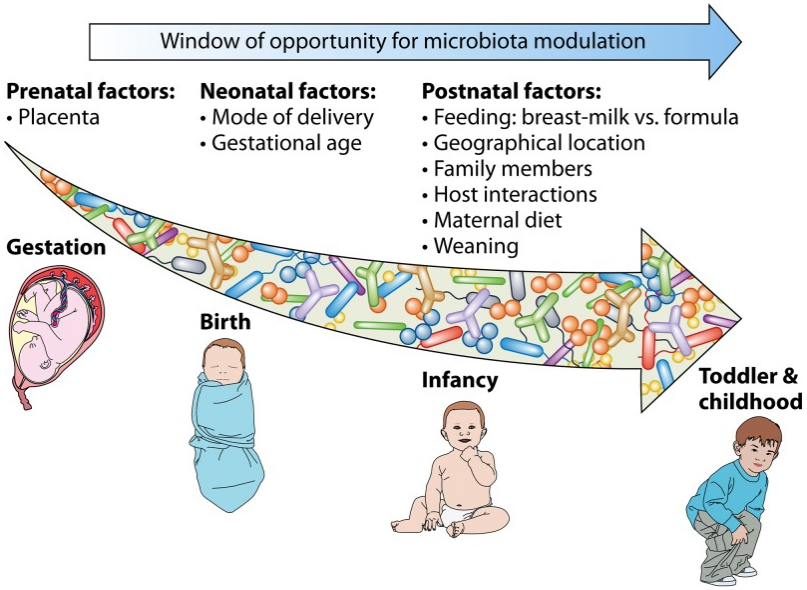

Fig. 3. Some of the factors affecting the modulation of the microbiota at every stage of life.(12)

The following are the main factors that may change the composition and function of the GM throughout life. They will be discussed in more detail.

7.1.3.1. Diet

After birth, diet is a critical driver of the formation of the infant's GM as it adjusts to the changing availability of nutrients (either enriched in genes to digest oligosaccharides found in breast milk, or later to digest polysaccharides and vitamins from solid foods).(16) Diet is considered to be one of the main modulators of the GM composition, the main one in adults.(2,3)

Long-term **dietary habits** profoundly influence the GM composition and especially the diversity of bacteria and the state of host metabolism and homeostatic systems. Thus, diets that affect a wide range of metabolic, hormonal, immune and neurological processes modulate all components of the MGBA, especially in early postnatal life.(11)

Vegetarian and fibre-rich diets are known to be associated with health, GM species diversity (predominantly *Firmicutes* and *Bacteroidetes*) and integrity of mucosal barrier function in the gut. The beneficial role of the **Mediterranean diet** in human health is also well known: it causes characteristic changes in the GM and is associated with anti-inflammatory effects leading to a decrease in the incidence of chronic diseases, such as cancer, autoimmune and NDDs. However, our society is exposed to a "Western lifestyle", characterised by an excessive intake of foods high in fat, cholesterol, animal proteins and a wide range of processed foods, which promote an inflammatory state leading to an increased risk of developing metabolic disorders, decreased immunity and increased susceptibility to infections. It has been correlated with an abundance of bile-tolerant genera (*Bacteroides*, *Bilophila*, *Alistipes*) and a suppression of *Firmicutes*.(3,16)

For all these reasons, diet can be seen as a **tool** to act on a dysregulated GM and improve disorders of the MGBA.(3) A **balanced diet** is essential to promote proper functioning of the GM.

7.1.3.2. Birth and Age

The early years of life represent an opportunity to promote optimal seeding and nourishment of the gut microbial ecosystem and, consequently, to guarantee a healthy state throughout life. This also coincides with a **critical period** for brain and CNS development, as many processes of morphological development, cellular and functional differentiation occur at this postnatal stage, in addition to the rapid increase in synaptic density.(2) Apart from the maternal microbiota which forms the initial inoculum, the **mode of delivery** and **lactation** are two important factors that determine the microbiota of each individual: vaginal delivery (avalanche of antigens) and exclusive breastfeeding (rich in probiotics and prebiotics) permit the maturing of the neonate immune system and help to the establishment of a correct GM; birth by caesarean (proliferation of bacteria from the nosocomial environment and from the mother's skin) is related to several metabolic and immunological problems and is very often practised unjustifiably. At this early stage, the maternal diet is also important for the neurological development of the offspring.(3,11)

The neonatal GM shows large interindividual differences, being more variable over time and between individuals than that of adults, and is characterised by a dominance of bacteria belonging to the phyla *Proteobacteria* and *Actinobacteria*. This microbiota develops in quantity and diversity over the first year, with the emergence and dominance of *Firmicutes* and *Bacteroidetes* phyla. The exact age at which a stable adult-like GM structure is formed is still unclear, but generally this happens at an age of around 2.5 to 3 years.(3,12) Despite this relative stability, **GM changes as the host ages** and impacts organismal ageing and lifespan.(11)

During **senescence**, the GM becomes unstable and highly susceptible to environmental factors; there are compositional changes and a significant **decline in diversity**. Although there is no definition of the GM profile characteristic of old age, a decrease in beneficial microorganisms and an increase in facultative anaerobic bacteria such as enterobacteria and in some opportunistic pathogens (*Clostridioides difficile* and *Klebsiella pneumoniae*) have been reported. These changes also manifest themselves in the form of reduced levels of SCFA.(3,16)

Such an altered microbiota is accompanied by a reduction in cognitive function and brain volume, and appears to contribute to the pro-inflammatory state of the elderly (**inflammageing**). These age-associated changes in both brain morphology and immune system alteration occur in parallel with increased oxidative and inflammatory stress, a weakening of BBB permeability, a generalised

disruption of homeostatic systems and the accumulation of amyloid plaques in the brain, leading to both impaired cognitive and behavioural function and the development of neural disorders such as **NDDs**. Besides the changes associated with the ageing process itself, other factors such as infections, pharmacological treatments (common and chronic in the elderly), changes in diet (low intakes of fibre and some vitamins and minerals are also common) and/or institutionalisation may introduce new and profound alterations to the GM.(3,11)

As a therapeutic strategy for the benefit of mental health, manipulation of the MGBA in these "**critical time windows**" (postnatal, adolescence, old age) could prevent the risk of neurodevelopmental disorders that can affect lifelong health and also slow down the onset of the neurodegenerative (ND) process as we age.(3)

7.1.3.3. Host Genetics

Different studies demonstrate that host genetics contributes significantly to the species richness and abundance of different taxa in the gut microbiome (where some phyla are more **heritable** than others), as well as contributing to variation in susceptibility to pathogens.(12,16)

7.1.3.4. Exercise

In humans, several studies have shown that **physical activity** enriched the diversity of GM, also increasing SCFA, both because of intrinsic adaptations to endurance training (decreased blood flow, increased GI transit) and because of the healthy lifestyles to which athletes are normally exposed.(16) Moreover, it has been observed that moderate exercise may have an effect on brain structures and functions; it may offer a potential reversal of ageing of brain structures.(3)

7.1.3.5. Antibiotics

The use of antibiotics is a two-edged weapon as it destroys both pathogenic and beneficial microbes, causing an alteration of the GM, either by direct or indirect mechanisms, and depending on the type of antibiotic, dosage and duration.(14,16)

The **loss of diversity** during antibiotic treatment is characterised by overgrowth of resistant opportunists (usually being enterobacteria) and other species with potential pathogenicity (*Enterococcus faecalis* and *Fusobacterium nucleatum*), while the abundance of fermenting species (bifidobacteria and butyrate producers like *F. prausnitzii*) is reduced.(3) Through these effects, antibiotics can play a role in the possible pathogenesis of many diseases, ranging from depression to autoimmune diseases.(11)

New scientific evidence suggests that other non-antibiotic drugs such as laxatives, antidiabetics or antidepressants may also affect the GM composition, potentially influencing behaviour and brain function.(3)

7.1.3.6. Geographical Impacts and Environmental Exposure

The proportion of each phylum of the human GM changes according to geological **location**, depending on atmospheric, genetic, dietary and other factors related to different **lifestyles**.

It is important to consider the contact with a rural or urban environment condition in the diversity of our microbiota, which is more diverse in children living in a rural habitat with a more traditional lifestyle than in more modern populations in developed countries. This decline in diversity is associated with an increase in the prevalence of common chronic metabolic disorders.(14)

Exposure to **pollutants** such as polycyclic aromatic hydrocarbons, pesticides or benzene derivatives may also alter the composition or metabolic activity of the GM, what may have physiological consequences; the MGBA may be a possible pathway of impact on mental health.(3)

In addition, other characteristics such as **pet keeping** or **smoking** have also been suggested to lead to significant differences in GM.(11,16)

7.1.4. DYSBIOSIS

The wide range of factors just seen can cause changes in the balance of the GM, thus altering its homeostasis and leading to a so-called state of **dysbiosis**. This condition is characterised by **quantitative** (fewer beneficial species) and **qualitative** (reduced variety) changes in the composition of microbiota, the results of which are potentially noxious to the host, as the state of physiological symbiosis between host and microbiota is broken. In any case, it is a controversial concept due to the lack of an accurate description of a "normal" or healthy microbiota,(3,12,17) and there is also the question of whether dysbiosis is the first event or whether it is the disease that causes a disturbance of the microbiota.(11)

Many studies describe **associations** (which not necessarily indicate causality) between the presence/absence of a range of microbial species and the disease, which helps to build hypotheses linking altered colonisation patterns and the aetiology of various pathological conditions (such as inflammatory bowel diseases, obesity, diabetes, allergy, cardiovascular and autoimmune diseases, etc.).(16,18) These pathologies are often associated with inadequate functioning of homeostatic systems and the basis for them is the presence of **oxidative and inflammatory stress**, which may be generated by the altered microbiota. Thus, the destruction of the balance of GM is directly related to increased intestinal permeability, which induces inflammation and oxidation in the gut and in all the organism. This eventually leads to the increased permeability of blood-brain barrier (BBB), influencing the oxidative and inflammatory state of the **CNS**. Within the possible impact of **gut imbalance on the MGBA**, given that beneficial actions of the commensal microbiota extend also to the CNS and seem to be essential for brain development and homeostasis, developmental disorders (autism, attention deficit hyperactivity disorder), psychiatric diseases (anxiety, depression, bipolar disorder), neurological diseases (epilepsy, headaches) and NDDs (AD, PD) are found.(3,11,19,20)

Several bottom-to-top directional pathways, activated by the products of the microbiota, are necessary for the proper development and physiological functioning of the brain. This is why dysbiosis, by modifying the activity of the gut and its ability to control its own microbiota, contributes to several NDDs, where changes in microbial molecules **levels** to be described below have been reported.(3,11,17)

In general, then, dysbiosis may cause a decrease in anti-inflammatory bacterial populations, excessive production of harmful molecules, intestinal barrier (IB) and BBB dysfunctions, and the development of various gut and brain disorders. Additionally, it is over the last decade that much evidence has been accumulated in favour of a significant association between dysbiosis, neuroinflammation and **neurodegeneration**.(17)

Although, until now, dysbiosis has been studied as the origin of several CNS problems, since this communication axis is bidirectional, the alteration of the IB, of the gut homeostasis and of the GM can also be seen as a **consequence** of several brain diseases.(11) These are issues that, among much literature, are still open.

7.2. THE MICROBIOTA-GUT-BRAIN AXIS

The concept of GBA, a **bidirectional** channel of communication between the "big brain" (**CNS**) in the cranium and the "little or second brain" (the enteric nervous system or **ENS**) in the abdomen, linked by neurons of the autonomic nervous system (ANS), as well as by circulating hormones and other neuromodulator molecules, is far from new and has long been visualised as the mediator of stress-related GI symptoms. Thus, motor, sensory and secretory functions of the GI tract are under control of signals generated in the CNS and, conversely, visceral messages from the GI tract can modulate brain functions (feelings, emotions, motivations and higher cognitive functions such as learning and memory capacities) in both normal and pathological states. In view of the importance that has been attributed to the GM in relation to host health, this axis has now been extended to include the **microbiota** (**M**GBA).(5,13,19)

The MGBA consists of the CNS (processing centre: brain and spinal cord), the ANS (it controls the glands and internal organs) and the ENS (complex network of neurons and glial cells that exists at the interface between the microbiota and the host; it directly controls the GI system), together with the neuroendocrine and neuroimmune systems and, obviously, the microbiota, resulting in a **complex interaction** between the gut and the brain.(21,22) The similar morphology, physiology, pharmacology, developmental patterns and neurochemistry shared between the distant CNS and ENS suggest that if bacteria can influence the ENS, they could have a similar impact on the CNS if they, or their messengers, could reach there.(2,5)

Tantalizing evidence to suggest that bacteria resident in the gut could impact on the "big brain" has emerged. There are multiple direct (such as vagus nerve) and, more frequently, indirect (neurotransmitters, SCFA, hormones, cytokines) pathways through which the GM can modulate the GBA. At present, the exact mechanism of communication between gut microbes and the brain is not yet fully understood and elucidated but, in general terms, a distinction can be made between **3 different pathways** or communication routes: **neural** (ENS, sympathetic and especially parasympathetic branches within the ANS), **endocrine** (e.g., hypothalamic-pituitary-adrenal axis) and **immune** (gut and neural-immune system).(5,21,23)

In turn, the CNS is said to affect the GM directly through stress mediator-induced virulence gene expression and indirectly through ANS-mediated control of gut function, modifying the intestinal neuro-immuno-endocrine dialogue and consequently the dialogue between the gut and the microbiota, as well as affecting the diversity and function of these microorganisms. In addition, the ENS can directly modulate microbial composition through changes in basic intestinal functions that it regulates like secretion, motility, permeability and immune defence.(2,3,11,13)

Achieving a proper MGBA will depend on the establishment of an **appropriate dialogue** of the microbiota with the **homeostatic systems** (nervous, endocrine and immune), which will be influenced by numerous genetic, environmental and lifestyle factors(3,11) and will condition the development and function capacity of these systems and consequently the health status.

The three different communication routes outlined will be discussed one by one below (7.2.1-7.2.3), mentioning their most characteristic "participants", the potential effects they may produce and the scarce mechanistic information that is known about them.

