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Review

From the Gut to the Brain: Is Microbiome a New Paradigm in Parkinson's Disease Treatment?

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Abstract: Parkinson's disease (PD) is recognized as the second most prevalent primary chronic neurodegenerative disorder of the central nervous system. Clinically, PD is characterized as a movement disorder, exhibiting an incidence and mortality rate that is increasing faster than any other neurological condition. In recent years, there has been a growing interest concerning the role of the gut microbiome in the etiology and pathophysiology of PD. The establishment of a brain-gut microbiome axis is now real, with evidence denoting a bidirectional communication between the brain and the gut microbiota through metabolic, immune, neuronal, and endocrine mechanisms and pathways. Among these, the vagus nerve represents the most direct form of communication between the brain and the gut. Given the potential interactions between bacteria and drugs, it has been observed that the therapies for PD can have an impact on the composition of the microbiome. Therefore, in the scope of the present review, we will discuss the current understanding of gut microbiome on PD and whether this may be a new paradigm for treating this devastating disease.

Keywords: parkinson's disease; microbiome; gut-brain axis

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1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease of the central nervous system (CNS)[1,2]. Currently, this movement disorder affects 10 million people worldwide, with 75,000 new cases per year [3,4]. According to the World Health Organization (WHO), PD's incidence and death rate is increasing faster than any other neurological condition, and its prevalence has doubled over the past 25 years [5].

PD is a complex and multifactorial disease, presenting distinctive pathological hallmarks, including the depletion of dopaminergic neurons in the substantia nigra pars compacta (SNpc). This depletion arises from the accumulation of a pathological misfolding alpha-synuclein (αSyn) protein, leading to the formation of Lewy neurites in the (remaining) neurons. αSyn is a monomeric protein and thus is expected to undergo genetic and post-translational modifications, including phosphorylation, ubiquitination, nitration, and oxidation, which can predispose it to misfold [2,6]. This misfolding makes this protein insoluble since it tends to form β-sheet-rich amyloid aggregates that accumulate and form intracellular eosinophilic inclusions [1,3]. Consequently, these changes can significantly impact the correct functioning of the central, peripheral, and enteric nervous systems (ENS) [1,3,7,8]. In fact, the presence of αSyn aggregates, combined with other factors, such as mitochondrial dysfunction, ubiquitin-proteasome system (UPS), synaptic dysfunction, e.g., contributes to neuronal degeneration and death, leading to the appearance of motor (slow movements, resting tremors, rigidity, postural instability, and bradykinesia) and non-motor (dementia, gastrointestinal dysfunction, behavioral and cognitive impairments, among others) symptoms [1,6,7,9–12].

Despite all this knowledge, the etiology of PD is still unclear, although genetic and environmental factors provide the most plausible explanation for the onset of this pathology [7]. From the clinical point of view, PD displays heterogeneity, with different onset and progression patterns [13,14]. Advanced age at diagnosis is the most critical risk factor, although young Parkinsonism (even if rare) cannot be excluded. Nevertheless, in addition to aging, other risk factors should also be considered, namely family history of the disease, gender (in which men are more affected than women), and environmental factors such as exposure to pesticides and rural living [1,6]. Notwithstanding, it is known that cellular and molecular alterations can also contribute to PD through high levels of oxidative stress, mitochondrial dysfunction, neuroinflammation, xenobiotic toxins, and altered dopamine metabolism [6].

At the moment, both pharmacological and non-pharmacological therapies are available for PD. The current gold-standard PD treatment is pharmacological, based on dopamine replacement. However, non-pharmacological alternative approaches such as deep brain stimulation (DBS), an established safe neurosurgical symptomatic therapy, can be used in certain patients in more advanced stages of the disease, in which the treatment had no effectiveness or in which the medication itself produced severe side effects [13,14]. Nevertheless, these treatments promote temporary relief, being ineffective all over time, leading to the necessity of increasing drug dosages (in case of pharmacological therapies) or DBS intensities (in case of non-pharmacological approaches), which in most cases results in the appearance of adverse effects such as sleep disturbance, impulsivity, and addiction [1,4]. Hence, addressing this remains a noteworthy constraint in managing PD and a significant obstacle in developing or repurposing innovative therapies to slow down or halt its progression [17]. Moreover, it poses the question of how we can effectively manage PD and determine the optimal target for its treatment?

Despite numerous other avenues of research, in recent years, significant emphasis has been placed on exploring the vast potential of the gut microbiome and its intricate interconnection. As a result, efforts have been made to comprehend how the gut microbiome may respond under PD conditions, specifically whether it may contribute to the development or progression of the disease or offer a new therapeutic target to address PD. Therefore, in the present review, we will explore the bidirectional association between the gut microbiome and PD, discussing the current understanding of the gut microbiome in the pathophysiology of PD and as a potential target for novel therapeutic approaches.

Gut Microbiome

The human body is over 99% microbial in terms of genes. Our gut holds almost 100 trillion microbes, meaning there are 10-100 times more bacteria than eukaryotic cells in the human body [10,18]. Nevertheless, bacterial composition and diversity differ according to different parts of the body [6]. The colonization of the gut is thought to begin immediately after birth, and it can be influenced by various factors such as the method of removing the fetus, namely vaginal or caesarean, or the type of breastfeeding (breastfeeding or formula), environmental stimuli, and antibiotic use [19–21]. Remarkably, this colonization is considered in the first 3-5 years of life. Although it is generally stable within healthy adult individuals throughout life, it is a dynamic entity whose composition is susceptible to change due to external disturbances [6,22]. Factors such as genetics, stress, environmental exposures, age, metabolism, geography, surgeries, physical activity, antibiotic intake, and diet can influence the stability of human gut microbiota [21,22].

Microbiota is the complex ecological community composed of all the microorganisms that reside in our gut, and it has been called microbiome to the entire genetic material (genome) of the microbiota [21]. Specifically, microbiota comprises bacteria, eukaryotic cells, viruses, archaeobacteria, fungi, and protozoa [23]. These microbiota constituents interact with each other and the host, impacting health through their metabolic activities and host interactions and in normal human physiology and disease contexts [6,18]. Bacteria predominately control the gut microbiome's metabolic activities, such as modulation of energy metabolism, nutrition absorption, and regulation of gut microbiota composition [24]. Thus, gut microbes can play a harmless or beneficial effect on the host, being essential to keep the gut homeostasis [4,25]. In addition, the microbiota interacts with the immune system, facilitating the normal development of immune functions during homeostasis and the emission of signals to promote the maturation of immune cells [25].

The adult microbiota harbors more than 1,000 phylotypes of bacteria at the species level, with Firmicutes and Bacteroides emerging as the two most abundant phyla, representing at least 70-75% of the total number of microorganisms [26]. Actinobacteria, Proteobacteria, and Verrucomicrobia can also be found, albeit in a smaller amount [22,27]. Scientific reports have been highlighting that it is essential to understand the stability of the microbiota within an individual over time to predict health status and to be able to develop therapies to correct dysbiosis, the term used to define any imbalance between the bacterial community and the host. Indeed, gut dysbiosis is associated with several pathologies, including systemic or neurological disorders [18,27]. Accordingly, recent studies have claimed that gut microbiome can be an important piece of evidence to understand human pathology and physiology better [3]. Along with that, even though there was variability in adult gut communities, certain bacterial populations were common across different groups of individuals. Thus, a concept called “enterotype” emerged, which has been used to categorize individuals according to the composition of their intestinal microbiota [7]. Based on this concept, it was then proposed that three distinct symbiotic host-microbial states could be formed, driven by groups of dominant genera, namely Bacteroides (this has recently been subdivided into two groups: B1 and B2), Prevotella, or Ruminococcus. However, it should be noted that this concept of enterotype remains to be fully addressed [7,18,20,22]. Nevertheless, several factors may also play a role in this distinction and characterization. For instance, there is considerable intra- and inter-individual diversity in the microbiota composition of healthy control subjects and in the context of multiple disorders, where intestinal dysbiosis has been described, indicating that the gut microbiota is not uniform across populations. Environmental factors (e.g., smoking, alcohol, and diet), which significantly influence the structure and composition of microbial communities, can be used to explain this heterogeneity [7,28]. Besides age, genetics, environment, and lifestyle be factors affecting microbiota (see Figure 1), these are also critical factors contributing to PD onset and modulation throughout life [27].

