Review

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Review

Dysbiosis of the Gut Microbiota Being a Contributing Factor to Parkinson's Disease: A Literature Review

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Abstract: Parkinson's disease (PD) is a prevalent neurodegenerative disorder characterized by symptoms such as tremors, dementia, and bradykinesia. Early non-motor signs, including constipation and hyposmia, suggest a potential connection between PD and the gut. The loss of dopaminergic neurons in the substantia nigra is a hallmark of PD, with unknown causes. While current treatments provide symptom relief, there are no therapies to slow the progression of the disease. The gut microbiota plays a role in inflammatory and immune responses and has been implicated in neurological disorders. The connection between gut microbiota and neurodegenerative disease remains unclear. In this paper, we aim to explore the potential link between gut microbiome dysbiosis in PD, focusing on constipation, which is an early symptom of the disease. Our review will examine the evidence that suggests the possibility of chronic intestinal inflammation that may contribute to the development of Parkinson's disease and implicate further connections with other neurodegenerative diseases.

Keywords: Parkinson's disease; Dopamine; α-Synuclein (αSyn); Gut microbiota; Gut microbiome dysbiosis

Acronyms: BBB (Blood Brain Barrier); COMT (catalyzed by catechol-O-methyl transferase); ENS (Enteric Nervous System); GALTs (Gut-associated Lymphoid Tissue); GI (Gastrointestinal); HPA (Hypothalamic-pituitary-adrenal axis); L-Dopa (Levodopa); LID (Levodopa induced dyskinesia); LPSs (Lipopolysaccharides); MAO (Monoamine oxidase); MEDI (Mediterranean diet); MIND (Mediterranean-DASH Intervention for Neurodegenerative Delay); NMSQ (Non-Motor Symptom Questionnaire); OSyn (oligomer α-Synuclein); PD (Parkinson's Disease); PSyn (phosphorylated α-Synuclein); RBD (REM sleep behavior disorder); SCFAs (Short chain fatty acids)

Introduction

Parkinson's disease (PD) comes second to Alzheimer's disease as one of the most prevalent neurodegenerative disorders in the United States. PD is characterized by notable symptoms such as tremors, dementia, and bradykinesia. However, the early unique non-motor signs are hyposmia and gastrointestinal disorders, such as constipation, which could indicate a potential for this disease to arise outside the central nervous system (CNS) [1]. The pathology of this disease is characterized by the loss of dopaminergic neurons in the substantia nigra, and the precise causes are not yet known. Current treatments alleviate PD symptoms; the most effective is carbidopa-levodopa, but no currently available therapies slow the progression of PD-related neurodegeneration [2]. Levodopa (L-Dopa) is a natural chemical converted to dopamine in the brain. The drug has to be combined with carbidopa to ensure that levodopa would not convert to dopamine outside of the CNS to ensure proper efficacy.

Our literature review explored the potential contribution of gut microbiome dysbiosis to PD, with constipation being one of the early onset symptoms. The dysregulation in the composition of

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the gut microbiota and their metabolites can lead to intestinal inflammation and disrupt the communication and integrity of the blood-brain barrier (BBB). Specifically, gut dysbiosis is believed to be a potential cause of developing a "leaky gut," leading to alterations in the BBB and subsequent neuroinflammation [3]. Amongst the various cytokines, IFNγ and TNFa have shown a correlation with the altered gut microbiome. The elevated levels of proinflammatory cytokines and chemokines, such as IL-6, TNF, IL-1B, and IL-2, may indicate peripheral manifestations of PD, leading to the deterioration of the intestinal barrier.

Consequently, this allows for systemic exposure to bacterial products like lipopolysaccharides (LPSs) found in the outer membrane [3,4]. This can induce an acute inflammatory response and trigger the production of several inflammatory cytokines in various cell types. In this context, Zonulin and alpha-1-antitrypsin are two factors that contribute to the promotion of increased gut permeability. a-synuclein is a protein in PD that affects neuronal function and communication by damaging dopaminergic neurons responsible for movement. The propagation of a-synuclein from the gut to the brain is thought to contribute to the development of PD. Due to intestinal inflammation and increased gut permeability, the accumulated aSyn moves from the enteric nervous system (ENS) to the CNS through the vagus nerve, leading to synucleinopathies impacting motor function [2,5–8].