7.2.1. Neural Pathway: Vagus Nerve, Neurotransmitters and SCFA

Afferent and efferent neurons, within the parasympathetic (vagal) and sympathetic (splanchnic and pelvic spinal pathways) branches of the ANS, convey the **neuronal signalling**. In the CNS, both spinal and vagal afferent inputs synapse with higher brain regions, particularly with the emotional motor system, consisting in the limbic system and in some paralimbic structures, which coordinate responses to emotion along the GBA. In particular, afferent vagal neurons have their cell bodies in the nodose vagal ganglion (**NVG**) and transmit sensory information to the nucleus of the solitary tract (**NTS**) in the brain stem regarding the presence of food, motor activity and degree of gut distension, whereas afferent spinal neurons have their cell bodies in the dorsal root ganglia (**DRG**) and contribute to transmit sensory inputs to the dorsal horn neurons of the thoracic and upper lumbar spinal cord, which then project with second-order neurons to the brain and modulate unconscious responses to visceral sensory inputs (arousal, orientation, autonomic responses, prototype behavioural and emotional responses, including pain).(19)

Direct neural communication between GM and the brain (in both directions) is mainly realised through the **vagus nerve** (VN), also called X cranial nerve, the longest and most complex one.(20,23) The contact between the VN and the GM (shown in **Fig. 4**) is not direct because its afferent fibres do not cross the epithelial layer. Still, they can sense specific signalling of the microbiota or the enteroendocrine cells (EECs; distributed throughout the GI mucosa representing just over 1% of the epithelial cells in the intestinal lumen), which recognise microbial products through Toll-like receptors (**TLR**; the first line of defence system against microbes). Additionally, **neuropod** cells, a subset of EECs, can synapse with neuronal circuits maintaining the communication with the plexuses of the ENS and consequently with the VN.(19,22)

Vagotomy studies in mice have shown that vagotomy blocks central signalling of *Lactobacillus* and *Bifidobacterium* species, resulting in the siege of their mood-modifying effects.(2,24) Moreover, it has been demonstrated that individuals who underwent a full truncal vagotomy for treatment of peptic ulcer disease have a decreased risk for certain neurological disorders such as PD when they enter old age.(18) This underlines the role of this neural pathway within the axis.

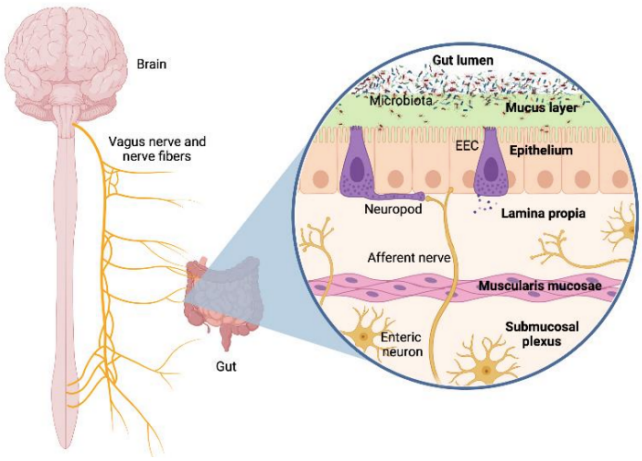

Fig. 4. Vagus nerve as the main direct neural pathway: sensory input travels through the ganglia from the intestine to the brain (afferent route). Involvement of EECs and neuropod cells is shown. EEC: enteroendocrine cell.(22)

Gut microorganisms, as a result of evolutionary adaptations, can secrete several **neuroactive substances** to determine the proper development and function of the ENS by affecting neuronal and glial cells in the gut. Some of these are inhibitory **neurotransmitters** such as serotonin (5-HT) and GABA, and some are excitatory such as dopamine, glutamate, and acetylcholine (ACh).

Hence, a lot of necessary neurotransmitters in the body are generated by the GM, exerting influence on the human body including the brain; e.g., low GABA levels are associated with psychiatric illnesses, and dopamine deficiency relates to addiction and PD. The neuroactive amino acids tyramine and tryptophan, SCFA and bile acids (which influence each other, with microbiota, in their composition) are other molecules of interest. Bacterial enzymes may also produce **neurotoxic metabolites** such as D-lactic acid and ammonia and, of relevance to AD, bacteria have also been shown to produce amyloid. All these substances can act directly on the ENS (via intrinsic primary afferent neurons and motor neurons) or indirectly through the EECs and VN, but it is improbable that they directly influence brain physiology because even if they enter the blood stream, most of them are incapable of crossing the BBB. These neurochemicals also enable intracellular communication between the members of the microbiota.(5,18–20,22–25)

The way this gut-brain communication may happen has been studied more extensively for **SCFA**, **5-HT** and other tryptophan metabolites, which have been shown to exert **major influences** on ENS and CNS development and functions including GI motility, mood and cognition.(2,11)

In recent years, research has explored the potential role of **SCFA** in regulating the brain neuron and glial homeostasis and thus their function capacity, and in supporting BBB integrity.(19,24,25) Many findings link butyrate to memory, cognition, mood and metabolism. For example, it can modify the expression of the brain-derived neurotrophic factor (BDNF) in the hippocampus and can change its activation in response to emotional stimuli, as well as control the progression of cognitive decline in ageing.(11,25) *F. prausnitzii* and *Roseburia* spp. are the main butyrate-producing bacteria. The decrease of these strains in the intestine is related to diseases such as Parkinson's, Crohn's, or ulcerative colitis. Additionally, it has been observed that NaB (sodium salt of butyrate) has therapeutic effects on Alzheimer's and Huntington's disease models,(22) and butyric acid has been shown to produce antidepressant-like effects in preclinical tests.(26) Moreover, these SCFA are inhibitors of histone deacetylases and can also elicit intracellular signalling by binding to their cognate G-protein coupled receptors to influence a range of physiological functions throughout the body. They are also involved in the regulation of the differentiation, recruitment and activation of immune cells.(19)

On the other hand, much research has linked GM with **5-HT** regulation in the gut, as it influences tryptophan (precursor of serotonin) uptake and, in that way, 5-HT synthesis.(13,22,25) One of the specialised neuroendocrine cells in the gut mucosa, called enterochromaffin cell (ECC), with mechanosensory and chemosensory functions that regulate motility and secretion, synthesises and contains more than 90% of the body's 5-HT, which synthesis and release is modulated by SCFA, by up-regulating tryptophan hydroxylase 1 (TPH1) expression in ECCs.(2) The fraction of free tryptophan reaching the circulation may cross the BBB to participate in 5-HT synthesis in the brain, since it is involved in mood, cognition, sleep and appetite control.(19,24,25)

7.2.2. Endocrine Pathway: Hypothalamic-Pituitary-Adrenal Axis and Hormones

GM functions as an endocrine organ by itself because it can produce and metabolise chemicals that are very similar to human **hormones**, affecting gut homeostasis, but also regulating a wide range of physiological processes in all host organs, such as the brain, reached via the bloodstream. In fact, bacterial products can stimulate EECs and produce neuropeptides such as neuropeptide Y (NPY), peptide YY, cholecystokinin, glucagon-like (GLP) and substance P, which act as **neurohormones** by binding to their corresponding receptors on the VN, or mediate other behavioural responses by binding directly to their cognate receptors in the brain.(13,26) Moreover, hormones can directly affect the richness and diversity of microbiota.(3,11,22)

GM also intervenes in the hypothalamic-pituitary-adrenal (**HPA**) axis, one of the central endocrine systems of the body that controls **stress** responses (via cortisol release), a process that promotes an **increase** in the **permeability** of the GI tract, also involved in regulating mood, emotions and the immune system.(22,25) Stress and the HPA axis can, conversely, affect the composition of GM, a fact tested with the early stress of separating a rat from its mother at birth.(23)

7.2.3. Immune Pathway: Microglia, Lymphocytes and Cytokines

Between the brain and the intestine there is a dynamic balance that the ENS and the HPA maintain, but a critical intermediary that interacts directly with these two is the immune system.

GM can modulate the CNS-resident immune cell function, so it is involved in the maintenance of a healthy brain. The immune response plays vital roles in brain development and homeostasis. It is carried out by myeloid cells, such as **microglia** and macrophages, and in less amount dendritic cells, but also by **lymphocytes**, which are in parenchyma and especially around it.(11)

GM contributes to the development and maturation of gut immune response; it regulates both innate and adaptive immunity. The microbial-associated molecular patterns (MAMPs) are the way microbiota presents itself to the immune system. One of these is **lipopolysaccharide** (LPS) from pathogenic bacteria, which stimulates dendritic cells, macrophages, and neutrophils via TLRs. The activated innate system components produce **inflammatory cytokines** such as tumour necrosis factor-alpha (TNF-α) and interleukins 1 (IL-1) and 6 (IL-6), which can act on the afferent nerves (VN) and also cross the BBB, interacting with the microglia (which eliminates dead neurons and promote inflammation) and altering its activation state and function (**neuroinflammation**). Another signalling pathway by which the microbiota affects the microglia is SCFA, which can promote certain lymphocytes T differentiation and inhibit cytokines such as IL-6 and IL-8, therefore exerting an anti-inflammatory action (neuroprotective factor).(20,22–24)

Nowadays, it is widely recognised that most neurological conditions, including some forms of depression, autism spectrum disorders, epilepsy, AD, PD and cerebrovascular diseases have **low-grade systemic inflammatory components**, indicative of a malfunctioning innate immune response and dysbiotic microbiota; the adaptive immune system can also be perturbed by bacterial proteins that cross-react with human antigens. Moreover, there have been extensive studies of the causal role of GM in inflammatory bowel disease (IBD), where there is an alteration of intestinal permeability and subsequent translocation of bacteria from the gut lumen to the systemic circulation, which is associated with an increased susceptibility to PD.(2,20,25,26)

These **TLR pattern recognition** receptors are part of the innate immune system, which is the first step in producing the cytokine response; they are also widely distributed in neurons.(23) These receptors have been found to be reduced in the brains of both GF-treated and antibiotic-treated mice, suggesting that gene expression in the brain is sensitive to microbiota manipulation. How microbe-TLR communication affects ENS structure and function, and how these changes relate to gut-brain signalling, has yet to be determined.(2)

For all the above mentioned, is understandable that a **balanced GM** is associated with an adequate immune system and inflammatory response. A healthy innate immunity can properly regulate the function of adaptive immunity cells (B and T lymphocytes), which are essential for **discriminating** between the pathogenic microbes, which have to be destroyed, and the GM components, such as food antigens, which have to be tolerated, thereby modulating the inflammatory response.(2,3,11)

A concluding sentence may be that, due to the existence of the MGBA, any changes in the GM may also lead to changes in brain function and thus affect, among others, host behaviour.
In **Fig. 5**, the 3 pathways explained above are illustrated:

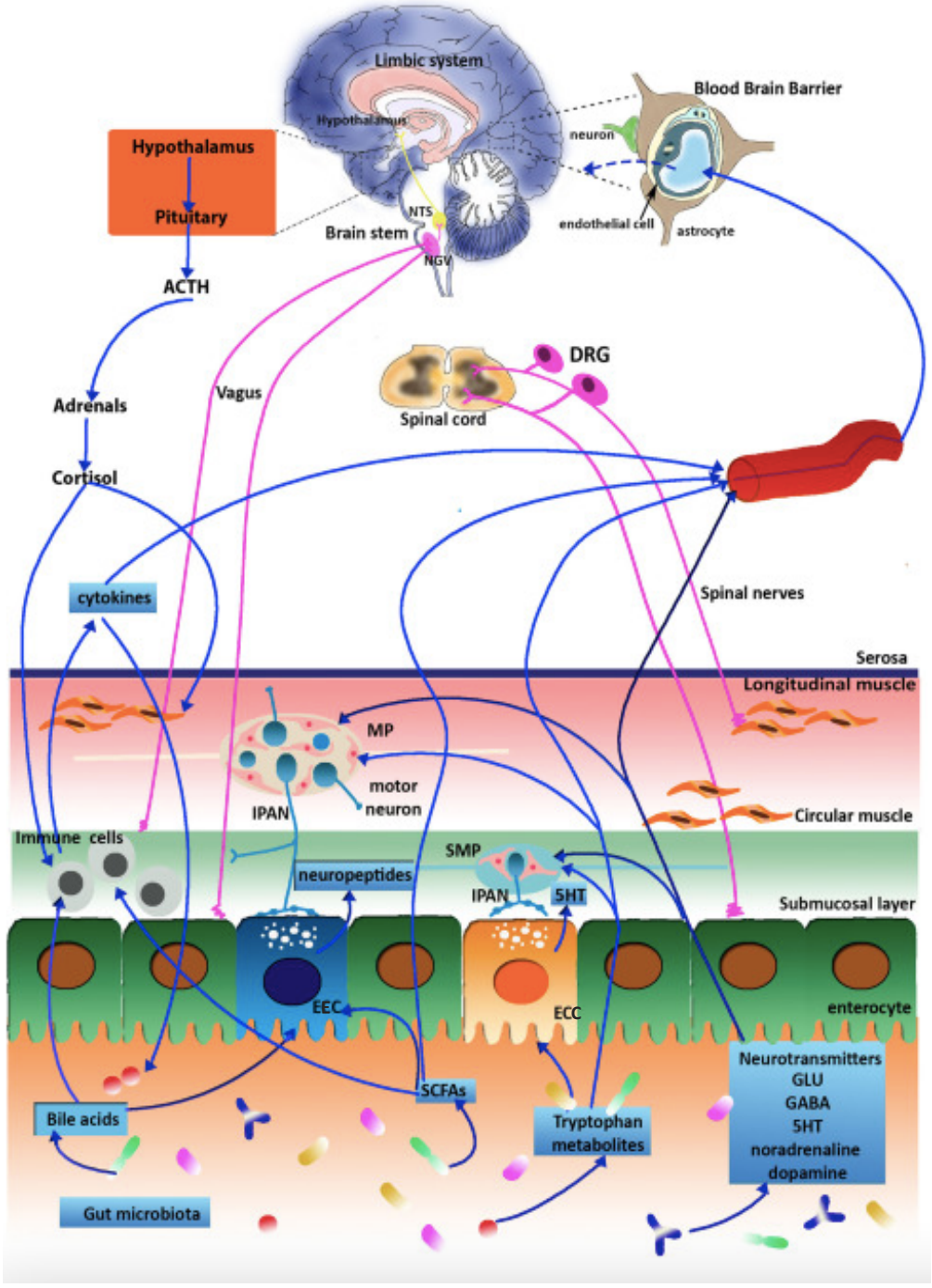

Fig. 5. Schematic representation of the microbiota-gut-brain axis: the gut microbiota -GM- signals to the central nervous system and to the enteric nervous system via **endocrine** (hypothalamic-pituitary-adrenal axis and cortisol release), **immune** (immune cells recognising microbial products and producing cytokines that can cross the blood-brain barrier) and **neural** (spinal and vagal afferent inputs synapsing with higher brain regions, ECCs releasing 5-HT, and GM producing neuroactive substances such as various neurotransmitters, SCFA or bile acids, which will either act at the enteric nervous system level, via the vagus nerve or through the bloodstream to the brain) **pathways**.
ACTH: adrenocorticotropic hormone; DRG: dorsal root ganglion; ECC: enterochromaffin cell; EEC: enteroendocrine cell; GABA: gamma-aminobutyric acid; GLU: glutamate; 5-HT: serotonin; IPAN: intrinsic primary afferent neurons; MP: myenteric plexus; NGV or NVG: nodose vagal ganglion; NTS: nucleus of the solitary tract; SCFAs: short-chain fatty acids; SMP: submucosal plexus.(19)