Age

Microbiota colonization begins immediately after birth and plays an essential role in developing the ENS and postnatal immune system [4,7]. This colonization process is primarily influenced by various perinatal conditions such as the type of birth, nutrition, and the use or non-use of medication in the initial stages [29]. During the first years of life, the gut microbiota experiences various compositional and functional changes, which can significantly impact individual susceptibility to immune-related diseases in adulthood [7]. In the case of neurodegenerative diseases, aging is a well-defined hallmark associated with the accumulation of mitochondrial DNA defects, oxidative damage, and neuromelanin [27,30]. Furthermore, it is also recognized that problems caused by the gut or gut bacteria that eventually lead to dysbiosis have a higher frequency in older people [3,27].

Genetics

PD is mainly considered an idiopathic condition, with only approximately 10-15% of cases having a hereditary component associated with several genetic mutations [31]. Extensive research has been focusing on SNCA (αSyn), LRRK2 (leucine-rich repeat kinase-2), PINK-1 (phosphatase and tensin [PTEN] homologue-induced kinase-1), and PARK genes [32,33]. SNCA gene encodes for αSyn, and its mutations impact the protein’s expression levels, thus becoming a risk factor for PD. This gene has been widely associated with increased opportunistic pathogens in the intestine of PD patients. Interestingly, mutations in SNCA are not the prevalent ones in PD patients. Indeed, LRRK-2 is the most frequent genetic cause of the autosomal dominant form of PD, accounting for 10–40% of familial cases in various ethnicities [34]. Mutations in this enzyme are also closely associated with inflammatory bowel diseases (IBDs) [34,35].

Regarding the PINK-1 gene, it plays a role in the removal of damaged mitochondria, being mutations in this gene associated with mitochondrial dysfunction seen in the autosomal recessive early-onset PD [36]. The PARK gene encodes the Parkin protein, which can undergo several mutations resulting in a cellular accumulation of damaged mitochondria [32]. In addition, mutations in this protein are also known to disrupt the ubiquitin-proteasome pathway, which ultimately triggers the accumulation of misfolded protein aggregates (e.g., αSyn) [32,37]. Curiously, exposure to pathogenic bacteria or bacterial metabolites was shown to cause epigenetic changes in the genes mentioned above. Hence, this interaction could be involved in most sporadic cases of PD. However, the relationship between genetic predisposition and the bacterial community in a PD context remains poorly understood [6].

Environment

Increased xenobiotic degradation in the gut, particularly herbicides and pesticides, has been found in the gut of PD patients [38,39]. Preclinical studies have demonstrated that exposure to these compounds can induce the death of dopaminergic neurons, leading to movement impairments in mice [6,40]. Still, considering the wide variety of pesticides in use, their long half-life, and the potential for accumulation in food chains, the associated risk may be even greater [41]. Therefore, more extensive studies should be conducted to approach this potential interconnection and how this may interplay with microbiome and neurologic diseases.

Lifestyle

Although the three factors mentioned above (age, genetics, and environment) have major effects on the pathophysiology of PD, lifestyle can also play a role in PD, in which diet, physical exercise, caffeine, and nicotine consumption could be contributors (Figure 2) [41]. Despite all the information regarding possible microbiome modulators and their impact on such disorders, a fundamental question remains: how can microbiome data analysis be correlated with a diseased state?

The main challenge in addressing this question lies in several perspectives, namely in the heterogeneity profile between patients and diseases and in the technological availability to deeply discriminate the inherent variances. The conjugation between advanced techniques (proteomics, metabolomics, transcriptomics) and bioinformatic tools will be crucial to analyzing and integrating large amounts of data, based on the idea that only then will it be possible to capture the multilayered structure of the data.

Microbiome Characterization Techniques

The study of the human microbiome diversity began in the 1680s, when Anton Van Leeuwenhoek compared the oral and fecal microbiomes and found that microorganisms vary not only from region to region but also between states of health or disease regardless of location [42–44]. The initial investigations into the microbiome, namely the identification and characterization of microorganisms, were conducted through methodologies focused on cultivating and isolating bacteria [45]. Nevertheless, it quickly became evident that all existing species' cultivation and phenotypic characterization were unfeasible, either due to a lack of favorable conditions or to slow bacterial growth [46]. Nevertheless, developing novel analytical and sequencing techniques for studying the human microbiome has become a priority [44]. Indeed, the study of the microbiome has been a significant focus of various diseases in recent years, with an increasing number of areas recognizing the microbiome as a *"Key Player"* in several pathological conditions [47–49]. Presently, a multitude of molecular biological analysis tools can be employed in the study of the gut microbiome, from conventional to more advanced techniques, as outlined in Table 1 [50–52]. Although the development of traditional methods has been essential to the initial investigations of the gut microbiome, the development of novel methodologies, such as microbiome shotgun sequencing, has made it possible to overcome the biases associated with traditional culture, thus enabling a better understanding of the composition, diversity, and interrelationships among the microorganisms constituting the microbiome, contributing significantly to our understanding of their role in states of health or disease [52].

Table 1. Techniques used to characterize the gut microbiota.

Technique	Description	References
Culture-based	Isolation/ Growing of bacteria on selective media	[51–54]
16S rRNA (16S ribosomal RNA) gene sequencing based on cloning	The process involves cloning the entire 16S rRNA amplicon, followed by Sanger sequencing and capillary electrophoresis for analysis.	[51,52,55,56]
Direct sequencing of 16S rRNA amplicons	High-throughput parallel sequencing of partial 16S rRNA amplicons.	[51,52,57]
Quantitative real-time polymerase chain reaction (qPCR)	16 S rRNA amplification and quantification. A substance in the reaction mixture exhibits fluorescence when is attached to a double-stranded DNA.	[51,52,58,59]
Denaturing gradient gel electrophoresis (DGGE) / Temperature Gradient Gel Electrophoresis (TGGE)	16S rRNA amplicons separated on a gel using a denaturant/ temperature gradient	[51,52,60]
Terminal restriction fragment length polymorphism (T-RFLP)	After the quantification of fluorescently labeled primers, the 16S rRNA amplicon undergoes digestion utilizing restriction enzymes. Subsequently, the resulting digested fragments are separated through gel electrophoresis.	[51,52,61]
Fluorescence in situ hybridization (FISH)	Oligonucleotide probes, labeled with fluorescent markers, bind to sequences that are complementary to the target 16S rRNA. The fluorescence generated during this hybridization process can be quantified using flow cytometry.	[51,52,62]
Microbiome Shotgun sequencing	Extensive parallel sequencing of the entire genome.	[51,52]
Metagenomics	Exploring high-resolution profiling of gut microbiota genomes and characterizing gene structures of uncultivated microbiota.	[51,63]
Metatranscriptomics	High-resolution gene expression profiling is achieved through sequencing messenger RNA (mRNA) or complementary DNA (cDNA). This approach is used to explore differential microbial gene expression under various physiological or environmental conditions, providing insights into microbial adaptation and responses.	[64,65]
Metaproteomics	High-resolution protein monitoring and profiling involve the identification of proteins and peptides, enabling the examination of differential microbial protein production in diverse physiological or environmental conditions.	[66–68]
Metabolomics	Metabolites undergo analysis to profile the metabolic activity of microbial hosts.	[67,69,70]
DNA microarrays	Oligonucleotide probes labeled with fluorescent markers undergo hybridization with complementary nucleotide sequences. The resulting fluorescence is detected using a fluorescence laser detector.	[51,71]