The gut-brain axis represents a reciprocal communication pathway linking the gastrointestinal (GI) tract and the CNS. Notably, the vagus nerve plays an essential role in establishing this connection. Ongoing investigations have proposed that the gut microbiota influences the axis, potentially contributing to the pathogenesis of PD. Experimental studies utilizing rodent models have elucidated the ability of aSyn to disseminate from the gut to the brain via the vagus nerve [8–10]. Furthermore, cohort studies have revealed a reduced incidence of PD among individuals who have undergone vagotomy. These findings underscore the alleged involvement of the gut-brain axis in the etiology and progression of PD.

The gut microbiota, comprising various microorganisms, is crucial in maintaining normal physiological function and impacting human health. Age, diet, genetics, antibiotics, probiotics, and stool transplants can alter the gut microbiota composition [11,12]. A typical early symptom of PD, constipation, contributes to gut dysbiosis. Pivotal bacteria such as *Prevotella*, *Akkermansia*, short-chain fatty acid (SCFA)-producing bacteria, and *Bacteroides* in the gut microbiota support the pathogenesis of PD. Decreased SCFA levels can increase endotoxin and neurotoxicity, affecting microglial activation and neuronal function [9–14].

Bacteroides species may contribute to inflammation in PD, while *Akkermansia's* increased levels can disrupt intestinal integrity and promote a-synuclein formation [10,14]. Reduced levels of *Prevotella* may lead to gut inflammation and oxidative stress, triggering neuroinflammation and neurodegeneration [15]. As manifested by their associations with disease duration, cognitive impairment, and potential implications for therapeutic advancements, these findings highlight the gut microbiota dysbiosis as a potential biomarker and therapeutic target for PD.

Lastly, researchers are investigating the potential of diets, such as the Mediterranean diet (MEDI) and ketogenic diets, as an intervention for individuals with PD [16,17]. Probiotic dietary supplementation and fecal microbial transplantation (FMT) have shown initial promise in clinical trials, particularly in alleviating PD-related symptoms [18,19]. Overall, these interventions hold the potential for managing PD symptoms and warrant further investigation in clinical settings.

Dopamine in Parkinson's Disease

Parkinson's disease is caused by the loss of dopaminergic neurons in the substantia nigra, a midbrain dopaminergic nucleus, resulting in decreased dopamine production [20]. The substantia nigra is involved in the control of movement, cognition, and the limbic system [20]. The substantia nigra is divided into the pars compacta and pars reticulata [20]. The pars compacta is the region of the substantia nigra that contains dopaminergic neurons [20]. The pars reticulata consists of GABAergic neurons [20]. The substantia nigra pars compacta appears dark due to the elevated levels of neuromelanin pigment within the cell bodies of the dopaminergic neurons [20]. The nigrostriatal

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pathway, extending from the substantia nigra to the putamen, contributes to the motor deficits associated with Parkinson's disease [20].