7.3. MICROBIOME-MEDIATED NEURODEGENERATION

NDDs are complex multifactorial diseases characterised by an accumulation of neurofibrillary tangles and senile plaques, increased oxidative stress, and consequent neuron and synapse degeneration. Currently, as stated before, nearly 50 million people worldwide suffer from **NDDs**, mainly dementia, and this number is expected to reach 152 million by 2050 due to the increase in average life expectancy. Neurodegeneration causes various cognitive impairments, affecting mostly learning and memory, with a progression in the symptom severity over time.(4,6,7)

It has been recently suggested that **host gut microbiome** and its dysregulation (aberrant MGBA) may also be involved in modulating gut barrier and BBB integrity, neuroinflammation and protein misfolding, and therefore that gut microbes are associated with the aetiopathogenesis of various NDDs.(5,6,27,28) In addition, certain factors may potentiate these effects. Among these, **ageing**, with its attendant deterioration in the abundance and diversity of GM, as well as in immune, gut barrier and BBB functions, may be a critical player and is especially relevant in this context given the age profile of many NDDs. Diet is another critical factor; in the elderly, **poor diet** has been associated with lower GM diversity, inflammation and disability.(5,11,29,30)

7.3.1. PROTEIN MISFOLDING AND MEMBRANE DAMAGE

ND pathologies are commonly characterised by the misfolding, oligomerisation and gradual accumulation in the CNS of toxic species such as amyloid beta (**Aβ**) and tau (**τ**) in AD and alpha-synuclein (**α-Syn**) in PD. These protein alterations (which can occur or not through genetic mutations) trigger neuronal degeneration and dysfunction, and drive the progression of each disease. They are able to propagate from affected cells to neighbouring cells by a well-documented intercellular transfer of protein inclusions, resulting in cellular defects such as transcriptional alteration, mitochondrial dysfunction, and an impaired protein/RNA quality control system, thus leading to synaptic failure and neuronal apoptosis.(6,7,30,31)

Most importantly, these abnormal proteins are capable of inducing membrane damage in the brain, another common feature in NDDs, by assembling monomers into non-selective ion **pores** and subsequently inserting them into a variety of cell membranes leading to cytoplasmic leakage and cell death.(6)

Microbial products, such as **endotoxin** and functional **amyloid**, promote the fibrillation and aggregation of prion-like proteins such as α-Syn, τ and Aβ. These, once aggregated and in the CNS, form **insoluble aggregates** (Lewy bodies, neurofibrillary tangles and amyloid plaques, respectively) that overwhelm the phagocytic capacity of microglia, thus promoting aberrant immune activation and neuroinflammation.(32)

In PD and AD individuals, an increased abundance of *Enterobacteriaceae* taxa (*Escherichia coli* and *Salmonella* spp.) is associated with increased concentrations of **LPS** and the bacterial amyloid protein **curli**. Several studies show that both of them promote the aggregation of peripheral α-Syn, τ and Aβ, thereby accelerating fibrillogenesis and neuroinflammation. On the other hand, SCFA derived from the GI microbiota can inhibit amyloid aggregation.(6,13,29)

7.3.2. NEUROINFLAMMATION AND OXIDATIVE STRESS

All NDDs also share the common ground of displaying an increased inflammatory response in the brain, known as **neuroinflammation**. CNS has a tightly regulated innate and acquired immune response, but in some specific cases, such as neurodegeneration, peripheral inflammation can

reach the brain parenchyma. This process in the CNS usually starts with the activation of microglia and astrocytes, as the first line of the brain defence, leading to the initiation of the pro-inflammatory cascade; cytokines (such as IL-1, IL-6, TNF-α), chemokines and other inflammatory molecules are produced and cause local inflammation affecting BBB permeability, while recruiting peripheral immune cells like macrophages and T cells across the BBB.(4,6,32)

Referring to microglia activation, **TLR4** recognise different MAMPs, with **LPS** being the most well-characterised bacterial-derived molecule in NDD models, but also **misfolded proteins**. Neuroinflammation has been shown to increase Aβ production and aggregation, leading to a vicious cycle known as the amyloid cascade hypothesis.(6,31,32)

Whilst this activation and the associated release of cytokines and pro-inflammatory factors is beneficial in the context of a short-term threat to the CNS, in the context of a chronic inflammatory stimulus, such as prolonged exposure to stress or to pathogenic protein aggregates leading to dysregulation of innate immunity in the CNS, the tolerogenic response is suppressed and **neuroinflammation** can spread indefinitely, promoting **neurodegeneration**.(32)

Lastly, neuroinflammation is closely related to **oxidative stress**, which is characterised by an excessive production of free radicals, glutamate, reactive oxygen species (ROS) and reactive nitrogen species (RNS), mainly done by microglia. They are toxic for neuronal cells and therefore lead to local tissue damage. GM can interfere with the oxidative state of the CNS through production of anti-oxidants (vitamins), SCFA, polyphenols, neurotransmitters, or even toxic products.(4,32) Some gut bacteria metabolites are found to be directly associated with the increase in **ROS levels**, one of the most important risk factors of neurodegeneration.(33)

7.3.3. Barrier Evasion and Permeability

The BBB is a multi-layered unit which acts as a semipermeable barrier to control the passage of molecules and nutrients between the circulatory system and the brain parenchyma.(31)

An increased BBB permeability, typical in the elderly and in several NDDs due to systemic inflammation, facilitate the **entry** of bacterial cells and molecules and peripheral inflammatory mediators into the brain, which subsequently would exacerbate local neuroinflammation via TLRs.(6,32,34) Consequently, neurogenesis and neurotransmission may be affected.(35)

Although they are disparate sites, detrimental changes in the GM composition result in increase of intestinal permeability and systemic inflammation, which negatively affects the **BBB integrity** or exacerbates existing inflammation in the CNS. Likewise, SCFA can enter the circulation and decrease BBB permeability by increasing the expression of endothelial tight junction proteins, especially **occludin** and **claudin-5**.(31) Under homeostatic conditions, however, the presence of a pathogen-free GM can actually promote the maintenance of an intact BBB.(32,36)

7.3.4. Gut Dysbiosis and Microbiota-derived Products Reaching the CNS

In the presence of gut beneficial microbes, homeostatic mechanisms such as neuropeptide synthesis, promotion of IB integrity and immune cell regulatory functions are maintained. When there occurs a condition of **gut dysbiosis**, there is a decrease of anti-inflammatory molecules (like SCFA, H_2) to that of pro-inflammatory molecules (LPS, amyloids), also with an alteration of beneficial bacteria to that of pathogenic in the gut.

This causes mucin degradation, dysregulation of the IB and allows infiltration of pathogenic microbes and their metabolites and endotoxins, promoting local recruitment of immune cells and triggering **systemic inflammation**, as shown in **Fig. 6**.(29,31,33)

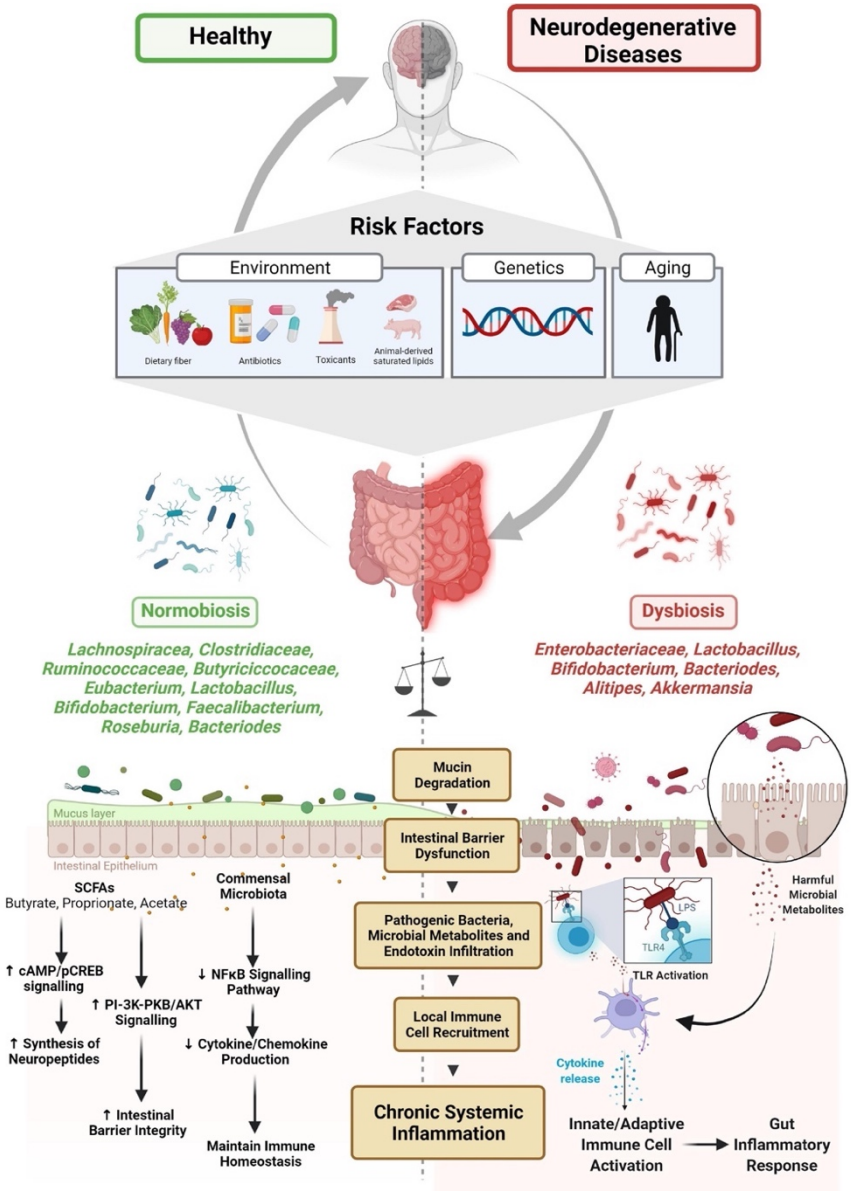

Fig. 6. Several risk factors have been associated with the **aetiology** of microbial dysbiosis and neurodegenerative diseases. The balance between normobiosis and dysbiosis within the gut microbiome is preserved by the presence or absence of high-abundant species (in green) and low-abundant species (in red). cAMP/pCREB: phosphorylation of the cAMP response element binding protein; LPS: lipopolysaccharide; NFkB: nuclear factor-kappa B; PI3K-PKB/ AKT: phosphatidylinositol 3-kinase-protein kinase B/Akt; SCFAs: short-chain fatty acids; TLR: Toll-like receptor.(29)

This increased intestinal permeability and inflammation leads to increased circulating levels of microorganisms such as spirochetes, herpes simplex virus type 1 or *Chlamydia pneumoniae*, from which **harmful products** such as β-N-methylamino-L-alanine (BMAA; neurotoxin linked to neurodegeneration, cognitive impairment, astrogliosis and accumulation of neurofibrillary tangles), LPS, microbial amyloid, bacteria cell fragments and pro-inflammatory mediators are derived.(6,35,37) These derived metabolites will contribute to disease **propagation in the brain** by facilitating various disease processes, including chronic intestinal and systemic inflammation (elevation of inflammatory mediators and protein aggregates), therefore disrupting the BBB permeability and bypassing it.(6,29–31,34) Some derived compounds produced by GM were found in the brain, confirming that leaky gut can affect the permeability of BBB.(4)

Apart from the probable mechanism of the propagation of NDD by the GM, based on the induction of systemic inflammation by a pro-inflammatory gut environment (the well-known "**leaky gut**" hypothesis), there is a second likely mechanism based on the VN. In this theory (which is not independent of the first), inflammatory mediators produced by GM such as α-Syn enter afferent enteric neurons and are transmitted **retrogradely via the VN** to the dorsal vagal nucleus in the CNS, where they may specifically alter the mitochondrial integrity of neurons.(6,32)

Intestinal disorders that can cause dysbiosis and high risk for NDDs showing an altered gut permeability due to GM alterations include IBD (Crohn's disease and ulcerative colitis), irritable bowel syndrome (IBS) and celiac disease. Colonisation of the gut by inflammatory bacteria such as *Helicobacter pylori* is an additional established origin of dysbiosis that can exacerbate many pathologies, including NDDs.(29,30,32)

7.3.5. Alzheimer's and Parkinson's Diseases

NDDs such as AD and PD, two of the most studied in the context of the MGBA and on which this study will focus, represent a heterogenous collection of disorders that feature deterioration of the central and/or peripheral nervous systems and affect an estimated 8% and 1% of the world's population, respectively. Predictions by the WHO estimate that by 2040, NDDs will bypass cancer to become the second leading cause of death globally.(3,13) In relation to the GM, in both diseases it has been observed that patients show an **altered intestinal microbial profile** (in diversity, relative abundances and metabolites) from healthy control study participants; however, the precise role and mechanisms have yet to be fully elucidated.(2,3,13,32)

The advent of high-throughput sequencing, metagenomics (functional analysis), metabolomics (metabolites) and other techniques has revolutionised the study of the GM and its metabolism, and a number of studies have been already performed among subjects with both PD and AD.(5) **Stool samples** are naturally collected, non-invasive and can be sampled repeatedly, making them the source of samples for most GM studies. However, among other limitations, fresh faecal samples generally cannot be analysed immediately (preservation at -80°C; possible degradation) and it is becoming increasingly clear that there may be significant differences in microbial composition between mucosa and faeces (estimation bias). Similarly, microbial profiling by **16S ribosomal RNA** (rRNA) **gene sequencing** is the most common method to study bacterial phylogeny and taxonomy, mainly because it consists of highly conserved (potential universal primer binding sites for whole gene or fragment amplification) and hypervariable (species-specific sequences to discriminate between different bacteria and archaea) regions.(38,39)