The microbiome is studied by analyzing the structure and dynamics of bacterial communities and the interactions that they can establish with each other [72]. Regardless of the chosen analytical technique, the study comprises four essential phases (Figure 1): 1) fecal sample collection, 2) DNA extraction, 3) DNA analysis (utilizing the most suitable technique for the intended purpose (techniques outlined in Table 1)), and 4) bioinformatic analysis of the results obtained. These analyses can be done to obtain a taxonomy profiling or else analysis of gene functions of the gut microbiome

[50,73]. Subsequently, correlations between the healthy and altered gut microbiome in a pathological context can be made [50,74,75].

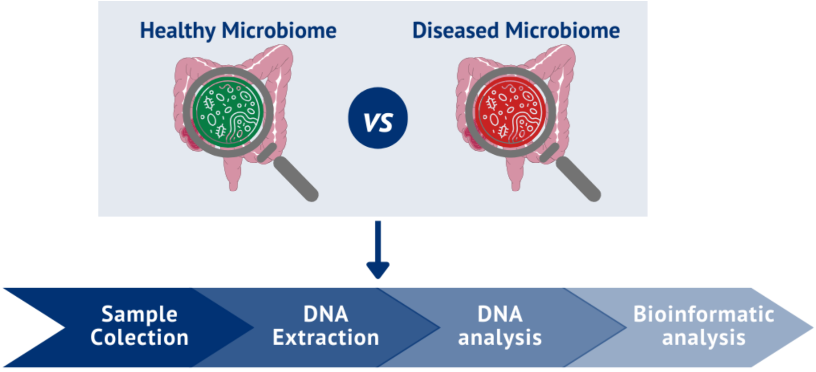

Figure 1. Phases of Gut Microbiome Study. The study of microbiome comprises four essential phases, namely (1) fecal sample collection, (2) DNA extraction, (3) DNA analysis, utilizing the most suitable technique for the intended purpose (techniques outlined in Table 1), and (4) bioinformatic analysis of the obtained results. These analyses can be done to obtain a taxonomy profiling or else analysis of gene functions of the gut microbiome [50,73].

Bearing this in mind, to perform microbiome research accurately, it is essential that, in combination with standard methodologies, appropriate pipelines (such as Quantitative Insights Into Microbial Ecology (QIIME) [76], and the Metagenomic Rapid An- notation using Subsystem Technology (MG-RAST) [77] should be established for research regarding the human gut microbiome [45,50,78]. This is crucial since methodological differences could lead to inconsistent data, thus limiting knowledge in this area [50,79,80]. Projections indicate that, in the forthcoming years, analyses of the gut microbiome could yield pivotal insights, facilitating the provision of personalized medicine for different diseases and thereby exerting a profound impact on individual human health [81–83].

Despite the substantial growth in this area in recent years, it is still impossible to have direct correlations between the microorganisms in our gut microbiome and their role in the onset and progression of specific pathologies, such as PD [52,84]. Consequently, there is a paramount need to allocate resources towards comprehending intestinal microorganisms' diversity and their bioactive metabolites' functionality in the context of diverse diseases, including PD [85,86]. This strategic investment is essential for the eventual utilization of the intestinal microbiome in diagnosing and treating PD.

Microbiome and PD: From Diagnosis to Treatment

Exposure to pathogenic bacteria, or bacterial metabolites, is one of the factors shown to cause epigenetic changes when they interact with (PD) genes [6]. It has been proposed that this interaction may be involved in most sporadic cases of PD. However, the relationship between genetic predisposition and the gut bacterial community in PD needs to be better understood [6]. These features lead to a particular interest in exploring the gut microbiome when studying PD. From a scientific and clinical perspective, this holds a vast potential for diagnosis, prognosis, and even for understanding disease pathogenesis, as dysbiosis can induce peripheral inflammation, which, in turn, may contribute to the pathophysiology of PD through humoral and neural pathways [34,87]. Actually, numerous studies have revealed a correlation between the prevalence of specific bacterial taxa and the longevity of the disease, severity, motor and non-motor symptom scores, and the use of antiparkinsonian therapies [6]. Additionally, thirteen studies conducted across three continents have demonstrated differences in the gut bacterial community between PD patients and healthy individuals [88].

This bidirectional communication between the brain and the gut microbiota, known as the Brain-Gut-Microbiome Axis (GBrA), refers to the interaction between the gut microbes and the CNS via metabolic, immune, neuronal, and endocrine signaling pathways and mechanisms (Figure 2) [89][90].

Still, one pathway is offering a more direct form of communication: the vagus nerve [3,4,6,89,91]. The vagus nerve is a complex bidirectional system that allows the communication between the hypothalamic-pituitary-adrenal axis hormones, the CNS, the autonomic nervous system, and the ENS [4,27]. Modifications in this axis have been postulated to impact the development of PD directly [3,4]. The discovery of αSyn aggregates in peripheral locations, such as the ENS, supports the concept of the "GBrA". The ENS, which innervates the gastrointestinal (GI) system and is located near the intestinal lumen, provides a significant region for interaction with bacteria [6,7]. From the gut to the brain, there is an ascending aggregation of αSyn and Lewy Bodies (LB) formation, leading to a progressive neurodegeneration [4]. Having mentioned this, a study conducted by Sampson *et al.* underscores the significance of GBrA in the pathogenesis of PD [92]. The research involving transgenic mice with PD reveals that alterations in the intestinal composition contribute to the disease's pathogenesis [92]. Notably, αSyn aggregates demonstrate a propensity to readily spread from the enteric nervous system to the brain, shedding light on the pivotal role of the GBrA in the progression of PD [4,92].

In this way, it can be assumed that the intestinal microbiome profoundly influences this entire neuronal network, facilitating the absorption of nutrients, vitamins, and medications while modulating the immune system [4]. In 2017, Stolzenberg *et al.* demonstrated a positive correlation between inflammation of the intestinal wall in pediatric patients and the expression of αSyn in the ENS [93]. This finding suggests an inflammatory response that precedes GI infections commonly associated with the pathogenesis of PD [94]. Additionally, it further indicates that the expression of this protein aggregates within the ENS is part of its normal immune defense mechanism, which supports the hypothesis of Braak et al. [6,93].