Dopamine synthesis begins with the precursor L-dihydroxyphenylalanine or L-DOPA, which can be synthesized via a direct or indirect pathway [21]. Following a direct synthesis pathway, L-DOPA can be synthesized from tyrosine, a non-essential amino acid [21]. The indirect synthesis of L-DOPA utilizes phenylalanine, an essential amino acid, which will be the pathway discussed for synthesizing dopamine [21]. L-phenylalanine is converted to L-tyrosine by the enzyme phenylalanine hydroxylase in the liver. Phenylalanine hydroxylase utilizes oxygen, iron, and tetrahydrobiopterin as cofactors, essential for catalysis [21]. L-tyrosine is then transported to the dopaminergic neurons in the brain via an active transport mechanism [21]. Once in the brain, L-tyrosine is converted into L-DOPA by hydroxylation of the phenol by tyrosine hydroxylase. A further decarboxylation reaction then converts L-DOPA into 3,4-dihydroxy phenethylamine (dopamine) via the enzyme L-3,4-dihydroxyphenylalanine decarboxylase at the pre-synaptic terminal [21]. Dopamine metabolism follows a few various pathways. One pathway resulting in dopamine metabolism is the conversion of dopamine to 3-methoxytryramine catalyzed by catechol-O-methyl transferase or COMT [21]. Monoamine oxidase (MAO) then converts 3-methoxytryramine to 3-methoxy-4-hydroxyacetaldehyde. Aldehyde dehydrogenase then oxidizes the aldehyde in 3-methyoxy-4-hydroxyacetaldehyde into a carboxylic acid yielding homovanillic acid [21].

Deficits in the release of dopamine involve several mechanisms that include defective synaptic vesicle exocytosis, impaired dopamine synthesis, and defective dopamine release [22]. The release of dopamine is facilitated by calcium-dependent exocytosis. In a normally functioning dopaminergic axon, SNARE complex is formed on the plasma membrane [22]. The formation of the SNARE complex on the plasma membrane allows for direct interaction with Parkinson's disease proteins such as a - synuclein and LRRK2, resulting in changes in the expression of the SNARE complex [22]. LRRK2-dependent phosphorylation of the Snapin protein coding gene inhibits SNAP25, which results in defective exocytosis of dopamine [22]. Mutations in these complexes lead to mitochondrial dysfunction, leading to insufficient axonal energy to produce an action potential and resulting in synaptic loss. This synaptic loss disrupts the release of dopamine in dopaminergic neurons [22]. In models with Parkinson's disease with mutations in the *SNCA* gene, which encodes for a – synuclein, inhibit the release of dopamine. The aggregation of a - synuclein in the synapse results in impaired dopamine release by blocking protein function [22].

a-Synuclein in Parkinson's

a-synuclein (aSyn) is a neuronal protein encoded by the *SNCA* gene that plays a physiological role in the pathogenesis of Parkinson's disease [23–25]. Abnormal accumulation and aggregation of a-synuclein results in the degeneration of dopaminergic neurons which are associated with Parkinson's disease [26]. Although the exact function of a-synuclein remains unknown, convincing evidence supports the involvement of a-synuclein in synaptic plasticity and neurotransmitter release [26]. To better understand the connection between neuronal activity and a-synuclein, researchers used cultured neurons and living mice [27] A study conducted hypothesized that neuronal activity regulates the release of a-synuclein from neurons [8,27] Researchers were able to conclude that depolarization of neurons led to increased exocytosis of pre-synaptic vesicles, which is important in a-synuclein release [27].

The underlying genetics contribute to the pathogenic role of aSyn in Parkinson's disease which include point mutations, duplication of *SNCA*, and polymorphic variants [26] Under native conditions, aSyn exists in a state between an unfolded monomer and folded tetramer, which prevents aggregation [26]. A decrease in the tetramer-to-monomer ratio leads to an increased level of aSyn in the unfolded monomer state, favoring aggregation [25,26]. As a result of conformational change, aSyn adopts a β-sheet-rich structure facilitating aggregation of the protein and accumulation in Lewy Bodies [25–27]. aSyn undergoes post-translational modification with phosphorylation being a significant pathological marker of Parkinson's disease. Evidence revealed a relationship between phosphorylation and aSyn degradation. Increased phosphorylation of aSyn due to inhibition of the

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ubiquitin–proteasome system suggests regulation of aSyn degradation [26] Failure to inhibit the ubiquitin-proteasome system can prevent phosphorylation of aSyn and dysregulation of aSyn degradation allowing for the accumulation of aSyn in Lewy bodies [26,27].