7.3.5.1. Alzheimer's Disease

AD, the most common form of dementia affecting almost 50 million population worldwide and the major cause of morbidity and death in older people, is a chronic age-related progressive NDD that is characterised by gradual memory loss and cognitive decline. As stated above, the main and initial pathological features of AD, apart from inflammatory processes and consequences of oxidative stress, are extracellular deposition of senile plaques of highly insoluble Aβ peptide in the brain and the formation of intracellular neurofibrillary tangles composed of highly phosphorylated τ protein. This abnormal accumulation of proteins leads to activation of microglia for the clearance of **Aβ and τ proteins**, but with subsequent ageing chronic neuroinflammation occurs, mediated by excessive innate immune activation, causing reduced phagocytosis of Aβ (accumulation), synaptic disconnection, progressive neuronal cell death, leading to atrophy and AD.(6,28,30–34) Decreased levels of **BDNF**, a protein that particularly facilitates neuronal survival, differentiation, synaptogenesis and brain development, are also associated with the pathogenesis of AD, impacting memory based learning.(34)

The most convincing evidence of **GM influence** on AD pathology is an experiment showing that restoration of GF mice with a complex AD mouse-derived microbiome increases pathological findings of AD.(32) Additionally, Zhang et al., in 2009, showed that AD patients had increased levels of serum LPS and monocyte activation with respect to the controls.(40) Decreased SCFA were found in gut and faeces among patients with AD and also in PD patients.(4)

Down to the molecular level, it has been reported that AD patients have reduced GM diversity compared to healthy controls, as well as compositional changes.(5,6,13,28–31,35,37) (**Table 1**)

7.3.5.2. Parkinson's Disease

PD is the second most common ND pathology, with features of impaired motor function, which may be related to the progressive loss of dopaminergic neurons in the substantia nigra. It is also pathologically characterised, as stated above, by the intraneuronal aggregation and deposition of phosphorylated **protein α-Syn**, which contributes to the generation of protein inclusions known as Lewy bodies. Excessive ROS production, mitochondrial dysfunction and microglia activation (as in AD) are also found. Motor symptoms such as bradykinesia, rigidity, tremor and postural instability, and non-motor symptoms such as cognitive dysfunction, sleep disorder, depression, anxiety, apathy and dementia are seen. It is worth noting that **GI dysfunction** (weight loss, gastroparesis, constipation) is recognised as the first symptom that appears before motor symptoms. The extensive inflammation and pre-accumulation of α-Syn in the gut also indicates that the intestine is related to pathogenesis of PD.(6,28,32,33)

Nowadays, accumulating evidence suggests a **strong association** between GM and PD pathogenesis. For example, it was observed that risk of developing PD decreases almost 50% in those individuals who have gone for truncal **vagotomy**, clinical evidence supporting the belief that the VN is a pathological route for α-Syn.(6,13,27,29,32,33) Moreover, colonisation of mice by faeces from PD patients further accelerated the disease process.(5,28)

In terms of composition of gut microbes, some of them have shown abundance alterations in PD patients compared to healthy controls.(5,13,28,29,32,35,37) (**Table 1**)

Table 1. Comparison of the main and most evidenced gut microbial changes in Alzheimer's and Parkinson's diseases (AD, PD). Data obtained from studies at different taxonomic levels. LPS: lipopolysaccharide.(5,6,13,28–32,35,37)

	Increased abundance	**Decreased abundance**
AD	**Genus**: *Escherichia* and *Shigella* (pro-inflammatory cytokines production inducers), *Lactobacillus*, *Streptococcus*, *Dorea* **Family**: *Ruminococcaceae*, *Enterococcaceae*, *Lactobacillaceae*, *Prevotellaceae*	**Species**: *Eubacterium rectale* (anti-inflammatory bacterium), *Bacteroides fragilis* (anti-inflammatory bacterium; gut leakiness reverser; short-chain fatty acids -SCFA- producer) **Family**: *Veillonellaceae*, *Bacteroidaceae*, *Lachnospiraceae*, *Fusobacteriaceae* **Class**: *Negativicutes*
PD	**Genus**: *Akkermansia* (mucin degrader), *Lactobacillus* **Family***: Enterobacteriaceae* (LPS producer), *Bifidobacteriaceae* **Phylum***: Proteobacteria* (proinflammatory)	**Species**: *Prevotella copri* (mucin and SCFA producer), *Faecalibacterium prausnitzii* (anti-inflammatory bacterium; gut leakiness reverser) **Family**: *Lachnospiraceae*, *Enterococcaceae* (SCFA producer; barrier integrity mediator)

Taken together, a mutual relationship is associated with altered gut microbes and pathogenesis of NDDs, with Alzheimer's and Parkinson's as representatives.(**Table 1**) The challenge now is to move towards a more mechanistic view and, as GI comorbidities are common in NDDs such as PD and AD, study the **potential of manipulating the GM** in clinical care to both treat and prevent these chronic conditions, since it is not yet clear whether the changes in the diversity and composition of GM are the cause or consequence of brain disorders.(1,2,4,28,30,32)

This prospective intervention will be considered in the following section.

7.4. MICROBIOTA AS A THERAPEUTIC TARGET: NEW APPROACHES

As life expectancy in developed nations continues to increase, and due to the consistently growing prevalence and the heavy burden of NDDs, the identification of the mechanisms underlying the pathogenesis, early disease biomarkers and both new drug targets and therapeutics to attenuate the progression of neurodegeneration are greatly needed.(13,31)

The **MGBA** is emerging as a particular area of interest and a potential new therapeutic **target** for effective treatment of brain-related disorders, including NDDs for which, currently, there seems to be no remedies as treatments are very **limited** and mostly **palliative** (section 7.4.1), aimed at treating the symptoms and improving the patient's quality of life but not at curing the disease. Since the GM constitute a changeable and experience-dependent ecosystem, and has been involved in the pathogenesis of various NDDs, it is not surprising that beneficial brain effects are now sought to be mediated through its **modulation**.(7,35,41–45)

Many studies have pointed out the significant role of various microbiome-based therapies in changing the composition of GM. However, the current pre-clinical studies and human clinical trials are still in the early stages and their efficacy in NDDs remains to be further validated.(46,47)

Below, the most widespread **drugs** and **diagnostic tools** for AD and PD (as representatives of NDDs) so far, and the pharmacological prospects that exist, will be analysed.

7.4.1. CURRENT STRATEGIES: DIAGNOSIS AND PHARMACOTHERAPY

For a long time, there have been four Food and Drug Administration (FDA) approved medications for the management of cognitive impairment and dysfunction in global activities in **symptomatic AD**. These include three cholinesterase inhibitors (**ChEIs**) named donepezil, rivastigmine and galantamine, which work to reverse the loss of cholinergic neurons thought to contribute to learning and memory dysfunction in AD by increasing synaptic levels of ACh inhibiting the enzyme that breaks it down, and an uncompetitive N-methyl-D-aspartate (**NMDA**) receptor antagonist named memantine that may act to reduce glutamate-mediated neurotoxicity that develops as neurons die during AD progression. The ChEIs are currently approved for use in mild-to-moderate AD whereas memantine is used in moderate-to-severe AD. However, in both cases, the effect size is quite small and the medication has no effect on long-term disease progression. In 2014, a fifth treatment option consisting of a fixed-dose combination with donepezil and memantine, which appears to have synergistic benefits, was also approved for the treatment of moderate-to-severe AD.(48–53)

In addition, on June 2021, FDA provided accelerated approval for the newest medication, aducanumab, a human **anti-Aβ** monoclonal antibody that selectively targets and helps to reduce amyloid deposits in the brain and may help slow the progression of AD, although it has not yet been shown to affect clinical symptoms or outcomes, such as progression of cognitive decline or dementia. Results of the phase IV clinical trial for aducanumab are expected to be available by early 2030. Then, **none** of the medications available at this time will **cure** AD.(50–53)

A person's quality of life may be impacted by a variety of behavioural and psychological symptoms that accompany dementia, such as sleep disturbances, agitation, hallucinations and delusions. Some other approved medications focus on treating these non-cognitive symptoms.(51)

Current **AD diagnosis** is primarily based on **neuropsychological** testing and assessments of detailed medical history and physical and mental status. A clinical diagnosis of AD requires

neuroimaging and monitoring accepted **biomarkers**, e.g., concentrations of Aβ-peptides as well as total and hyperphosphorylated τ proteins in the cerebrospinal fluid (CSF) and more recently in serum (more easily accessed), where changes in these levels develop decades before the onset of clinically significant AD. Amyloid oligomers and plaque accumulation can also be imaged with positron-emission tomography (PET). Magnetic resonance imaging (MRI) is another helpful, non-invasive approach for identification of functional abnormalities.(52–54)

PD treatment is also symptomatic, focused on improvement in motor (e.g., tremor, rigidity, bradykinesia) and nonmotor (e.g., constipation, cognition, mood, sleep) signs and symptoms. Disease-modifying pharmacologic treatments are not available either.

Since most symptoms of PD are caused by a lack of dopamine in the brain, **levodopa**, the metabolic precursor of dopamine, is often regarded as the gold standard of Parkinson's therapy. Concurrent administration of the peripheral decarboxylase inhibitor **carbidopa** prevents the decarboxylation of levodopa to dopamine outside the brain and encourages it to occur once it crosses the BBB. Other therapeutic groups that sometimes accompany levodopa, increasing its effectiveness, are **dopamine agonists** (such as bromocriptine, pergolide, pramipexole and ropinirole) which mimic the role of dopamine in the brain, catechol-O-methyltransferase (**COMT**) inhibitors (such as entacapone and tolcapone) and monoamine oxidase B (**MAO-B**) inhibitors (such as selegiline), being two enzymes that metabolise levodopa peripherally and dopamine centrally, respectively, **anticholinergic** medications (such as trihexyphenidyl) which restore the balance between the chemicals in the brain, or the antiviral drug **amantadine**. Approaches such as surgery on the deep brain structures involved in motion control (thalamotomy, pallidotomy), deep brain stimulation (through the implantation of small electrodes in the same structures) and levodopa-carbidopa enteral suspension can help individuals with medication-resistant tremor, worsening symptoms when the medication wears off, and dyskinesias. Aerobic exercise and physical, occupational and speech therapies complement pharmacologic treatments.(55–58)

Diagnosis of PD can take some time and is based on medical history and a physical and neurological examination, during which a neurologist will ask the subject to perform tasks to assess the agility of arms and legs, muscle tone, gait and balance. History can include **prodromal features** (e.g., rapid eye movement, sleep behaviour disorder, hyposmia and constipation, which precede physical manifestations and can lead to an early diagnosis), characteristic movement difficulty (e.g., tremor, stiffness, slowness) and psychological or cognitive problems (e.g., cognitive decline, depression, anxiety). Examination typically demonstrates **bradykinesia** with **tremor**, **rigidity**, or both. Absence of other possible causes of parkinsonism, such as the use of tranquilizer medications o stroke, and responsiveness to Parkinson’s medications, are other conventional methods for its diagnosis.(55–59)

There is no laboratory or imaging test such as PET or MRI that is recommended or definitive for PD. However, in 2011, the U.S. FDA approved an imaging scan called the **DaTscan** (dopamine transport scan). This technique allows to see detailed pictures of the brain’s dopamine system and can serve to confirm doctor’s diagnosis.(57–59)

The **biopharmaceutical industry** continues to work to explore new targets for early detection, developmental treatments and, ultimately, cures for NDDs, taking advantage of promising advances in digital technology tools. The 261 drugs currently in development for NDDs include 85 for AD and 64 for PD. But although the pipeline of new treatments is strong, the battle to find successful treatments and cures for NDDs is still considered a **challenge**. R&D for these types of diseases is complex as little is known about its biology and pathologic pathways involved; there

is a high rate of treatment failure and many NDDs have heterogeneous conditions, meaning there are multiple root causes that make it difficult to diagnose and treat patients.(49,60,61)

The current drug design strategy is based on the modulation of different targets to interfere with the ND process. In addition, given that these are multifactorial diseases, multi-targeted drug design is another method that is being worked on and has potential.(45,49,54) For example, the majority of AD-therapeutic approaches are focused on reducing levels of toxic forms of Aβ and τ, but most of the drugs that showed promising results in *in vivo* studies were not able to clear human clinical trials and failed, creating an urgent need to develop new and different strategies, leading to truly effective disease-modifying therapies.(48,49,52–54)

AD and PD are the most diffuse **incurable** dementias; therefore, the identification of a definitive therapeutic intervention is a major challenge of our time.(62)

7.4.2. Microbiome-based Diagnostics

Early diagnosis is still one of the main challenges in the management of NDDs as it could be essential to act at the earliest disease stages.(52,53,63)

Since, as discussed earlier, the human GM is associated with various disease states, and in view of the rapid advances in sequencing technology and computational biology which have opened the door to new and massive amounts of information, diagnostic **profiling of GM** is a very promising tool for earlier and non-invasive diagnosis.(64–66) These diagnostic technologies could link the composition of a person's microbiome to specific diseases, enabling early disease prevention, prognosis and treatment. Even so, as microbial signatures change in response to many types of factors, it frequently becomes difficult to establish clear connections.

Although initially hypothesised for GI disorders, **GM-derived biomarkers** have also been considered for psychological and NDDs, reporting powerful predictivity and differential diagnosis ability.(63,67,68) Therewith, it has been suggested that GI biopsies could serve as biomarkers for early stages of PD based on detection of α-Syn aggregates. However, even with early detection, clinical interventions that would halt the progression of PD remain out of reach.(32)

Despite promising results published and increasing global efforts recently made to develop and commercialise microbiome-based diagnostics, more research is still needed to limit interstudy inconsistencies and enhance **reproducibility** before considering a clinical application.(63,68)

7.4.3. Diet-based Strategies and Faecal Microbiota Transplantation

As introduced above, in the absence of a definitive treatment for AD and PD, where most therapies are limited to better symptom control but do not stop or slow disease progression, recent studies have focused on the role of the human microbiome in regulating multiple neurochemical pathways through the GBA and on looking for new and promising therapeutic approaches through the rational modulation of GM composition, in order to prevent or delay their onset or to counteract their progression.(13,62)

Preclinical and human studies on GM modulation through **oral bacteriotherapy** and **faecal microbiota transplantation** (FMT), listed in **Fig. 7**, showed anti-inflammatory and antioxidant effects, upregulation of plasma concentration of neuroprotective hormones, restoration of impaired proteolytic pathways and amelioration of energy homeostasis with a consequent decrease in the molecular hallmarks of NDDs, apart from a noticeable improvement of behavioural and cognitive performances.(46,47,62)

Moreover, as seen in section 7.1.3, some **environmental factors** are known to influence the GM and thus the onset and progression of NDDs. Focusing on the most important ones, recent meta-analysis demonstrated that regular **exercise** performed by elderly people is protective against AD and PD, slowing down the decline of cognition. Meta-analysis on the consumption of Mediterranean **diet** (MD) revealed better cognitive scores, and multiple preclinical and clinical studies provided evidence for a favourable relationship of MD with reduced risk of AD and later onset of PD. Lastly, **stress** exposure has been found to be clinically associated with accelerated onset, development and incidence of AD and PD. Therefore, these feasible changes in lifestyle could be considered as potential therapeutic treatments for various NDDs.