Microbiome *vs* αSyn – Potential Contributions of the Microbiome to PD: Braak's Hypothesis

Interestingly, PD not only affects the brain, but recent studies have also demonstrated that the disease extends to other organs, namely belonging to the GI system [34]. In 2003, Braak and colleagues postulated a "dual-hit hypothesis" suggesting that αSyn aggregation, triggered by a neurotrophic agent or an unknown pathogen, propagates in a prion-like manner from the ENS and the olfactory bulb to the CNS via the dorsal motor nucleus of the vagus nerve [4,27,91,95,96]. This invasion creates a pro-inflammatory environment, increasing the permeability of the intestinal barrier and leading to reactive oxygen species (ROS) accumulation. Consequently, this will result in a dysregulation in homeostasis, activating various immunological mechanisms that might promote αSyn aggregation [34,97]. Based on such postulation, Braak *et al.* proposed a six-stage progression system for PD in the brain and in surrounding olfactory regions based on the propagation patterns of αSyn, which can be further correlated with several characteristic hallmarks of the disease [34,98]. With this in mind, Holmqvist *et al*. conducted studies that provided direct evidence supporting Braak's hypothesis [8]. This study observed that all forms of αSyn (monomers, oligomers, and fibrils) were actively transported from the gut to the brain through the dorsal motor of the vagus nerve. To support this theory, the same authors employed human brain lysate from PD patients containing various forms of αSyn and recombinant αSyn in an *in vivo* model (Adult wild-type Sprague Dawley rats) [8]. In both situations, it was deduced that αSyn was transported from the gut to the brain via the vagus nerve [8]. Following such pieces of evidence, S. Kim *et al.*, upon injection of misfolded αSyn into the gut of healthy mice, discovered an accumulation of this protein in both the vagus nerve and the brain. This finding points to the fact that PD may originate in the gut in specific subsets of patients [91,99]. Yet, this assumption remains under debate.

Given the divergent nature of the symptoms associated with PD, these findings support the hypothesis that this disease may be divided into two variants. One variant postulates that PD originates in the gut, explaining why some patients initially present intestinal discomfort problems. The other variant suggests that the disease initiates in the brain, being justified by the early onset of neurological symptoms [91,100].

Gut Microbiome and Neuroinflammation

The cell structure of microbes varies, presenting a tendency to initiate signaling pathways for pattern recognition receptors that can cause inflammation [4]. Inflammation is widely recognized as a prominent pathophysiological characteristic of PD [9]. Patients with PD have been observed to display heightened levels of several inflammatory molecules (IL-1β (Interleukin-1 beta), IL-6 (Interleukin-6), INF-γ (interferon-γ), MCP-1 (Monocyte chemoattractant protein-1) and TNF-α (Tumor necrosis factor- α)) in their bloodstream, cerebrospinal fluid (CSF), and brain [101]. (Neuro)Inflammation is widely recognized as a prominent pathophysiological characteristic of PD, being a major contributor to disease progression and severity, so it can be assumed that it typically develops due to protein aggregation and dopaminergic cell death [9,27,88]. Indeed, several studies have demonstrated the ability of αSyn to stimulate the production of pro-inflammatory cytokines in both microglia and monocytes [6]. Nevertheless, it should be noted that the same inflammatory markers are not always implicated in PD. Even so, some specific cytokines and chemokines are frequently dysregulated (in the brain, CSF and blood) such as IL-6, TNF (Tumor necrosis factor), IL-1 (Interleukin-1), CRP (C-reactive protein), IL-10 (Interleukin 10), CCL5 (Chemokine (C-C motif) ligand 5), INF-γ, and IL-2 (Interleukin-2) [4,27]. This inflammatory process may be triggered by a breakdown in intestinal barrier function (caused, for example, by bacterial infections, oxidative stress, and dysbiosis), leading to increased systemic exposure to inflammatory microbial products, causing αSyn deposition, a characteristic of intestinal hyper-permeability in PD (Figure 2) [4,6,88,102]. This may be related to the etiology and symptomatology of PD [95]. Furthermore, systemic inflammation can disrupt the blood-brain barrier (BBB) permeability, resulting in inflammatory cytokines or systemic immune cells' entrance into the CNS. As a result, the association between low-grade inflammation in the gut and PD has gained considerable attention [27,88].

The GI tract is coated on the inside by the intestinal mucosa, which is a physical and immunological barrier that separates the external environment from the host’s bloodstream. Numerous factors can compromise this barrier's proper functioning and permeability, including bacteria and their metabolic by-products. For instance, disruption of the bacterial balance causes an increase in permeability, subsequently triggering an inflammatory intestinal response, which leads to a state of (neuro)inflammation [6]. Indeed, comparative studies have shown that individuals with PD exhibit an overabundance of a pro-inflammatory profile (such as *Streptococcus, Bifidobacterium, Lactobacillus, Akkermansia,* and *Desulfovibrio*) in their gut microbiota, along with increased expression of genes related to pro-inflammatory cytokines and chemokines in their intestinal tissue, compared to control subjects [27,88,103]. In addition, a significant population of neurons near the intestinal epithelium directly connects to the brainstem via the vagus nerve [27]. In a study conducted by Sampson *et al*. in 2016, oral administration of microbial metabolites into germ-free mice promotes neuroinflammation (αSyn-Dependent Microglia Activation) and subsequently led to the manifestation of characteristic motor symptoms of PD [34,92]. Nevertheless, the role of inflammation is still a matter of discussion in PD since studies defend that it can exert a dual role, being either neurotoxic or neuroprotective. [9].

Molecular Mediators: Toll-Like Receptors (TLR)

The vagus nerve is frequently involved in how the microbiota influences the CNS, specifically through neuroimmune and neuroendocrine mechanisms [89,104]. This communication is facilitated by microbe-derived molecules known as modulators that interact with enteroendocrine cells (EECs), enterochromaffin cells (ECCs), and the mucosal immune system to propagate signals [89,105]. TLRs are innate immune receptors expressed in microglia, immune and non-immune cells [106]. They are capable of recognizing various exogenous and endogenous stimuli to mediate inflammatory responses [106,107]. Most studies indicated an increased expression of TLRs in the brain and peripheral blood cells of PD samples (TLR2, TLR4, and TLR9 having the most prominent roles) [106,107]. We can hypothesize that by increasing both TLRs and inflammatory cytokines (whose function is to serve as endogenous damage-associated molecular patterns (DAMPs)), αSyn may directly impact microglial cells [89,108]. The signaling of TLRs triggers NF-κB activation, which is essential for an increase in nitric oxide production and dopaminergic neuron apoptosis [6,109]. In fact, according to some researchers, when gut dysbiosis occurs, the barrier becomes dysfunctional, activating TLRs, which subsequently recognize bacterial lipopolysaccharide (LPS) [110]. The presence of LPS impairs the function of the intestinal barrier and promotes the production of various pro-inflammatory cytokines that will cross the BBB, thereby inducing a state of neuroinflammation and consequently leading to neuronal cell death in PD [6,110].

Molecular Mediators: LPS and Lipopolysaccharide-Binding Protein (LBP)

Maintaining the integrity of the intestinal barrier is essential for regulating how luminal substances travel across the gut wall, both through paracellular and transcellular pathways. As previously mentioned, a compromised intestinal barrier increases the susceptibility of systemic circulation of inflammatory microbial products such as LPS [27,88]. This leads to intestinal inflammation and oxidative stress, further disrupting mucosal permeability and triggering the aggregation of αSyn in the ENS [4]. Actually, LPS is the major component of the outer surface membrane specific to gram-negative bacteria [111]. Studies regarding the blood and plasma of PD patients have shown increased systemic exposure to LPS, supporting the hypothesis that PD is associated with intestinal barrier dysfunction [88]. Given that oscillations between LPS and LBP levels occur, there have been indications assuming this is a potential marker for dysbiosis [27]. For instance, a study by Paul M. Carvey *et al.* showed that prenatal exposure to LPS leads to long-term loss of dopaminergic neurons in SNpc of a PD rat model. [112]. Such finding is in line with the study of Adam Jangula and colleagues, which revealed that the expression of αSyn enhances LPS-induced BBB permeability in preclinical models of PD (Snca−/− (KO) mice) [113].