aSyn propagation from the gut to the brain contributes to the etiology of Parkinson's disease [28]. The connection between pathological aSyn and gut-brain axis follows Braak's hypothesis. Braak's hypothesis suggests that progressive Parkinson's disease is a result of pathogens entering the nasopharyngeal cavity and migrating from the gastrointestinal tract to the brain resulting in loss of dopaminergic neurons [5,28,29]. The imbalance of gut microbiota promotes intestinal inflammation and impairment in the mucosal barrier [6]. The endotoxins from the entering pathogens cause misfolding and accumulation of aSyn resulting in inflammation [6]. The accumulated aSyn spreads from the enteric nervous system and migrates to the central nervous system via the vagus nerve spreading synucleinopathies from the brainstem to the substantia nigra causing motor dysfunction that contribute to the clinical manifestations of PD such as delayed gastric emptying and severe constipation [5,6,29].

In recent research conducted, researchers aimed to investigate three different aSyn species including oligomer aSyn (OSyn) and phosphorylate aSyn (PSyn) [6]. OSyn was found to display toxic effects on the nervous system and contribute to the pathogenesis of PD [6]. Researchers found that aSyn resided in both PD and control group with variations in distribution within intestinal mucosa, while OSyn excretion occurred from the mucosal layer [6,7]. Enteroendocrine cells lining the intestinal tract aid in the propagation of defective aSyn [7]. OSyn is an abnormal aggregate of aSyn [6]. Increased excretion of Osyn from mucous layer into the gut causes toxic protein accumulation and exhibits a correlation between the gut-brain axis retrograde theory [30]. In PD patients, the increased production of Osyn in the gut and propagation to the brain via the vagus nerve leading to motor dysfunction and severe constipation [6,31].

Gut-Brain Axis

The gut-brain axis has a reciprocating relationship with the central nervous system (CNS), sympathetic nervous system (SNS), ENS, hypothalamic-pituitary-adrenal (HPA) axis, and gut microbiota [12,32]. These systems combined contribute to the altered gut, production of neuromodulatory components, vagus nerve activation, and immune pathways. They are probiotic strains that induce changes in neurotransmitter pathways, including serotonin, dopamine, and norepinephrine [32]. A well-maintained and consistent composition of the gut microbiota is vital for preserving intestinal barrier integrity and reducing inflammation in healthy individuals [3]. This, in turn, positively influences brain development and behavior through the intricate network known as the microbiota-gut-brain axis [3]. Multiple pathways exist connecting the brain and the intestine, with the vagus nerve facilitating the most direct route [2]. The vagus nerve originates from the dorsal motor nucleus in the medulla oblongata and extends throughout the abdominal region, innervating the visceral organs [2]. When stimulation within the intestine occurs, the stimulus can initiate afferent signaling via the vagus nerve. This signaling process is crucial in neuroimmune inflammatory reflex circuits, vital for maintaining peripheral immune regulation [2]. Additionally, emerging evidence suggests that the vagus nerve may serve as a direct pathway through which substances and signals from the intestine can be transmitted to the brain [2]. This highlights the potential bidirectional communication between the gut and the CNS mediated by the vagus nerve [2].

Given the bidirectional communication between the CNS and ENS, there is a postulation of whether the onset of Parkinson's disease is due to "body-first" or "brain-first" [2,14]. One would automatically think of the "brain-first" aligning with the pathology of PD since it would indicate that the misfolded a-synuclein would arise in the CNS and inevitably impact the dopaminergic neurons present in the substantia nigra, and eventually descend towards the rest of the body, causing subsequent symptoms such as motor impairments and constipation. On the other hand, the body-first phenomena would indicate that an external pathogen would enter the gastrointestinal system and initiate the a-synuclein misfolding, suggesting that the progressive neurodegeneration could be peripheral [14]. Then, this misfolding would continue to occur until it would progress into the dorsal

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motor nucleus of the vagus nerve to the medulla oblongata, eventually reaching the cerebral cortex [14]. Another way an external pathogen may enter the host is through the olfactory bulb, which suggests a two-hit hypothesis that would trigger the onset of PD more substantially [14]. Returning to the gastrointestinal tract obtaining a pathogen, the chronic inflammation that could be associated with it will eventually cause dysbiosis of the gut and lead to increased intestinal permeability. Since intestinal permeability will increase, it increases the chances of other bacteria and toxins crossing the ENS, potentially causing a-synuclein misfolding [14].