Understanding the specific mechanisms by which key environmental factors can modulate neurodegeneration will have major therapeutic implications.(42)

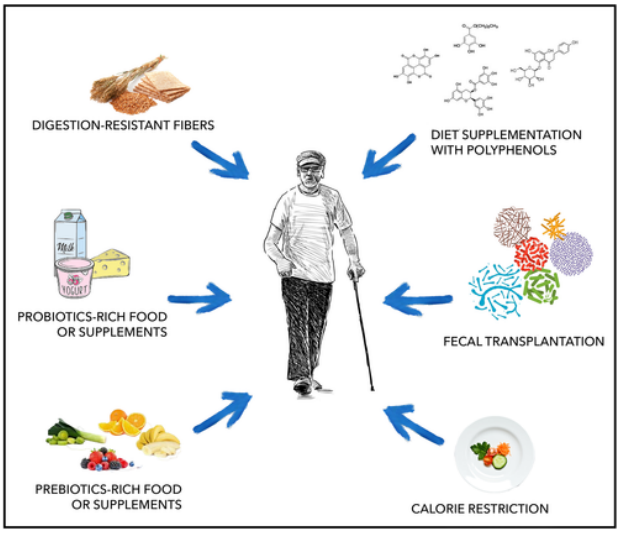

Fig. 7. Potential diet-based strategies (diet modifications and oral bacteriotherapy) and faecal microbiota transplantation used to modulate gut microbiota composition, favouring abundance of beneficial bacterial groups.(62)

7.4.3.1. Diet-based Strategies

As discussed in section 7.1.3.1, a diet rich in saturated fat, carbohydrates and highly processed foods may have detrimental effects on health contributing to the reduction of microbiota diversity, neuroinflammation and cognitive impairment. On the other hand, **healthy dietary patterns** show neuroprotective properties and delayed neurocognitive decline. On this regard, the MD is rich in many components considered helpful for mental health, among them vegetables, legumes, fruits, cereals and a high intake of unsaturated fatty acids and polyphenols.

In addition, **oral bacteriotherapy** through diet has been recently identified as an effective and accepted practice for the prevention and treatment of GI disorders with prominent gut inflammation. Beneficial effects of lactic acid bacteria and bifidobacteria in CNS-related diseases have also been reported.

Then, **diet-based** therapeutic interventions, usually cheaper and easier to handle than drug-based therapies, include: administration of **probiotics** ("live microorganisms that, when ingested in adequate amounts, confer a health benefit on the host", according to WHO) through specific supplements or probiotic-enriched foods, foods rich in **prebiotics** (substrate, mainly non-digestible fibres, selectively used to feed healthy bacteria), also **synbiotics** (combination of pro- and prebiotics) and **postbiotics** (metabolites of bacterial fermentation, including SCFA), supplementation with polyphenols, calorie restriction, and consumption of digestion-resistant fibres.(13,41,62,69,70)

The aim of these therapies would be to decrease the population of pathogenic endotoxin-producing bacteria, through **replacement**, and increase the population of butyrate-producing bacteria in order to re-establish a normal GM thus reducing systemic and neuro- inflammation and enhancing brain function and neurogenesis. Therefore, certain strategies should be adjusted depending on the underlying disequilibrium in gut bacteria.(13,70)

Although much work has been done on this approach, it presents many technical and pragmatic **difficulties**, such as ensuring the survival of the probiotic until it reaches the site of action or selecting the appropriate strains. *Lactobacillus* and *Bifidobacterium* strains, which have well-defined safety profiles, are widely used as probiotics in the clinical setting; both genera have been shown to have significant beneficial effects on human health, especially with regard to neurodegeneration.(41,46)

Recently, it has been demonstrated that transgenic AD mice treated with probiotics, as compared to untreated AD mice, have better cognitive performance and reduced number of Aβ plaques in the hippocampus. Other studies have indicated that high intake of probiotics, prebiotics and other nutrients decreases the risk of onset of AD. Probiotics can also ameliorate PD through a variety of mechanisms, including stabilizing symptoms of anxiety and depression, reducing symptoms of GI complications and inhibiting the growth of pathogenic bacteria. Research on prebiotics, synbiotics and postbiotics is more limited.(47,69)

Other than this, some **clinical trials** have revealed the effectiveness of probiotics in regulating GI disorders and in preventing or inhibiting cognitive or emotional disorders, thus suggesting the **potential of probiotics** for the treatment of NDDs. Even so, the specific mechanisms underlying their therapeutic properties still need further study.(47)

7.4.3.2. Faecal Microbiota Transplantation

Besides dietary interventions, FMT is another and **more promising** therapeutic option against NDDs that targets GM, but in a **less selective** and specific way. It involves the transfer of stool from a healthy donor into the GI tract of a patient aiming to **restore** the normal diversity and functionality of the microbial population. It can be administered directly via colonoscopy (84-93% efficacy; bowel preparation is necessary to remove pre-existing bacteria) and, less commonly, through flexible sigmoidoscopy or enema. For patients with ileus, severe colitis or contraindication to colonoscopy, FMT can be administered via nasoenteric tubes (prior administration of a proton pump inhibitor to increase survival of transplanted bacteria), esophagogastroduodenoscopy or capsule ingestion, a convenient and more recent modality (different routes are shown in **Fig. 8**).

This concept of FMT was introduced approximately 1700 years ago by a Chinese medical scientist named Ge Hong, when he orally administered human faecal material (then called "yellow soup") to treat patients with severe diarrhoea or gastroenteritis. Currently, this method is considered a **valid treatment** for the recurrent infection of *C. difficile* (CDI), Crohn disease and ulcerative colitis, and it was successfully tested in intestinal conditions including other IBD, diarrhoea, IBS and constipation. It is now being investigated for its possible application in **extraintestinal** conditions such as NDDs, given the existence of the GBA.

In general, FMT is considered a **safe** and efficacious therapeutic procedure with minor and transient side effects due to the introduction of live microorganisms and associated metabolites. However, **careful measures** should be taken to maintain the viability of the diverse microbial population, and the donor and the faecal material should be properly screened to avoid contamination of the patient with pathogenic microorganisms that could lead to serious infections. Other inherent major risks mainly associated with endoscopies and sedation, apart from **transmission of infections** such as bacterial translocation, include the possibility of aspiration

with bowel perforation, high-grade fever, worsening IBD, bleeding, cytomegalovirus (CMV) reactivation and pneumonia. In addition, some mild but more likely to occur GI symptoms have been reported following FMT, including constipation, diarrhoea, fever, abdominal discomfort, flatulence, bloating, belching, vomiting, nausea and borborygmi. FMT recipients should always be **informed** about these potential adverse effects before the procedure.(43,46,62,70–72) (**Fig. 8**).

There are still many obstacles and a **lack** of fundamental data. The exact and optimal microbial composition of the samples to be transplanted is still under investigation, and inter-subject variability and possible long-term effects need to be considered with further studies.(62,71)

Referring to **donor selection**, an issue that the expert PhD students contacted have stressed as being of extreme importance, they can be selected from intimate partners, family members or unrelated volunteers (**Fig. 8**), and fixed conditions are generally considered to prevent their recruitment: being obese; having IBD or IBS; taking antibiotics within three months; having an underlying infection; having a history of recent travel to diarrhoea-endemic areas; and a long etc.

A potential donor will always be **screened** for infective diseases by blood and stool tests and will be able to provide fresh stool within one month after screening. Although the ideal amount of stool weight has not been standardised, approximately 50-60 g of stool (stored for up to eight hours at 4°C with no decrease in bacterial survival) is recommended for each treatment, which is dissolved in approximately 150 mL of normal saline. The mixture is then filtered to remove large particles and drawn into 60 mL catheter-tipped syringes, which shall be labelled and stored at -80°C. On the day of infusion, it should be thawed in a 37°C warm water bath and infused within four hours from thawing (**Fig. 8**).

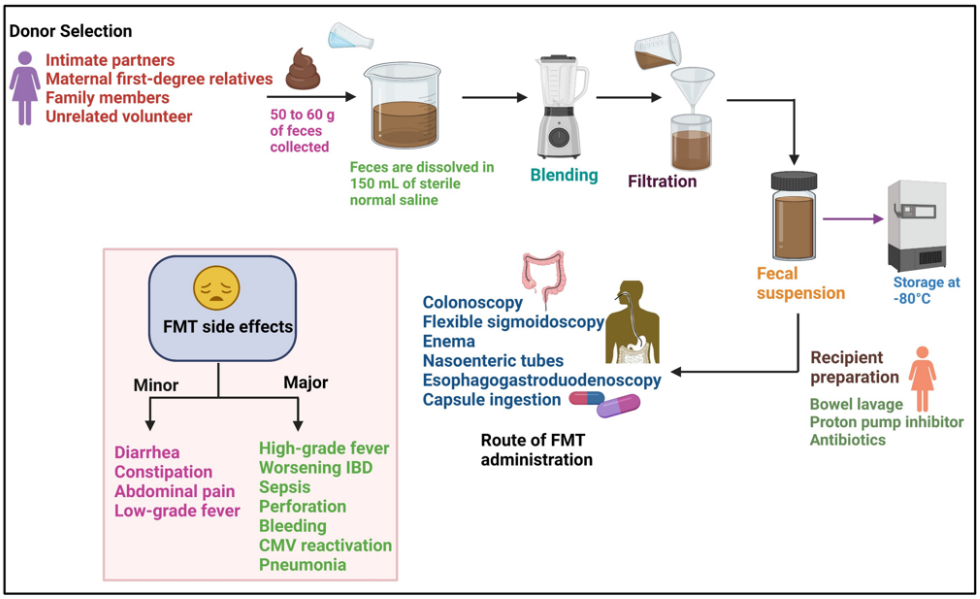

Fig. 8. Schematic diagram of the faecal microbiota transplantation (FMT) procedure and the associated adverse events. CMV: cytomegalovirus; IBD: inflammatory bowel disease.(72)

Another challenge, of both oral bacteriotherapy and FMT, would be to define when to apply them and which are the best clinical and biological markers to assess their impact. Having seen that dysbiosis is potentially implicated in the origin of systemic and CNS inflammation in NDDs, interventions to modify GM may be implemented even in the premotor phase of the disease, and a change in inflammatory markers could be a reasonable indicator of their effectiveness.(13,70)

In the following section 7.5, an own **revision** is presented of the latest experimental trials that have tested FMT as a treatment for NDDs; their most remarkable results and the possibility of a near clinical application as a **microbiota-based therapy** will also be discussed.

7.5. SYSTEMATIC REVIEW AND META-ANALYSIS

Background: **FMT** is currently considered the most effective GM intervention. To evaluate the indication of FMT for patients with NDDs, more specifically with **AD** and **PD**, the available latest literature on FMT is **summarised**. In addition, suggestions for future directions are provided.

Methods: In April-May 2022, a leading database, PubMed (Medline), was thoroughly searched, using the appropriate keywords, for studies and case descriptions on FMT in AD or PD (both as symptomatic and progression treatment) in human or animal models. The search was filtered by the previous five years. Furthermore, the ClinicalTrials.gov website was consulted for registered planned and ongoing trials with FMT in AD and PD.

Results: Of the nearly 100 identified studies, all observational studies to determine whether gut dysbiosis is clinically correlated with symptoms and progression of AD and PD, or reviews with information previously presented were discarded. Studies involving additional treatments other than FMT, or animals with different pathologies other than AD and PD were also excluded. Finally, 10 studies were comprised in this analysis (including animal studies and case reports), taking into account the scientific progress they represented, the variety of models used and the experimental explanation offered. Only studies where the full-text articles could be accessed were included.

Four of the studies were based on **animal models** (2 for AD and 2 for PD), more specifically, on commonly used mice models. A fifth interesting animal study, taken from the online library of *Alzheimer's Association* (73), was also added to assess the importance of the donor's age. They all suggested a positive effect of FMT on AD and PD (**Table 2**), what makes it an encouraging treatment option for humans. To conduct the studies, after the behavioural tests, all mice were deeply anesthetised, euthanised and their brains were quickly dissected to collect tissue samples.

In addition, the 3 **human case reports** (first cases) studied to assess the efficacy and safety of FMT on AD and PD patients supported this positive effect of FMT (**Table 3**). Moreover, there are also 3 recent preliminary uncontrolled **case series** that reinforce this efficacy and safety of FMT in PD: Kuai et al. (74) assessed the impact of FMT on 11 patients with PD and constipation and all of them were reported to have complete remission of constipation and very mild side effects; Xue et al. (75) administered FMT via two different routes to 15 PD patients (10 by colonoscopy; 5 by a nasogastric tube) and concluded an acceptable safety in both cases and a clear superiority of colonoscopic FMT in terms of efficacy; Segal et al. (76) conducted another study where in 5 of 6 PD patients motor, non-motor and constipation scores improved four weeks following the FMT, with only one patient experiencing an adverse event that did not require treatment.

Both the single and group cases involved subjects who were already taking medication for their dementia but showed very limited effect. In addition, all studies were conducted under informed consent from both the healthy donors (selected with a well-defined protocol) and patients, and routine examinations were completed to ensure the absence of contraindications of FMT. It is also important to note that faecal samples from patients were collected before and after FMT for 16S rRNA microbiota analysis, which showed taxonomic and significant SCFA levels differences and an increase in microbiota diversity at least during the first weeks post-FMT. Different genera with anti-inflammatory properties such as *Bacteroides* were the most enriched taxonomy after FMT; these were reported to be associated with cognitive function.

Lastly, seven **clinical trials** with FMT as treatment (only one for AD and six for PD) are currently planned or ongoing (**Table 4**). Although none of them have results posted yet, it is expected that the bulk of evidence on the efficacy of FMT in NDDs will grow.

7.5.1. Animal Models

Table 2. Faecal microbiota transplantation studies in different mice models. Evaluating the efficacy in Alzheimer's and Parkinson's diseases.