Metabolic Mediators: Short-Chain Fatty Acids (SCFAs)

With the help of EECs and ECCs, SCFAs play a crucial role as neuroactive molecules in the communication between host and microbes [6,89]. The concentration and distribution of SCFA types significantly influence gut health and homeostatic functions, such as mucin production, tight-junction formation, glucose homeostasis, and immunomodulation [4,114]. From a physiological perspective, these compounds are produced in the gut through bacterial metabolism of carbohydrates and protein, serving as an energy source for microbial growth and proliferation [6,88]. The three main types of SCFAs are acetic, propionic, and butyric acid [88]. Nevertheless, in environments with high pH levels, SCFAs may exist as salts, which limits their penetration through the bacterial cell wall [89].

One of the primary mediators contributing to the impact of the gut on PD symptoms is the reduced concentrations of a specific SCFA, butyrate [95]. Such decreased levels of these SCFAs in the intestine severely affect the integrity and function of the intestinal barrier, promoting inflammation and increasing the risk of αSyn deposition [6]. Thus, when a host has a diet low in fermentable fiber, the microbes nourish themselves on mucus glycans using alternative energy sources, which are, in turn, less favorable. As a result, fermentative activity decreases, reducing SCFA production [89]. Butyrate exerts its effects through multiple mechanisms, including activating specific receptors and inhibiting the histone deacetylase (HDAC) enzyme [115]. By inhibiting HDAC, butyrate can regulate gene expression and epigenetic activity, influencing various cellular processes (such as proliferation and apoptosis) [115] . Moreover, butyrate enhance mitochondrial performance and prevent cell death by blocking a key mediator (mitochondrial complex I) [95,116]. Additionally, it is thought that the microbiota may influence the synthesis of molecules such as neurotransmitters (e.g., gamma amino butyric acid) and the products of fermentation (e.g., the short chain fatty acids butyrate, propionate, and acetate) promoting the functioning of the nervous system [6,117]. While contrasting fecal SCFA levels in PD patients and control subjects, decreased concentrations of SCFAs, particularly butyric acid, were found in PD patients, which were linked to microbiota alterations [88]. In fact, several bacterial taxa reportedly reduced in PD are SCFA producers [2,3,27,88]. These molecules are important as an energy source in the body, as well as by stimulating colonic blood flow, capturing fluids and electrolytes, influencing inflammatory responses, and proliferating the mucosa [88,89].

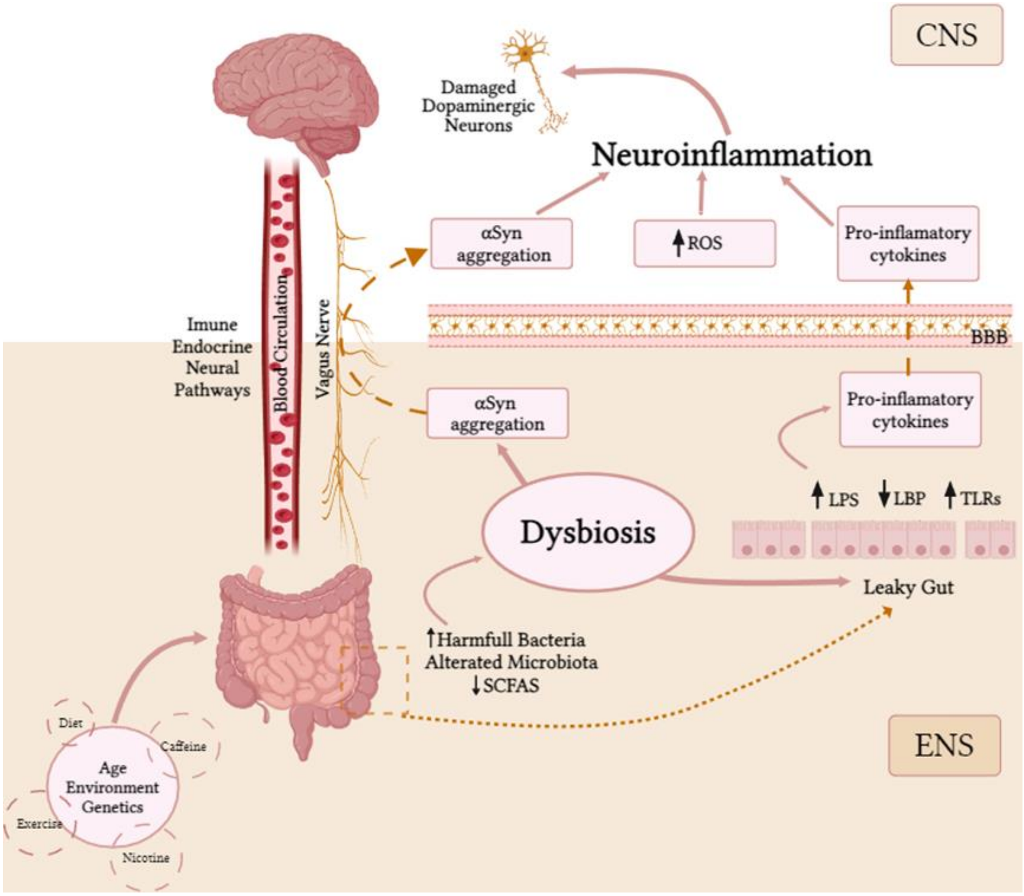

Figure 2. Gut-Brain Axis Model.

Thus, through the actions of SCFAs on tight junction protein expression, they can modulate the permeability of both intestinal and blood-brain barriers, exhibiting anti-inflammatory and antioxidant properties [6,27].

Age, environment, and genetics are the three major factors modulating the microbiota. In addition, there are other lifestyle factors that appear to play a role in PD pathophysiology. Diet can modulate our microbiome through the availability of macro- and micronutrients. For example, there are certain compounds that may trigger the PD onset/pathology and others that have neuroprotective properties [118,119]. Exercise has been associated with beneficial alterations in microbiome composition, impacting energy homeostasis and regulation [4,120]. In addition to medication, it is clinically recommended to prescribe activation therapies, such as physiotherapy, which is a widely employed therapeutic approach [41]. Caffeine consumption is one of the most studied nutritional habits and is highly correlated with a decreased PD risk, suggesting its potential to modulate the pathophysiology of the disease [91,95,121,122]. As regards nicotine, there are a few studies that have consistently demonstrated an inverse association between smoking and the risk of developing PD [91,95]. Former smokers and smokers had a lower risk of developing PD compared to non-smokers [122]. These factors will cause an alteration in the microbiota, increasing harmful bacteria and decreasing SCFAs, which will lead to a state of dysbiosis, this causes the integrity and function of the intestinal barrier to be affected, increasing metabolites such as LPS and TLRs and decreasing LBP, promoting an increase in various pro-inflammatory cytokines that in turn will cross the BBB reaching the CNS. This state of dysbiosis will also cause an aggregation of αSyn, which via the vagus nerve (Braak's Theory), will reach the CNS. In the brain, this aggregation of αSyn increased pro-inflammatory cytokines, and increased ROS will lead to a state of neuroinflammation and consequently lead to death of dopaminergic neurons. Parkinson Disease (PD); Short-chain fatty acids (SCFAs); bacterial lipopolysaccharide (LPS); lipopolysaccharide-binding protein (LBP); Toll-like receptors (TLRs); Blood-Brain Barrier (BBB); Enteric Nervous System (ENS); Central Nervous System

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(CNS); alpha-synuclein (α-Syn); Reactive Oxygen Species (ROS). Figure generated using BioRender.com (accessed on March 2024)).