Notably, abnormal accumulation of a-synuclein, an essential constituent of Lewy bodies, has been identified within the ENS [10]. A study on healthy rodents involved injecting human a-synuclein fibrils into the gut tissue, which induced a-synuclein to spread to the vagus nerve and brainstem [10]. Furthermore, some studies involved the targeted expression of human a-synuclein in the vagus nerve by injecting adeno-associated viral vectors to which a-synuclein aggregates spread progressively from the medulla oblongata to other brain regions [8]. In addition, a rodent study revealed that dysbiosis of the gut has effects preceding the formation of a-synuclein in mouse brains [9]. These findings support that the vagus nerve is a conduit for transmission from the GI tract to the brain [8]. Additionally, cohort studies performed in Northern Europe yielded compelling outcomes revealing that individuals who underwent truncal vagotomy had a lower risk of developing PD compared to the control group that was age and gender-matched [8]. Furthermore, experimental manipulations in mice showed that cervical vagotomy performed before the injection of a-synuclein into the gastric wall prevented a-synuclein formation in the vagus's dorsal motor nucleus [8]. As supported by the experimental evidence, these findings substantiate that a-synuclein can propagate from the gut to the brain, providing clarity on crucial aspects of this pathological process [8,10].

Studies have highlighted that the gut microbiota can regulate the gut-brain axis through endocrine, immunological, and direct neuronal processes, contributing to the idea that PD is a pathological condition that extends from the gut to the brain [8,10]. Aligning with the occurrence of gastrointestinal dysfunctions that precede the typical motor symptoms in individuals with PD, it becomes progressively apparent that the impact of microbiota community changes on the interactions within the gut-brain axis is also implicated in PD development [3]. During the pathogenesis of PD, gut microbiota dysbiosis can elicit persistent inflammation within the intestinal epithelium, which could inevitably induce neuroinflammation through the microbiota-gut-brain axis [3]. These inflammatory responses and fluctuations between the population levels of some bacteria contribute to microbiota dysbiosis, which will disrupt the integrity of the intestinal barrier, referred to as the "leaky gut" [3]. Since there is increased permeability of the intestinal barrier, this will promote the entry of pro-inflammatory byproducts such as lipopolysaccharides (LPSs) and cytokines into the systemic circulation [3]. Consequently, these molecules breach the BBB, accessing the substantia nigra (SN), contributing the neuroinflammation, and potentially leading to the demise of dopaminergic neurons, which is a hallmark feature of PD [3].

Gut Microbiome and Parkinson's

The gut microbiota contains a collection of microorganisms, such as bacteria, viruses, fungi, and archaea, that live in a symbiotic relationship with the host. These microorganisms have many crucial functions, such as promoting normal motility, developing the gastrointestinal epithelium and enteric nervous system (ENS), and contributing to the development of priming the immune system to maintain a barrier towards pathogenic bacteria and digestion of nutrients. Its composition can be altered throughout one's lifetime from various factors such as diet, genetics, use of antibiotics or probiotics, energy intake, and stool transplants [11,12]. Normal physiology and the host's vulnerability to illnesses are impacted by the metabolic processes and interactions of the gut microbiota [33]. At the bacterial level, unique differences are caused by changes in pH, immunological factors, and digestive enzymes. The microbiota directly impacts human health through the production and release of diverse components, including vitamins, essential amino acids, and lipids [33]. This ecosystem is susceptible to changes in one's external environment, such as diet, sleep, chronic noise, and sedentary behaviors. These behaviors could contribute to the onset of several