NDD	ANIMAL MODEL/ EXPERIMENTAL DESIGN	USED TECHNIQUES (before and after sacrificing mice)	RESULTS SEEN IN FMT-TREATED MICE and conclusions reached	REF [LOC]
AD	**APPswe/PS1dE9** transgenic (Tg) mouse model, aged 6 m. All males. It is one of the most extensively used Tg mouse model of AD, which express the Swedish mutation of APP together with PS1 deleted in exon 9. They are characterised by an early-onset age-related increase in Aβ-levels with Aβ plaques starting at 4-6 m of age, also with glial activation and deficits in cognitive functions.(77) Three groups (n = 8 per group): AD model group (Tg), Tg + FMT and WT mice (control group and FMT donors). FMT was done once daily for 4 w.	MWM (spatial learning and memory ability) and ORT (discrimination of a familiar from a new object) behavioural/cognitive function tests. Faecal DNA extraction and 16S rRNA gene sequencing. NMR spectroscopy for SCFA levels. Congo red staining for compact amyloid plaques; ELISA for Aβ levels; WB for τ, COX-2 and CD11b integrin (inflammation-related proteins); IHC and WB for PSD-95 and synapsin I (synaptic plasticity-related proteins).	FMT reduced brain deposition of amyloid plaques, phosphorylation of τ protein, levels of Aβ40 and Aβ42 and neuroinflammation (↓COX-2 and CD11b). It also improved cognitive function (better performances in both tests), increased synaptic plasticity (↑PSD-95 and synapsin I) and reversed the alterations of GM composition and SCFA (↑butyrate, similar acetate and propionate). FMT therapy prevented AD-like pathology in a mouse model, suggesting that it might be a potential therapeutic strategy.	(78) (2019) [China]
AD	36-w-old **5XFAD** transgenic mice. 5XFAD Tg mice overexpress five FAD mutations: 3 in the APP 695 gene and 2 in the PS1 gene. These mice exhibit amyloid deposition and gliosis at 2 m of age, and progressive neuronal loss (Aβ42 cerebral accumulation) accompanied by cognitive and motor deficiencies at 6 m.(79) Sixteen, 36-w-old 5XFAD Tg mice were treated with faecal slurry from healthy WT donors of similar age (n = 8; Old Tg-FO) or from younger (8-10-w old) healthy WT donors (n = 8; Old Tg-FY) for 7 d. The control 5XFAD recipient mice (30-32 w old; n = 7) received normal saline (Old Tg-Control).	EPM (anxiety-related behaviour) and NOR (recognition and spatial memory) memory/cognitive tests. Thioflavin S staining for Aβ plaque loading.	FMT improved spatial and recognition memory in Old Tg-FY and enhanced recognition memory in Old Tg-FO mice (better performances in both tests). Both FMT-treated groups demonstrated a decrease in the number of quantifiable amyloid plaques, especially Old Tg-FY mice. By reducing amyloid pathology (↑clearance of cortical Aβ) cognition is improved, thus exhibiting the efficacy of FMT as a therapeutic in AD. Results also indicated the importance of the **donor's age** in decreasing amyloid pathology and improving cognition; they were better with Old Tg-FY mice (younger healthy donor, with fewer inherent alterations of ageing).	(73) (2020) [Australia]
AD	**ADLP^{APT}** transgenic mouse model. ADLP^{APT} mice carry three human transgenes, including APP, PS1 and τ, with six mutations. These mice exhibit accelerated neurofibrillary tangle formation in addition to amyloid plaques, neuronal loss and memory deficit at an early age.(80) Fresh faecal matters of WT mice were orally provided to Tg mice for 16 w.	Behavioural tests: Y-maze (short-term spatial learning and memory), CFC (long-term spatial learning and memory function) and open-field test (anxiety-like behaviours). Faecal DNA extraction and 16S rRNA gene sequencing. ELISA for Aβ levels; WB for τ aggregates; IHC for Iba1-positive microglia and GFAP-positive astrocyte (glial markers).	Frequent FMT attenuated the cognitive and memory impairment (better performances in all tests), Aβ plaque burden and both Aβ40 and Aβ42 levels, τ pathology and reactive glial activation (↓glial markers).	(81) (2020) [Korea]

PD	**MPTP** employed to create a subacute PD-like model in eight-w-old male **C57BL/6** mice. C57BL/6 is a common inbred strain of laboratory mouse. MPTP is a prodrug to the neurotoxin MPP^+ which causes permanent symptoms of PD by destroying dopaminergic neurons in SN. Three groups: the normal control (n = 15 mice) without any treatment, the MPTP + PBS group (n = 15 mice) and the MPTP + FMT group (n = 15 mice), which received FMT from normal control mice for 7 d.	Behavioural tests: pole descent test (evaluation of bradykinesia) and traction test (evaluation of muscle strength and equilibrium). Faecal DNA extraction and 16S rRNA gene sequencing. GC-MS for SCFA levels. HPLC with a fluorescence detector to quantify the striatal neurotransmitters dopamine and serotonin and their metabolites (DOPAC, HVA and 5-HIAA); IHC for microglia and astrocytes markers; ELISA for TLR4, TBK1, NF-κB and TNF-α levels.	FMT improved motor function and alleviated physical impairment (improved performances in both tests), increased striatal neurotransmitters (and metabolites) and decreased neuroinflammation and gut inflammation possibly by suppressing TLR4/TBK1/NF-κB/TNF-α signalling pathway (decreased levels). FMT also improved GM dysbiosis (higher microbial diversity similar to WT mice), restored normal SCFA levels, and decreased the activation of brain glia in the SN. In contrast, it could be seen that FMT from PD mice had a pathological effect on healthy recipients.	(82) (2018) [China]
PD	Eight-w-old male **C57BL/6** mice injected with **MPTP**. Same model as above. Four groups (10 mice per group): the sham + PBS, the sham + FMT, the MPTP + PBS, and the MPTP + FMT. PBS or FMT (from healthy normal mice) treatments lasted 7 d.	Pole descent test (evaluation of bradykinesia) and traction test (evaluation of muscle strength and equilibrium) as behavioural tests. Faecal DNA extraction and 16S rRNA gene sequencing. GC-MS for SCFA levels. WB for α-Syn, PI3K, AKT, TLR4, and NF-κB levels; ELISA for TNF-α levels; IHC and IF staining to analyse dopaminergic neurons and microglial activation in SN.	FMT ameliorated motor disfunction and alleviated physical impairment (better results in both tests), maintained dopaminergic neurons and restored the TH (dopaminergic neuron marker) level in MPTP mice. It also inhibited the activation of microglia in SN. FMT could protect mice against PD via suppressing α-Syn expression (↓α-Syn levels in SN compared to MPTP+PBS mice) and inactivating the TLR4/PI3K/AKT/NF-κB signalling pathway in the SN and striatum (decreased levels, also of TNF-α). It also alleviated the disturbance of GM composition and SCFA.	(83) (2021) [China]

Aβ: amyloid beta; AD: Alzheimer's disease; AKT (PKB): protein kinase B; APP: amyloid precursor protein; CFC: Contextual Fear Conditioning; COX-2: cyclooxygenase 2; d: day/s; DOPAC: dihydroxy-phenylacetic acid; ELISA: Enzyme-Linked ImmunoSorbent Assay; EPM: Elevated Plus Maze; FMT: faecal microbiota transplantation; GC-MS: Gas Chromatography-Mass Spectrometry; GFAP: glial fibrillary acidic protein; GM: gut microbiota; 5-HIAA: 5-hydroxyindoleacetic acid; HPLC: high-performance liquid chromatography; HVA: homovanillic acid; Iba1: ionised calcium binding adaptor molecule 1; IF: immunofluorescence; IHC: immunohistochemistry; LOC: location; m: month/s; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MWM: Morris Water Maze; NF-κB: nuclear factor-kappa B; NMR: Nuclear Magnetic Resonance; NOR: Novel Object Recognition; ORT: Object Recognition Test; PBS: phosphate buffered saline; PD: Parkinson's disease; PI3K: phosphatidylinositol 3-kinase; PSD-95: postsynaptic density protein 95; PS1: presenilin 1; REF: reference; sham≅ placebo; SCFA: short-chain fatty acids; SN: substantia nigra; α-Syn: alpha-synuclein; τ: tau; TBK1: TANK-binding kinase 1; Tg: transgenic; TH: tyrosine hydroxylase; TLR4: Toll-like receptor 4; TNF-α: tumour necrosis factor-alpha; WB: Western Blot; w: week/s; WT: Wild-Type.

7.5.2. Human Case Reports

Table 3. First human attempts. Clinical application for faecal microbiota transplantation, based on microbiota-gut-brain axis, in Alzheimer's and Parkinson's diseases.

NDD	AGE	SEX	DIAGNOSIS	ADMINISTRATION ROUTE/ FMT FREQUENCY/ AMOUNT	DONOR	CLINICAL OUTCOME	LOC	REF
AD	82	M	AD (gradual 5-year decline in memory and cognition), rCDI (several courses of antibiotics failed previously)	Colonoscopy Single FMT 300 ml FMT infusion	His 85-year-old healthy wife	Remission of CDI symptoms and negative stool test 2 m later; MMSE score increased from 20 (mild cognitive impairment) to 26 (normal cognitive function) after 2 m; improvement in memory retention after 4 m; significant improvement in mood (MMSE score 29) after 6 m No adverse effects	USA	(84) (2020)
AD	90	F	AD (gradual 5-year decline in memory and cognition) and severe CDI (treated unsuccessfully with several antibiotics)	Colonoscopy Single FMT; second FMT when diagnosed with a recurrent severe CDI (GI symptoms) Stool suspension (60 g)	27-year-old male healthy volunteer	Severe GI symptoms improved, and a stool test for CDI was negative; improvement in cognitive function tests after 3 m: MMSE from 15 to 20, MoCA from 11 to 16 and CDR from 1 to 0,5 but also a new positive CDI stool test (solved 1 w after the second FMT); improvement in mood and daily living activities Nausea was noted for 3 hours after the first FMT; diarrhoea, fever and abdominal pain after 3 m	Korea	(85) (2021)
PD	71	M	PD for 7 years (resting tremor and bradykinesia); intractable constipation for >3 years (defecation needing more than 30 minutes)	TET tube (colon), inserted into the ileocecal junction 3 times (during 1 w) 200 ml of prepared faecal microbiota suspension injected through TET tube	26-year-old male healthy volunteer	Constipation cured even after 3 m (end of the follow-up period) with a decrease of Wexner constipation score from 16 to 10 and a decrease of defecation time from >30 to 5 minutes; UPDRS decreased but became similar to pre-FMT at 3 m; motor symptoms such as leg tremors relieved for 2 m No adverse effects	China	(86) (2019)

AD: Alzheimer's disease; (r)CDI: (recurrent) *Clostridioides difficile* infection; CDR: Clinical Dementia Rating; F: female; FMT: faecal microbiota transplantation; GI: gastrointestinal; LOC: location; M: male; MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment; m: month/s; PD: Parkinson's disease; REF: reference; TET: Transendoscopic Enteral Tubing; UPDRS: Unified Parkinson's Disease Rating Scale; w: week/s.

7.5.3. Clinical Trials

Table 4. Clinical trials at different stages testing faecal microbiota transplantation in Alzheimer's and Parkinson's diseases. Data accessed until 05/2022, from clinicaltrials.gov.

Study Title [NCT number]	Recruitment status/ First and last update posted/ Phase	NDD	Objectives	Study Design	Intervention/ treatment	Arms	Eligibility criteria	Outcome measures	LOC	REF
Oral Fecal Microbiota Transplant Feasibility Study in Alzheimer's Disease [NCT03998423]	Terminated (to minimise risk, as SARS-CoV-2 has been detected in faecal material) 06/2019-07/2020 Phase 1	AD	1: Asses the safety and feasibility of an oral FMT intervention in people with and without AD 2: Demonstrate the effects of FMT on the composition and function of the GM	Interventional; 5 participants; randomised; parallel assignment; open label	Biological: FMT FMT route: capsules	*Dose of 30 capsules (22,5g) of oral FMT 1: single dose (week 0) 2: double dose (week 0 and 8) 3: triple dose (week 0, 8 and 24)	45 years and older; all sexes; healthy volunteers accepted	Adverse events; 16S rRNA sequencing of recipient stool samples pre- and post- FMT; cognitive and neuropsychological screening tests like MoCA; CSF biomarkers; etc.	United States	(87)
Fecal Microbiota Transplantation for Parkinson's disease [NCT03808389]	Recruiting 01/2019-12/2021 Phase N/A	PD	1: Investigate FMT effects on symptoms and PD progression	Interventional; 40 participants; randomised; parallel assignment; double-blind	Biological: through FMT donor or via autologous FMT FMT route: nasojejunal	1: treatment group: FMT with donor stool 2: control group: FMT with own stool	50-65 years; all sexes; healthy volunteers not accepted	Neurological clinical examination and standardised clinical scoring scales including MDS-UPDRS, NMSS and MoCA; questionnaires; microbiome sampling; serum inflammation markers; GI biopsy; etc.	Belgium	(88)
A Trial of Fecal Microbiome Transplantation in Parkinson's Disease Patients [NCT04854291]	Recruiting 04/2021-04/2021 Phase N/A	PD	1: Asses the safety and efficacy of FMT intervention in PD patients with abnormal GM composition	Interventional; 48 participants; randomised; parallel assignment; double-blind	Biological: FMT Other: placebo FMT route: intracaecal infusion	1: experimental: FMT from a healthy donor 2: placebo comparator: carrier solution (NaCl + glycerol mixture)	35-75 years; all sexes; healthy volunteers not accepted	MDS-UPDRS I-III at 6 months (symptom changes); biospecimens; imaging data; etc.	Finland	(89)
Efficacy and Safety of Fecal Microbiota Transplantation in the Treatment of Parkinson's Disease With Constipation [NCT04837313]	Recruiting 04/2021-08/2021 Phase N/A	PD	1: Evaluate the efficacy and safety of FMT in the treatment of constipation symptoms in patients with PD receiving a steady dose of levodopa 2: Analyse intestinal flora diversity in patients with PD with constipation	Interventional; 30 participants; single group assignment; open label	Biological: FMT FMT route: N/A	1: experimental: FMT performed	18-75 years; all sexes; healthy volunteers not accepted	Change of several scales of PD: GSRS, Wexner Constipation Score; etc.	China	(90)