Microbiome: A New Source for PD Biomarkers?

As previously indicated, in addition to the fact that there is no effective treatment to halt the progression of the disease, when patients are diagnosed, they already have a high level of degeneration of the dopaminergic neurons [75,123]. Consequently, there is an urgent need to develop sensitive and practical biomarkers that might allow to detect PD at earlier stages, particularly within the symptomatic prodromic phases of the disease [124–126].

Numerous molecules in the CSF, including αSyn, DJ-1, amyloid-beta, tau, and lysosomal enzymes, are currently under investigation as potential biomarkers for PD [7,127–129]. Recently, Siderowf *et al.*, show that α-Syn seed amplification assays (SAAs) might be able to distinguish PD patients and healthy subjects profiles, which can be tested, in a near future, as a potential biomarker [130]. Up to date, no definitive and specific biomarkers have been identified. Nevertheless, an in-depth analysis of gut dysbiosis, might be an important step to successfully originate specific PD biomarkers.

Current research indicates that the intestinal microbiota and the GI environment exert influence and may be used as non-motor biomarkers for PD [92]. As previously noted, alongside the characteristic motor symptoms, individuals afflicted with PD commonly experience GI dysfunctions, primarily involving the intestinal tract [131,132]. Constipation, a common GI disorder in PD, may be partly caused by changes in gut microbiota composition and its byproducts [133,134]. Consequently, constipation, dysbiosis, modified intestinal permeability, enteric accumulation of αSyn, and GI inflammation are evident in individuals, indicative of PD at various points in their lives [135–137]. It is hypothesized that by profiling the microbiota of PD patients and those at a heightened risk of developing PD, alterations in the GI system could be employed as potential biomarkers for the early detection of PD [138]. However, more studies are needed to fully support this possibility.

The genomic characterization of fecal samples offers a potential avenue for identifying biomarkers associated with PD [139,140]. Qian and colleagues successfully identified twenty-five genetic markers within the intestinal microbiota, which is beneficial for PD diagnosis and to distinguish this pathology from others, such as Alzheimer's disease (AD) and multisystem atrophy (MSA) [140]. Furthermore, investigations into the fecal metabolome of individuals with PD, in comparison to healthy controls, revealed a diminished concentration of SCFAs and noteworthy alterations in the metabolism of amino acids, lipids, and vitamins [141,142]. Therefore, bacterial metabolites and perturbed metabolic pathways may also serve as biomarkers for PD [142]. Despite these promising findings, to date no studies have specifically evaluated the fecal metabolome as a diagnostic biomarker for PD. While the obtained developments are encouraging, replication in larger population samples is imperative to ascertain their reproducibility. Additionally, an emphasis on characterizing the intestinal microbiota, not only through gene sequencing but also at the level of the fecal metabolome, is essential to uncover novel possibilities for biomarkers associated with PD. Additionally, exploring biomarkers, underlying causes of the disease, and symptom management strategies offer potential clinical applications in the future. Pursuing these avenues holds the potential not only to enhance our understanding of PD but also to contribute to the development of effective and early interventions aimed at symptom management, slowing down the disease progression in the future and could make it possible to identify the patients who would most benefit from experimental therapy and to quantify the effectiveness of future drugs [143].

Microbiome: Are We Looking for a New Therapeutic Target?

The existing therapies for PD, including the gold standard, levodopa, predominantly alleviate symptoms with limited efficacy and lack substantial prophylactic effects [135,144,145]. Given the prevalence of GI dysfunction in over 80% of PD individuals, it is plausible to hypothesize that alterations in our microbiota may influence PD. Therefore, this suggests the potential role of a compromised GBrA in the pathogenesis of the disease [146,147].

In contrast to predictive biomarkers, which might indicate or demonstrate a causal relationship, therapeutic interventions need a direct connection between microbes and disease states [148]. If imbalances in the microbiome were considered, as synonymous of disease, restoring a healthy microbiome emerges as a compelling therapeutic alternative [149]. In this context, diverse approaches have been investigated to model the microbiome and reinstate equilibrium. Indeed, microbiome-based therapies encompass various strategies, including live biotherapeutics, fecal microbiota transplant, microbiome mimetics, dietary interventions, prebiotics, probiotics, symbiotics, antibiotics, and phage therapy [148,150,151].

Since the intestinal microbiome is significantly influenced by diet, dietary interventions also emerge as a potential therapeutic target for preventing, modifying, or delaying PD [152–155]. Notably, nutritional interventions (such as probiotics) can restore the composition of the intestinal microbiota, thereby improving the prognosis of the disease [135,156].

As mentioned above, patients with this disease often exhibit a dysregulated gut microbiome characterized by reduced SCFA bacteria and increased LPS bacteria [152,157,158]. Addressing this imbalance, a treatment method could involve using prebiotics, such as fibers, which can stimulate the growth of SCFA-producing bacteria, increasing SCFAs in the colon [159,160]. In addition, it was noted that administering prebiotics to individuals diagnosed with PD may potentially modify the course of the disease. Therefore, assumptions have been made postulating that an increase in SCFA levels might mitigate issues such as intestinal permeability to endotoxin, colonic inflammation, and neuroinflammation, ultimately contributing to a reduction in the loss of dopaminergic neurons [7,157,161].

Furthermore, Perez-Pardo *et al.* discovered that a dietary intervention combining two membrane synthesis precursors—uridine and docosahexaenoic acid (DHA)—which enhance phospholipid synthesis, along with prebiotic fibers, exhibited a neuroprotective effect in a PD model-induced by rotenone [162]. Their study demonstrated partial alleviation of both motor and non-motor symptoms induced by rotenone and the restoration of DAT levels in the striatum [162–164]. This suggests that the combination of these nutritional elements may benefit the progression and symptomatology of PD. Subsequent investigations have further corroborated this view, demonstrating that diet, probiotics, and prebiotics can potentially normalize dysbiotic microbiota [7,165–167]. Therefore, these interventions could hold promise as potentially beneficial strategies in treating PD.

Another potential use of microbiota-targeted intervention is in treating PD patients to optimize the efficacy of current PD drugs since a better understanding of the influence of GM as a predictive biomarker for drug metabolism will give us information on the bioavailability of the medication, its responsiveness, and the direct results of the treatment [4,168–170]. Notably, research demonstrated that eradicating *Helicobacter pylori (H. pylori)* improved levodopa absorption, a key drug used in PD treatment, resulting in reduced motor symptoms [171–173]. In PD patients, an *H. pylori* infection correlates with ongoing motor deterioration [172,173]. Therefore, *H. pylori* infection interferes with the pharmacokinetics of levodopa, leading to increased delays and periods of ineffectiveness [174]. These findings provide a compelling rationale for considering microbiota-targeted interventions to optimize levodopa therapy, particularly for individuals requiring high and frequent doses of levodopa who may experience significant side effects [175]. Such interventions could potentially enhance the therapeutic outcomes and alleviate the life-altering impact of levodopa-related side effects in PD patients.