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neurodegenerative diseases, such as Parkinson's disease. One of the predominant contributing factors that is also correlated with the inevitable dysbiosis of the gut is aging [32]. Throughout aging, there is a potential for a decrease in bowel motility, a sign of constipation [32]. Constipation is one of the early symptoms presented in PD. Chronic constipation could result in low-grade inflammation of the intestines, causing a leaky gut, ultimately leading to changes in the blood-brain barrier and neuroinflammation. A study conducted on PD and control groups utilized the Wexner Constipation Scoring System to evaluate constipation severity [15]. The results revealed a significant association between the two groups, indicating a statistically significant positive correlation [15]. This study confirmed an increased prevalence of constipation among PD patients, particularly in severe grades, thereby establishing a link between constipation and the severity of PD symptoms [15]. These findings suggest that individuals experiencing severe constipation are more susceptible to developing PD, accentuating the potential utility of constipation as a preclinical biomarker [15]. Several bacterium levels, persistently noted across studies, have been associated with PD, highlighting the critical role of the gut microbiota in the pathogenesis of the disease [4,10,14,15]. Such bacterium includes decreased *Prevotella* and short-chain fatty acid (SCFA)-producing bacteria and increased *Bacteroides* and the *Verrucomicrobiaceae* genus, *Akkermansia* [1].

Prevotella, a gram-negative bacterium, is thought to maintain mucosal integrity and produce neuroprotective short-chain fatty acids (SCFAs) that have the potential to exert a protective influence on dopaminergic neurons, shielding them from degradation [15]. Additionally, Prevotella is involved in the protection of dopaminergic neurons via the secretion of hydrogen sulfide [15]. Hydrogen sulfide is a gaseous neurotransmitter in the gut [15]. One consistent finding across studies is a decreased abundance of the bacterium *Prevotella* in PD patients across various populations [1,9–11,13–15,33]. The mean abundance of *Prevotella* was reduced by 46.6% in PD patients compared to healthy controls [4]. Decreased levels of *Prevotella* could increase inflammation and oxidative stress in the gut, which would inevitably trigger neuroinflammation and neurodegeneration in the brain. Moreover, *Prevotella* has been found to correlate with the occurrence of REM sleep behavior disorder (RBD) along with the progression of motor symptoms in PD over a two-year period [10]. In a separate study, individuals with an increased abundance of *Prevotella* exhibited lower rates of constipation, indicating a decreased probability of experiencing subthreshold parkinsonism [10]. Subthreshold parkinsonism refers to the presence of mild Parkinsonian features that do not meet the criteria for a proper PD diagnosis [10]. These significant findings could suggest that a reduction in *Prevotella* abundance could serve as a biomarker for PD diagnosis and a target for disease-modifying interventions [15].

Studies have consistently demonstrated reduced short-chain fatty acid (SCFA)-producing bacteria, including *Faecalibacterium prausnitzii* and *Roseburia* from the *Lachnospiraceae* family in PD patients [9,13,14]. Acetate, propionate, and butyrate are the three principal SCFAs (Short Chain Fatty Acids) produced by gut bacteria [12]. They typically cause activities such as anti-tumorigenic, anti-inflammatory, and anti-microbial effects, change gut integrity, induce reactive oxygen species, and change cell proliferation and function. SCFA-producing bacteria function to maintain epithelial integrity, possess anti-inflammatory properties, and promote gastrointestinal motility while regulating ENS function [10]. Several ways could lead to the depletion of this bacteria, including diet, physical activity, sleep, antibiotics, and exposure to environmental toxins such as metals. The decrease in SCFA levels may lead to an increase in endotoxin and neurotoxic occurrence, which have been linked to the onset of PD [10]. Recent studies implicated that SCFAs can promote microglia's maturation and inflammatory capabilities [34]. Microglia does not express the SCFA receptors. However, they express the associated responsive genes, such as histone deacetylases, which could modulate the gene expression [34]. Increased microglia activation and the production of pro-inflammatory cytokines could alter neuronal function and increase cell death in PD patients [34]. Moreover, decreased SCFA levels may contribute to the emergence of gastrointestinal motility disorders, including constipation in PD, since one of these bacterium's functions is to promote gastrointestinal motility [10]. There is a significant association in PD patients that low SCFAs are

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substantially correlated with poor cognition and lower BMI, and these individuals were found to have poorer postural instability-gait disorder scores as well [10].