Fecal Microbiota Transplantation As a Potential Treatment for Parkinson's Disease [NCT03876327]	Completed 03/2019-08/2020 Phase 2/3	PD	1: Explore the potential usage of FMT in treating constipation and motor symptoms in PD patients 2: Increase understanding of the potential relationship between the identities of intestinal microbial communities and PD	Interventional; 10 participants; non-randomised; parallel assignment; open label	Biological: FMT FMT route: N/A	1: experimental: PD patients that will receive FMT 2: no intervention: PD patients that will not receive FMT 3: no intervention: healthy people living with PD patients that will not receive treatment	50 years and older; all sexes; healthy volunteers accepted	Scoring system questionnaires like MDS-UPDRS III; etc.	Israel	(91)
Effect of Fecal Microbiota Transfer on Progression of Parkinson Disease [NCT05204641]	Enrolling by invitation 01/2022-01/2022 Phase 1/2	PD	1: Asses the impact of FMT on clinical symptoms and progression of PD 2: Identify pathogens that may affect the course of the disease	Interventional; 120 participants; randomised; parallel assignment; double-blind	Biological: through FMT donor or via autologous FMT FMT route: colonoscopy	*Pre-treatment with rifaximin/ assessment of levodopa-benserazide/ assessment in clinical scales 1: active comparator: FMT from healthy donor 2: placebo comparator: auto transplant	45-75 years; all sexes; healthy volunteers not accepted	Scales like MDS-UPDRS or Constipation Assessment Scale; composition of GM assessed before and after the procedure; etc.	Poland	(92)
Study of the Fecal Microbiome in Patients With Parkinson's Disease [NCT03671785; new modified protocol of NCT03026231 which was withdrawn]	Active, not recruiting 09/2018-12/2021 Phase 1	PD	1: Characterise the intestinal microbiome in subjects with PD 2: Determine safety and trends in improvements in diversity of colonic microbiome following FMT	Interventional; 12 participants; randomised; parallel assignment; single-blind	Biological: PRIM-DJ2727 (twice filtered and lyophilised faecal microbiota product from 3 healthy donors) Other: placebo oral capsule (lactose) FMT route: capsules	*Each dose of enteric coated capsules consists of 60g of stool or lactose and will be administered orally twice-weekly for 12 consecutive weeks 1: experimental: PRIM-DJ2727 2: control: placebo group	55-75 years; all sexes; healthy volunteers not accepted	Microbiome diversity with Shannon Diversity Index or number of taxonomies per participant; motor function with MDS-UPDRS; cognitive dysfunction with MoCA tests; anxiety and depression scales; changes in intestinal transit time; etc.	United States	(93)

AD: Alzheimer's disease; CSF: cerebrospinal fluid; FMT: faecal microbiota transplantation; GI: gastrointestinal; GM: gut microbiota; GSRS: Gastrointestinal Symptom Rating Scale; LOC: location; MDS-UPDRS: Movement Disorder Society Unified Parkinson's Disease Rating Scale (parts I, II, III); MoCA: Montreal Cognitive Assessment; N/A: not applicable; NaCl: sodium chloride; NMSS: Non-Motor Symptoms Scale for Parkinson's disease; PD: Parkinson's disease; REF: reference.

Conclusions and future directions: After this analysis, it can be concluded that preliminary literature suggests that FMT may be a promising treatment option, both in terms of symptom improvement and disease progression, for both NDDs studied. These **beneficial effects** may be related to changes in the GM and an increase in SCFA; the reduction of pro-inflammatory gut bacteria may decrease neuro- and systemic inflammation and brain oxidative stress.

On the basis of all animal studies summarised, it can be assumed that while significant reduction in cerebral amyloid plaques, neurofibrillary tangles and reactive gliosis correlates to improve cognitive and memory function in **AD**, a suppression of TLR4/TBK1/NF-κB/TNF-α signalling pathway and α-Syn expression correlates to improve motor and physical impairment in **PD**. Several limitations are encountered when using animal studies, though: the GI and physiological differences between humans and animals preclude the direct extrapolation of the results to humans; behaviour and cognition tests in animal models may be subjective or difficult to interpret and not reproducible; immunoreactivity of animal models could be very different; etc.

On the other hand, with the results obtained in case reports and preliminary human trials, it has also been shown, by means of physical and mental examinations and questionnaires, that this therapy remitted several signs and symptoms and improved cognitive functions without causing significant damage to the recipients. However, the reliability of conclusions in humans might be undermined by the **lack of a blind design**; neuropsychiatric diseases analysed with subjective outcome measures are prone to strong placebo effects after such an invasive treatment.(94)

Other **limitations** in both the human and animal studies included are related to the often complex study design, including different FMT procedures, modes of delivery and required pre-treatments (they all need to be improved to further minimise the potential side effects), the choice of different donors (the best donor for each disease is still unknown), the lack of long term follow-up (6 months is short knowing that NDDs are long-standing diseases) or appropriate control groups. Another significant limiting factor is the **small sample size**, which potentially limits the generalizability of the results. Moreover, referring to GM analysis, results can be affected by a wide variety of factors, starting with sample collection and DNA extraction and ending with the influence that the recipient's medication, diet or age, among others, may have.

Alterations detected between pre- and post-FMT suggest potential for more targeted FMT-based therapies in NDDs and lend themselves to taking a closer look into the mechanism of MGBA and on how FMT manages to re-establish a balanced GM. Only then can we move toward developing an urgently needed standardised **protocol** for FMT. The required number of FMTs for each NDD (as they are progressive, a single FMT as it is usually done in the recurrent CDI would not be sufficient) and the effective interval between multiple FMTs would then need to be evaluated too.

Finally, not only in AD and PD, but a large number of clinical trials using FMT in NDDs, and more generally in mental and nervous system diseases, are currently **underway**. It therefore remains to be seen whether positive findings from animal studies can be confirmed in humans.

To sum up, although some evidence is available, the rationale for the clinical application of FMT is currently based on a small number of case reports and animal models. More and larger well-designed double-blinded randomised placebo-controlled **trials in human patients** are still **needed** to assess longer-term maintenance of efficacy and safety and to further elucidate the effect of FMT in the cure of NDDs, so that it can qualify as a widely recommended therapeutic measure. Future studies should carefully weigh the potential benefits of FMT against the potential risks, always respecting the inclusion and exclusion criteria for both patients and donors.

8. CONCLUDING REMARKS

Having found answers to all the initially proposed objectives, the principal **conclusions drawn** are summarised below:

- Human microbiota (microbial cells harboured by each person) reaches its highest density in the intestinal compartment, where bacterial cells predominate, with *Firmicutes* and *Bacteroidetes* as the main phyla. This GM interacts with the host in a dynamic way, providing key functions in the homeostasis of the organism, thus contributing to both health and disease. Even though a healthy GM has not been defined, in general, it is associated with high taxonomic diversity and gene richness. The infinite combinations of different genetic and environmental factors (diet, exercise, antibiotic usage, etc.) which can modify the composition of GM are responsible for its uniqueness in each individual.
- There are several neural, endocrine and immune pathways, with both direct and indirect routes, through which GM can modulate the bidirectional GBA. Although some of them have been broadly studied (e.g., VN, SCFA, HPA), there are still many molecular mechanisms to be further explored.
- It has recently been suggested that the dysregulated gut microbiome (dysbiosis) may be involved in modulating the integrity of intestinal and blood-brain barriers (facilitating the arrival of GM-derived products to the brain), neuroinflammation, oxidative stress and protein misfolding, all of which are characteristic hallmarks of NDDs.
- Altered intestinal microbes have been detected in Alzheimer's and Parkinson's patients (the most common NDDs), an association that opens the way to propose the GM as a diagnostic tool and a therapeutic target for this type of pathologies, which are expected to increase in prevalence as life expectancy rises, and for which there are currently no effective pharmacological or non-pharmacological cures.
- Preclinical and human studies based on modulation of GM through dietary modifications, oral bacteriotherapy and FMT (intervention studied more extensively in this project) have shown reversal effects on multiple neurodegeneration features, apart from cognitive and behavioural improvements.

After all the research done on this topic, it is noted that there is still some way to go but that the goals are starting to become clear. Analytical parameters and validated protocols are still needed to routinely assess the human GM in the clinical setting (markers for diagnosis, predictions for a personalised treatment, monitoring the outcome, etc.), and further research is also needed on technologies that allow its analysis (in which inconsistencies are sometimes observed); functional analysis of the gut microbiome, using metagenomics and metabolomics, are emerging and may be more important than taxa detection. It is imperative to further develop diet-based strategies while promoting FMT which, apart from promising outcomes, requires a short operation time (low hospitalisation cost). Moving forward with testing in other animal models (such as *Drosophila*) and with all clinical trials started, not only for AD and PD but for all NDDs, is the right path.

As a final conclusion, although the study of the relation between GM and NDDs is just beginning and the elucidation of pertinent mechanisms and of specific guidelines for GM modulation is still lacking, the success seen in the first results (coupled with a multi-omics approach and gradually reduced limitations) justifies the **hope** that exists for their early detection and effective treatment in the near future. We are on the verge of a **paradigm shift** in the way NDDs are approached.

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ANNEXES

In this appendix, as previously announced, a summary of the explanation received during the arranged visit to the *Alzheimer Center Barcelona* (ACE) and a transcript of an interview with a FMT expert are presented. In both cases it will be expressed in Spanish or Catalan, in order to maintain the originality of the conversations without any translation.

I. VISITING THE ALZHEIMER CENTER BARCELONA (ACE)

ACE (*Alzheimer Center Barcelona*) is a non-profit organisation at the service of people with Alzheimer's disease and other dementias, and their families. What began around 30 years ago as a center to apply a novel psychostimulation programme for people with cognitive deterioration, today is an entity that **diagnoses** more than 7500 people a year, **attends** more than 250 people in its day hospital and day center, has a team of **researchers** dedicated to understanding the origins of Alzheimer's disease, and a clinical trials unit working to find an effective treatment to halt or cure cognitive deterioration. They have participated in more than 130 clinical trials and have the largest Alzheimer's genetic collection in Europe.

After reading a public interview from 2017 with Agustín Ruiz (95), geneticist and research director of the ACE Foundation, about the role of the microbiota in our **brain health**, I thought it would be interesting to learn more about this center. In this mentioned interview, he made contributions such as: "Los microorganismos que habitan nuestro intestino pueden emitir factores a la sangre capaces de provocar inflamación a muy larga distancia", "modificando el microbioma también se pueden hacer modificaciones del perfil proinflamatorio o antiinflamatorio que tiene un sujeto y que eso repercuta luego en el desarrollo, la modulación o la prevención de algunas enfermedades. Ésa es nuestra esperanza, realmente", "Por el momento, estamos empezando a guardar material de pacientes con enfermedad de Alzheimer durante todo el proceso, desde el sujeto sano al que se queja de problemas de memoria, sujetos que ya tienen un deterioro cognitivo ligero antes de llegar a la demencia, con el objetivo de comparar el perfil de microbiota de todos ellos y ver si encontramos alguna pista de cómo intervenir".

In the visit I was granted at the center, where I was able to talk to both Xavier Morato (Clinical Trials deputy director) and Amanda Cano (PhD in genetics and molecular biology), I extracted some explanations of different topics of interest:

- About their different **areas and functions**

Tenemos 4 centros, cada uno con diferentes funciones o formas de abordar la enfermedad. Este es el hospital de día, donde se reciben a todos los pacientes, tanto a los nuevos como a los que seguimos hace años, para que se visiten anualmente con neurólogos y neuropsicólogos y les hagan tests cognitivos (como el test Mini-Mental, con pruebas como reproducir una figura en un papel, hacer la cuenta atrás desde 100, etc.), miren si hace falta cambiar la medicación, si ha evolucionado la enfermedad y se necesita estimulación cognitiva, etc. En la primera visita se les hace un estudio más amplio y después el seguimiento ya depende de cada caso.

En el mismo hospital de día está la parte de 'Clinical Trials', en este momento con 15-16 ensayos en activo de diferentes fármacos y fases. Si los pacientes cumplen con los requisitos de un ensayo clínico, se les explica y se les ofrece, y si quieren participar pasan a formar parte. Encontramos también la parte de tratamientos, con camillas para intravenosos, enfermería, farmacia para preparar todo lo necesario para los ensayos, laboratorio de bioquímica, etc. Por último, en el piso de arriba es donde se realiza la aféresis terapéutica: se reemplaza el plasma del paciente por un

plasma fresco, con albúmina renovada, involucrada en procesos de limpieza; se vió que mejoraba el estado cognitivo.

A parte del hospital de día, tenemos 2 centros de día para ofrecer terapias conductuales a los pacientes (con neuropsicólogos, estimulación cognitiva, musicoterapia, terapias ocupacionales…). Hay un centro para pacientes más jóvenes y otro para más ancianos, ya que se les trata diferente (actividades adecuadas a su estado cognitivo). Muchas veces se trata de personas que no son autónomas, y así también es una manera de que tengan donde estar ocupados.

Finalmente, hay el centro de investigación. Con todas las muestras que se recogen de hospital de día, se hacen los estudios de genética (1-5% de casos de Alzheimer se sabe que tienen origen genético; del otro 95% no se sabe la causa pero se pretende encontrar otras posibles mutaciones que puedan estar involucradas), se hacen proteinogramas, lipidómica, metabolómica, etc.

- On their latest involvement in **microbiota-related studies**: FACEHBI project

Participamos en un estudio longitudinal que empezamos hace 8 años de personas con quejas subjetivas de memoria e iniciamos un programa con el objetivo de hacer chequeos periódicos de su estado cognitivo. Es una cohorte de 200 pacientes, que después de haberles hecho una batería de pruebas neurológicas, los clínicos han determinado que todavía no tienen ningún proceso de demencia, ni siquiera un deterioro cognitivo leve. La intención es hacerles un seguimiento con tests cognitivos cada año y recolección de muestras biológicas y análisis de biomarcadores cada 2, para ver si realmente esas quejas subjetivas se acaban traduciendo en algún tipo de demencia.