While the prospect of interventions targeting the microbiota in PD is promising, it is crucial to acknowledge that additional clinical trials are necessary to ascertain the actual benefits in this context. Presently, there are 39 clinical trials underway (including those exploring the relationship between PD and gut dysbiosis and potential therapeutics), as outlined in Table 2 (Search conducted on the National Library of Medicine's Clinical Trials Registry Platform with the terms "Parkinson's" and "Gut Microbiome"). However, it's essential to note that pre-clinical and clinical studies investigating gut therapeutic strategies for diverse PD symptoms come with certain limitations. Importantly, small sample sizes, insufficient duration for therapeutic intervention, variations in disease severity among patients, inadequacies in outcome measures, and a lack of standardization in key reading variables are some of those limitations [135,176]. Furthermore, the substantial heterogeneity in the microbiome across individuals raises concerns regarding gut microbiota variability in PD patients [177,178]. This emphasizes the significance of individualized approaches during clinical assessment, diagnosis, and treatment selection in pursuing more effective outcomes [179].

Table 2. Summary table of clinical cases registered (Clinical Trials.gov: access on February 04, 2024)

Clinical Trial Title	**Study Type**	**Sample Size**	**Study Phase**	**ClinicalTrials.gov Identifier**
Ketogenic Diet Interventions in Parkinson's Disease: Safeguarding the Gut Microbiome (KIM)	Interventional	50	Not yet recruiting	NCT05469997
Characterization of Fecal Microbiome Changes After Administration of PRIM-DJ2727 in Parkinson's Disease Patients	Interventional	0	Withdrawn	NCT03026231
The Microbiome in Parkinson's Disease	Observational	210	Unknown status	NCT03129451
Microbiome and Diet in Parkinson's Disease - a Randomized, Controlled Phase 2 Trial (PD-Diet)	Interventional	40	Not yet recruiting	NCT06207136
Levodopa Response and Gut Microbiome in Patients With Parkinson's Disease	Observational	38	Completed	NCT04956939
Prebiotics in the Parkinson's Disease Microbiome	Interventional	20	Completed	NCT04512599
Observational Small Intestine and Blood Fingerprint (SmIle) Study in Parkinson's Disease	Observational	100	Not yet recruiting	NCT06003608
Deep Brain Stimulation Therapy and Intestinal Microbiota	Observational	30	Unknown status	NCT04855344
Single-center Pathophysiological Study of the Role of Inflammation, Changes in the Intestinal Epithelial Barrier and the Intestinal Microbiota in Parkinson's Disease	Interventional	77	Terminated	NCT04652843
A Pilot Study to Explore the Role of Gut Flora in Parkinson's Disease	Observational	100	Unknown status	NCT04148326
Study of the Genome, Gut Metagenome and Diet of Patients With Incident Parkinson's Disease	Observational	138	Terminated	NCT04119596
Resistant Maltodextrin for Gut Microbiome in Parkinson's Disease: Safety and Tolerability Study	Interventional	30	Active, not recruiting	NCT03667404
Microbiota Modification for the Treatment of Motor Complication of Parkinson´s Disease	Interventional	14	Completed	NCT04730245
Constipation and Changes in the Gut Flora in Parkinson's Disease	Observational	80	Recruiting	NCT05787756
AADC/TDC in Advanced Parkinson's Disease	Observational	50	Recruiting	NCT05558787
A Trial of Fecal Microbiome Transplantation in Parkinson's Disease Patients	Interventional	51	Completed	NCT04854291
Gut Microbiota and Parkinson's Disease	Observational	50	Unknown status	NCT03710668
Determining the Microbiota Composition of the Middle Meatus in Parkinson's	Observational	48	Completed	NCT03336697
Study of the Fecal Microbiome in Patients With Parkinson's Disease	Interventional	15	Completed	NCT03671785
Parkinson's Disease Biomarkers in Nerve Cells in the Gut	Observational	60	Recruiting	NCT05347407
Efficacy and Safety of Fecal Microbiota Transplantation in the Treatment of Parkinson's Disease With Constipation	Interventional	30	Recruiting	NCT04837313

Increased Gut Permeability to Lipopolysaccharides (LPS) in Parkinson's Disease	Observational	43	Completed	NCT01155492
Fecal Microbiota Transplantation for Parkinson's Disease	Interventional	49	Completed	NCT03808389
Microbiota Intervention to Change the Response of Parkinson's Disease	Interventional	86	Recruiting	NCT03575195
Microbiome Composition and Function Contributes to Cognitive Impairment and Neuroinflammation in Parkinson's Disease	Observational	100	Recruiting	NCT05419453
Gut Microbiota in the Progression of Alpha-synucleinopathies	Observational	490	Recruiting	NCT05353868
Gut Health and Probiotics in Parkinson's (SymPD)	Interventional	60	Active, not recruiting	NCT05146921
Effects of Probiotics on Peripheral Immunity in Parkinson's Disease	Interventional	88	Enrolling by invitation	NCT05173701
Gut Microbiota Across Early Stages of Synucleinopathy: From High-risk Relatives, REM Sleep Behavior Disorder to Early Parkinson's Disease	Observational	441	Completed	NCT03645226
Meridian Activation Remedy System for Parkinson's Disease (MARS-PD)	Interventional	88	Recruiting	NCT05621772
Mediterranean Diet Intervention to Improve Gastrointestinal Function in Parkinson's Disease a Randomized, Controlled, Clinical Trial (MEDI-PD)	Interventional	46	Completed	NCT04683900
MOVIN' CARE for PD (Risk Management) (jcpdmcP)	Interventional	308	Active, not recruiting	NCT06147284
Fecal Microbiota Transplantation As a Potential Treatment for Parkinson's Disease	Interventional	10	Completed	NCT03876327
Efficacy of Probiotics for Parkinson Disease (PD)	Interventional	300	Not yet recruiting	NCT06118294
MOVIN' CARE for PD (Mind-body Interventions) (jcpdmcI)	Interventional	154	Active, not recruiting	NCT06078046
Establishment of a Human Tissue Bank for Studying the Microbial Etiology of Neurodegenerative Diseases	Observational	0	Withdrawn	NCT01954875
The Sunnybrook Dementia Study (SDS)	Observational	1600	Recruiting	NCT01800214
Metabolic Cofactor Supplementation in Alzheimer's Disease (AD) and Parkinson's Disease (PD) Patients	Interventional	120	Completed	NCT04044131
N-DOSE: A Dose Optimization Trial of Nicotinamide Riboside in Parkinson's Disease	Interventional	80	Recruiting	NCT05589766

Gut Microbiome-Drug Interaction for the Treatment of PD

Notably, the efficacy of pharmaceutical drugs exhibits considerable variability among individuals, owing to the intricate nature of the human body [180,181]. The complexity of this system is further underscored by the pivotal role played by the gut microbiome [182]. Recent research has illuminated the significant influence exerted by gut bacteria on the modulation of drug mechanisms and associated side effects [183,184]. Importantly, these interactions between gut microbes and pharmaceutical agents are bidirectional [185]. In other words, drugs can induce alterations in the composition and functionality of the gut microbiota, subsequently impacting the metabolism and utilization of these drugs within the body [186,187].