*Dentro de las muestras biológicas que se tomaban en estos pacientes, unas eran **heces**. Era una colaboración con IRSI La Caixa que son los que realmente analizaban el microbioma. Nosotros solo, cada dos años, recibíamos una muestra de heces de los que querían participar, y la procesábamos para mandarla a su departamento de microbiología. Pero el estudio de las heces se paró el año pasado porque no vieron nada relevante en los primeros resultados. […] Esto ya es una apreciación personal, pero este tipo de pacientes que tienen quejas subjetivas, probablemente muchos de ellos no desarrollen demencia. Si ya en tu población de estudio de la relación microbioma-demencia no tienes claro si va a acabar desarrollando demencia o no, podemos pensar que quizás no sea la población más adecuada. Como para estudios así necesitas financiación, por el momento están en fase de buscar presupuestas y encontrar proyectos. Están valorando si cambiar el protocolo, cambiar de población…*

- On the processing of the **stool sample**

*Los pacientes participantes tienen un kit de recogida estéril con un empapador donde ellos depositan la muestra, y con un guante la cogen y la trasladan al bote. Se les proporciona un teléfono de una empresa de envíos de muestras biológicas para que avisen en el momento que lo tengan preparado, para que así no pasen más de 2 horas hasta que nos llega al centro y la congelamos. En el momento de procesarla, la dejamos en la nevera un rato y después vamos a las campanas de flujo laminar. Una vez allí, con una espátula de plástico que se desechará, se coge un trocito de muestra y se introduce en uno de los criotubos de unos 2 ml y se cierra. Lo limpiamos con alcohol y ya lo podemos dejar a -80ºC. La única diferencia entre las alícuotas que servirán para estudios de DNA y las que son para RNA es que en los tubos de RNA se les añade 1 ml del buffer conservador para evitar degradación. A -80ºC puede estar durante meses, así que en este último estudio normalmente lo recogíamos durante un año y lo enviábamos una sola vez todo junto a los colaboradores. Ellos lo analizarían teniendo en cuenta su hipótesis: el microbioma afecta al estado inflamatorio general de los pacientes, inflamación que se acaba traduciendo en un proceso **neuroinflamatorio**, consecuentemente **neurodegenerativo**.*

Añadir que, en general, es difícil que los pacientes accedan a donar heces dado que les resulta desagradable, mientras que la extracción de sangre está socialmente más aceptada.

- On **other** significant **biological samples** collected

[…] Como muestras biológicas distintas, aparte del análisis de sangre y de saliva, a algunos de estos pacientes, si accedían, se les hacia una punción lumbar para obtener el LCR (líquido cefalorraquídeo); se miraban niveles beta amiloide 42, proteína tau y fosfotau y, en función de los valores, se obtenía una puntuación que ayudaba a los médicos como diagnostico diferencial (cuando no lo tienen claro solo a través de tests cognitivos).

A pesar de ser una muestra con relevancia clínica dada su conexión con el cerebro, los análisis de biomarcadores hace años que se intentan trasladar a plasma (difícil, aún queda una trayectoria importante para afinar las técnicas de diagnostico), ya que LCR es una técnica muy invasiva y que no todos los pacientes pueden tolerar (evitar si hay problemas de coagulación, óseos, etc.).

- On their **future projects** for further study of the "microbiota-Alzheimer's axis"

*Ahora mismo estamos pendientes de financiación. A lo mejor se vuelve a recolectar muestra en un futuro muy próximo. Ya se nos han propuesto bastantes proyectos, y realmente no tenemos intención de frenar el estudio por este camino que representa la conexión con la microbiota. Disponemos de recursos y de material muy interesante para avanzar en este nuevo enfoque terapéutico, y las ideas que tenemos sobre la mesa nos dan grandes **esperanzas** dentro de los objetivos del centro.*

II. INTERVIEWING A FAECAL MICROBIOTA TRANSPLANTATION (FMT) EXPERT

In recent years, **FMT** has emerged as the most appropriate treatment for recurrent *Clostridioides difficile* infection, with overall cure rates of between 85 and 90%. For this reason, a multidisciplinary working group has been created in Catalunya to study and develop this new therapy. Recently, this group, with the aim of establishing recommendations for the rigorous practice of this therapeutic modality, has published a position paper on **donor selection**, a key point in the process to ensure the safety of the recipient.

This public consensus document (96) has been led and coordinated by physicians from Hospital Universitari de Bellvitge and Hospital Clínic de Barcelona, including **Andrea Aira Gómez**, microbiologist and pre-doctoral researcher at the Microbiology Department of Hospital Clínic de Barcelona. As a result of this document, which contains interesting details that are not mentioned in this project, and of the contact established with one of its authors, a series of questions to ask her were prepared. The literal content of the **interview** is presented below:

Pel que fa a la selecció del donant, existeixen tant criteris d'inclusió com d'exclusió? He llegit que us guieu més pels d'exclusió, a què es deu?

Realment el fet que hi hagi criteris d'exclusió ja implica que la resta són inclusius, però ens basem més en l'exclusió: no ens importa tant que una persona tingui una dieta vegana o mediterrània però sí que s'exclouria a una persona que no tingui una dieta equilibrada o hàbits de vida saludables. La prioritat és excloure els donants que puguin suposar un risc per a la salut del receptor tant per motius infecciosos, com per malalties metabòliques, genètiques, hàbits de vida no saludables i que no només puguin fer que aquella microbiota no sigui "ideal" sinó que a més es puguin transmetre qualsevol d'aquestes malalties.

Els criteris són sempre fixes davant de qualsevol possible donant, situació o patologia?

Hi ha alguns criteris que són fixes com el fet de tenir certes malalties, tenir edat fora del rang establert etc., però hi ha alguns aspectes de l'analítica que poden sortir alterats i s'han de revisar en conjunt. No pel fet de tenir una determinació alterada (una mica) en l'analítica s'exclou el donant si la resta està "Ok".

S'ha vist gaire afectat el tema de seleccionar donants amb l'aparició de la pandèmia? Què ha comportat aquest possible perill "extra"?

Primer de tot, ha sigut molt difícil trobar donants perquè en l'època del COVID no hi havia accés als hospitals; a més, s'han adaptat els protocols per cribrar els donants per SARS-CoV-2 tant per PCR a saliva com per PCR de femta i serologia. En el cas de la serologia, a l'estar tots vacunats, ja no ens dona informació rellevant, però al principi era un indicador de saber que el donant no havia agafat el COVID durant el període de donació.

És el mateix donant qui recull la mostra i la porta al centre perquè sigui emmagatzemada en les condicions convenients? Com sabeu que ha fet adequadament la recollida? Quina preparació prèvia a obtenir la mostra ha d'haver fet el donant?

Sí, el donant rep una bossa de donació (és una nevereta portàtil) preparada amb un document d'informació sobre la recollida de la mostra i un recipient específic per a la recollida (es diuen Fecotainers i estan dissenyats específicament per recollir femtes). La recollida és molt senzilla i nosaltres els hi hem facilitat un servei de "taxi" que permet que els hi recullin la mostra a casa, però també poden venir personalment al laboratori a portar la bossa. El donant no ha de fer cap preparació prèvia per recollir la mostra, només es demana que s'entreguin deposicions completes i sense contaminants com orina o sang; si no, en aquests casos, es rebutjaria la femta.

En quines condicions es mantenen les mostres al banc de femtes? Existeix un temps màxim o temperatures requerides? Quin procediment i quins terminis s'hauran de seguir una vegada vol utilitzar-se mostra d'aquest banc?

Les mostres, una vegada processades, es congelen a -80ºC amb un crioprotector per poder mantenir la viabilitat de la microbiota. El temps que es poden tenir congelades és d'1 any i aquest temps és el que es recomana, encara que trobaràs alguna guia que diu que pot estar més temps. Una vegada es sol·licita una mostra per fer un TMF, es descongelen durant la nit a 4ºC i al matí ja es poden utilitzar. No poden passar més de 6h una vegada es descongela abans d'utilitzar-se i mai congelem/descongelem més d'una vegada.

Encara no està clara la quantitat òptima de femta a trasplantar? A diversos estudis he llegit uns 50-60 g normalment dissolts en 150-200 ml aproximadament. És així?

Com que no hi ha un "gold standard" cada guia recomana coses diferents, però sí que està consensuat que a partir de 50g és molt més efectiu que amb menys quantitat. Nosaltres sempre utilitzem 50g per tractament, pel que si una mostra que ens arriba pesa 100g obtenim 2 tractaments. El volum a utilitzar per homogeneïtzar depèn de la ruta d'administració per al TMF, nosaltres els preparem per fer colonoscòpia i en aquest cas utilitzem 250ml de sèrum fisiològic. Si es volgués fer per via sonda nasogàstrica s'utilitzaria menys volum ja que no hi cap tant al tub digestiu superior.

De la mateixa manera, se sap quina és la millor via per trasplantar-la? La via oral mitjançant càpsula sembla més còmode pel pacient, però és prou eficaç i segura (més efectes adversos?) o

són millor altres vies més invasives? Això depèn de les condicions del pacient (si, per exemple, ja se sap que alguna via no serà ben tolerada per alguna patologia o lesió que pateix actualment)?

Excepte en l'administració en ènema que s'ha vist que és menys efectiva (degut a la poca retenció), la resta han mostrat eficàcia molt semblant. Fins ara, la manera més extensa ha sigut la colonoscòpia però és cert que l'administració per càpsules orals és més còmoda pel pacient. Està limitat a la possibilitat del centre de processar la mostra i poder-la emmagatzemar. En casos de pacients que no poden deglutir, sempre s'indica per colonoscòpia, i en algun cas en el que el pacient tingui el colon molt fràgil o no es pugui fer la preparació de colonoscòpia, s'indica millor la via oral o sonda nasogàstrica.

De què depèn l'eficàcia d'un TMF? És necessari un pretractament amb antibiòtics o fent un rentat d'intestí? Per què?

Encara no se sap exactament de què depèn l'eficàcia. En el cas de C. difficile sembla que és molt "fàcil" que funcioni ja que en aquest cas el colon del pacient està molt disbiòtic i sembla més fàcil que la nova microbiota s'hi "instal·li", però està en estudi.

Creus que és un mètode amb resultats poc extrapolables d'animal a humà? Per exemple en el cas de les malalties neurodegeneratives que és el que jo estic mirant, hi ha resultats que només s'han vist de moment en animals i no se sap fins quin punt podria extrapolar-se als humans.

Encara queda molt per estudiar i primer s'ha de veure si la relació microbiota-cervell és en una direcció, en l'altre o en ambdós costats.

Quins veus que siguin els efectes adversos més comuns? Creus que serien similars usant el mateix mètode però en altres patologies?

En general no s'ha reportat encara cap cas d'efecte advers sever en pacients després d'un TMF, però sí que són comuns efectes lleus com nàusees, vòmits, diarrees o dolor abdominal, que és autolimitant i acostuma a ser poques hores després d'haver-se realitzat el procediment.

Existeix un protocol molt estandarditzat sobre com dur a terme un TMF? O aquest varia segons cada hospital, i difereix encara més segons el país?

Les guies clíniques europees (ECCMID2022) i americana (IDSA 2022) defineixen els protocols que es poden seguir, però hi ha alguns detalls que varien segons els centres degut a l'accés que tinguin del material, etc.

Cal que se signi un consentiment informat? Això és necessari tant pel donant com pel pacient que rebrà el trasplantament? S'informa sempre tant a un com a l'altre de tots els riscs que estan assumint? Passa gaire que "es tirin enrere"?

Sempre en qualsevol procediment clínic s'ha de signar un consentiment informat, tant el donant per utilitzar les seves mostres com el pacient. Sempre es poden "tirar enrere" abans del procediment sense cap compromís, ja que és totalment voluntari pel donant i el pacient pot decidir si vol sotmetre's a aquest tractament. Nosaltres no hem tingut cap cas de ningun pacient que signi el consentiment i després no participi, de fet crec que el 95% dels pacients als que se'ls hi ha ofert aquest tractament han dit que sí.

Normalment, en les patologies en que ho poseu en pràctica ja sigui assistencialment o en investigació, feu una única "dosi" de TMF? A vegades, si no es soluciona, proveu dosis repetides?

Sempre se'ls hi fa un seguiment als pacients després de que rebin un TMF? Durant quant de temps?

Quan es tracta d'un TMF assistencial (on l'única aplicació actualment establerta és per C. difficile) és 1 única dosi; si el pacient fracassa, es pot proposar de fer un altre TMF i s'ha vist que en aquests casos funciona repetir-ho. En el cas de la recerca depèn de l'estudi, hi ha alguns que proven més d'1 dosi. Sempre es fa seguiment dels pacients i el temps depèn del que s'estableix a l'estudi i, en el cas assistencial, del seguiment que tingui previst per part de l'hospital.

Té alguna contraindicació el TMF?

Depèn del pacient, però exemples són estar embarassada, tenir alguna patologia que no permeti l'administració del TMF, o tenir alguna malaltia que comporta estar prenent antibiòtics de manera crònica sense poder-los parar (en aquest cas el TMF seria un fracàs perquè els antibiòtics alterarien la microbiota administrada i no tindria el mateix efecte).

Consideres que el TMF és una teràpia gaire selectiva? O a mesura que avanci el coneixement dels probiòtics o un nou mètode que s'estudia actualment sobre encapsular directament bacteris específics, el TMF quedarà substituït?

S'ha de partir de que el TMF no només conté certs bacteris (com els probiòtics) sinó que conté milers de microorganismes incloent bacteris, fongs, virus, etc. i que és una comunitat equilibrada i establerta amb funcions organitzades. L'estudi de probiòtics i còctels sintètics pot ser beneficiós però és molt difícil aconseguir la complexitat que es troba en un TMF. De fet, no sabem encara què és el que fa que funcioni tan bé, si són certs microorganismes, si és la combinació d'aquests, si són els seus metabòlits o el conjunt...

Amb quines patologies esteu investigant actualment, a part de la infecció recurrent per *C. difficile* (on ja és tractament de primera elecció)? Es preveu que s'estengui a moltes altres branques de la medicina més enllà de l'aparell digestiu? Saps en quin altre àmbit s'està provant o hi ha intenció de fer-ho?

*En el nostre hospital comptem amb un banc de femta per tal de tenir disponibles tractaments de TMF tant de manera assistencial com per recerca. Assistencialment ja ho realitzem com a tractament per la infecció de C. difficile (està en les guies clíniques com a tractament d'elecció en infeccions recurrents), però també col·laborem amb altres grups per estudiar el TMF en infeccions d'orina recurrents i en la descolonització de bacteris multirresistents del tracte digestiu de pacients ingressats. De totes maneres, em consta que hi ha multitud d'estudis amb moltes patologies (incloses del **sistema nerviós**) que no només són del tracte digestiu.*

Amb el coneixement que tens del tema, veus futur a l'hora de tractar mitjançant TMF malalties neurològiques com podrien ser les neurodegeneratives, moltes de les quals no tenen actualment cap tractament efectiu? Ho veus viable a curt termini?

*Crec que a curt termini és massa arriscat. Cal estudiar molt la relació entre la microbiota i tot el que comporta una malaltia neurodegenerativa, ja que no totes són de component genètic o immunològic. El TMF és un **tractament potencial** però s'ha d'anar amb cura per tenir molt en compte el cost-benefici que suposa per a aquests pacients.*