The spectrum of pharmaceuticals demonstrating discernible connections to the microbiota is extensive. However, the specific alterations incurred by various drugs on the microbiota exhibit distinctive patterns. Consequences may encompass a diminution in microbial diversity and an augmentation in the proliferation of particular potentially pathogenic bacteria, such as *Proteobacteria, Bifidobacteriaceae, Ruminococcaceae, Verrucomicrobiaceae, and Christensenellaceae* [188–192]. Nevertheless, the mechanisms through which medications impact the microbiota predominantly operate through indirect mechanisms, wherein alterations are introduced to various facets of the gut environment, including nutrient composition, thereby exerting an influence on bacterial growth [193]. Notably, certain medications undergo direct metabolic transformations by bacteria, thereby dictating their subsequent distribution and functionality within the body [184]. An illustrative instance of this phenomenon is evident in the case of levodopa. In this context, bacteria directly metabolize levodopa, ultimately shaping its trajectory and effects within the organism.

Following oral administration, the absorption of levodopa is imperative through the small intestine for it to traverse the BBB and gain access to the brain [194,195]. Within the brain, the human enzyme aromatic amino acid decarboxylase (AAAD), also called DOPA decarboxylase, catalyzes the conversion of levodopa into therapeutically active dopamine [196–198]. The pivotal determinant of the drug's efficacy lies in the bioavailability of levodopa to the brain [199]. Consequently, levodopa is frequently co-administered with catechol metabolism inhibitors, exemplified by carbidopa, to impede its metabolism at sites other than its intended action, thereby optimizing its availability for therapeutic impact [196,200]. This is crucial, as certain gut bacteria and peripheral human enzymes can metabolize the drug before it crosses the BBB [105,201]. This preemptive metabolism could substantially diminish the availability and consequently undermine the efficacy of levodopa [201]. In light of this, the interplay between levodopa and *H. pylori*, as previously mentioned, underscores the intricate relationship between pharmaceutical agents and gut microbiota [174].

Recent research has brought another dimension of microbial involvement in levodopa metabolism, specifically through microbial decarboxylases in the gut [202]. Notably, a newly identified bacterial metabolism of levodopa, facilitated by tyrosine decarboxylases (tyrDCs), has been determined, with *Enterococcus faecalis* (*E. faecalis*) predominantly carrying out this process [169]. Intriguingly, the potential to enhance the drug's efficacy is evident through manipulating this microbial metabolism. Mutation of tyrDCs in *E. faecalis* has been shown to impede the bacterial conversion of Levodopa into dopamine, presenting a promising avenue for improving the drug's therapeutic effectiveness [169,203].

Nevertheless, considering the role of the intestinal microbiome in drug metabolism, coupled with the substantial impact of medications on the microbiome's composition, the possible interaction between PD medication and the microbiome's composition is not surprising. Actually, insights derived from these studies support the plausible existence of a clinically significant relationship between the microbiota and drug metabolism in individuals with PD. Consequently, mapping the human microbiome becomes imperative to unravel the underlying mechanisms governing these intricate microbiota-drug interactions [204]. Such exploration is pivotal not only for elucidating the dynamics of this interaction but also for unveiling how it influences the efficacy of medication in the context of PD.

Future Perspectives

Although high-throughput sequencing technology has allowed outstanding advances in gut microbiota research, much remains to be unraveled. There is plenty of indirect evidence to support the active role of the microbiome in PD, but there is limited conclusive support. And the real question remains unanswered: Is GM dysregulation a cause or a consequence of PD? It is tough to demonstrate the exact molecular and cellular pathways through which the microbiome may promote the pathogenesis of PD. This is because the microbiome is highly heterogeneous between individuals. Most of the existing studies on gut microbes in PD are cross-sectional studies, which cannot sufficiently indicate the causal relationships between gut microbes and PD pathogenesis, resulting in a significant gap in the literature and much divergence in the results. Therefore, considering the high heterogeneity of gut microbes between different individuals, it is necessary to emphasize more longitudinal research to advance our knowledge about the mechanisms underlying the correlation between gut microbes and PD so that in the future, we can use GM as a biomarker and so that more targeted treatment strategies based on the gut microbiome can be developed (either alone or as an adjuvant for existing therapies) (Figure 3).

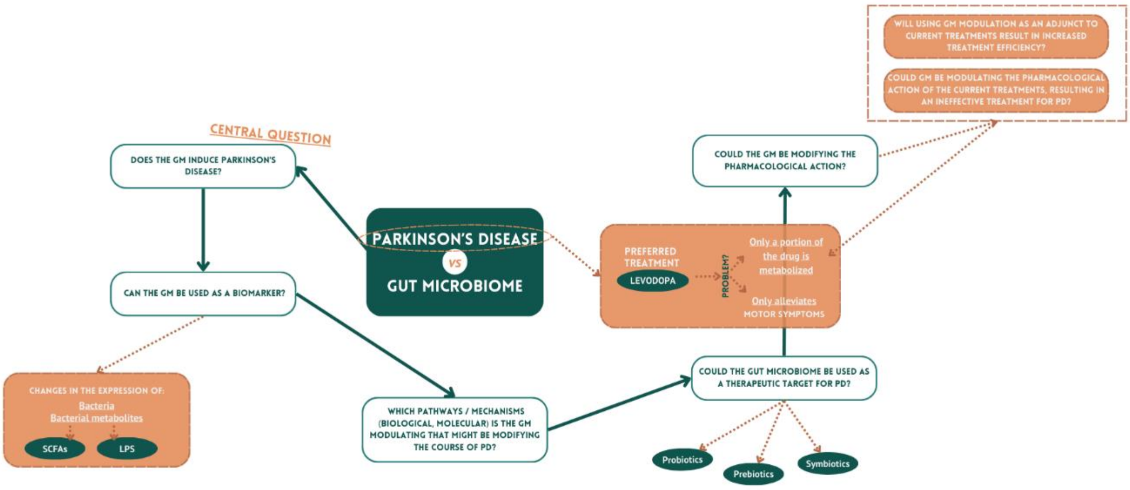

Figure 3. Gut microbiome and Parkinson's disease. The gut microbiome is an incredibly complex ecosystem, having a denoted and multifaceted impact on people's health. Indeed, it is nowadays well accepted that it might play a role in the pathogenesis of numerous diseases, including neurodegenerative diseases such as PD. This appreciation of the importance and complexity of the gut microbiome leads to several questions that are currently important to address, with the correct application of technological assays to the same set of samples in hopes of capturing multiple layers of information about the microbiome's involvement in disease. Parkinson's Disease (PD); Gut Microbiome (GM); Short-chain fatty acids (SCFAs); bacterial lipopolysaccharide (LPS).

Conclusions

PD is a highly heterogeneous disease with an unclear etiology. Whether or not the microbiome is the initial cause of PD remains uncertain, but it has been hypothesized to play a key role in the distinct stages of the disease. This is supported by various pro-inflammatory mediators associated with bacteria, which might contribute to or facilitate a neuroinflammatory state in PD. The GBrA assumes particular importance in PD, namely in the formation of αSyn aggregates and in the bidirectional communication and transport of αSyn via the vagus nerve. Therefore, even though it is an exciting perspective, there are still questions that should be addressed: is microbiome a cause or a consequence of PD? Can microbiome be a potential source of biomarkers or a therapeutic target for PD? Can the microbiome be a critical modulator of the current/available treatment pipeline?

Considering this, future randomized controlled trials are needed to evaluate the potential impact of microbiomes in the different stages of PD and distinct treatment regimens. By doing so, new concepts in the pathophysiology and therapeutic setting of the disease might be established.

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