The *Bacteroides* genus, which falls within the *Bacteroidetes* phylum, exhibited an exceptional abundance among patients diagnosed with the non-tremor subtype of PD [4]. It is worth emphasizing that PD is a clinically heterogeneous disorder. Individuals with the non-tremor subtype experience more rapid disease progression and display heightened a-synuclein pathology in the ENS neurons compared to those with the tremor subtype [4]. Specific genera within the *Bacteroidetes* phylum can produce diverse pro-inflammatory neurotoxins, including lipopolysaccharides (LPSs) and toxic proteolytic peptides [4]. Consequently, this suggests a potential role for *Bacteroides* species in contributing to the inflammatory processes observed in PD. Furthermore, an intriguing positive correlation was observed between the abundance of *Bacteroides* and the plasma levels of the pro-inflammatory cytokine TNFa in PD patients [4]. Notably, *Bacteroides* have been demonstrated to stimulate immune cells, such as macrophages and monocytes, to release TNFa through LPS-mediated pathways [4].

Akkermansia, which is a bacterium that is a member of the *Verrucomicrobiaceae* family, predominantly gram-negative, primarily inhabits the mucus layer of the large intestine, where it plays a vital role in maintaining intestinal integrity and facilitating the digestion of the intestinal mucus [10,35]. *Akkermansia* has been linked to certain health benefits such as improved immune function, accelerated wound healing, and protection against obesity [10]. The degrading feature must accompany the pro-inflammatory pathways since the gut barrier's breakdown would increase resident immune cells' exposure to pathogens, causing abnormal aggregation of a-synuclein formation in the ENS. Increased levels of *Akkermansia* increase intestinal permeability and intestinal inflammation, which would expose the intestinal neural plexus to toxins such as lipopolysaccharide (LPS), which would lead to the abnormal aggregation of a-synuclein and generation of Lewy bodies [10]. Furthermore, a study revealed a modest correlation between the abundance of *Verrucmicrobia* and IFNγ. IFNγ is a pro-inflammatory cytokine derived from type I helper T cells [4]. The dysregulation of the gut microbiota has been associated with abnormal immune responses within the gut and throughout the body, which would lead to the excess production of inflammatory cytokines in the bloodstream, and that would trigger motor impairments and neurodegeneration by inducing neuroinflammation in a PD mouse model with the overexpression of a-synuclein [4]. Therefore, since increased levels of *Akkermansia* are pronounced in PD, this could indicate the contribution to the disorder's progression.

These crucial bacteria strongly suggest the active involvement of gut microbiota in the pathogenesis of PD, as evidenced by their correlations with disease duration, cognitive impairment, and potential implications for treatment development. It is imperative to conduct further research to investigate the specific microbiota differences and their functional roles that contribute to the progression of PD. To gain comprehensive insights, the design of a longitudinal study aimed at monitoring disease progression and characterizing alterations in the taxonomic composition of the gut microbiome would prove highly advantageous. Such an approach would provide valuable information regarding the dynamic relationship between the gut microbiota and PD, enabling a deeper understanding of the disease mechanisms and potentially paving the way for targeted therapeutic interventions.

Immune System and the Gut Microbiota

The gut microbiome is a dynamic environment that balances the immune system. Regulating immune homeostasis is one critical benefit gut microbiota provides to the organism [36]. When changes occur within the body, homeostatic balance becomes disrupted, and similar results occur with alterations to gut microbiota [36]. Various gut microbiota cytokine interaction patterns have been discovered and are specific to a stimulus or cytokine specificity [37]. Microbial metabolic pathways link together specific microbiota species and various cytokine levels. Differences in cytokine levels are associated when more significant or lesser percentages of particular bacteria are present [37]. Such taxonomic associations include the typical gut microbiota, *Dorea Formicigenerans*,

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where higher levels correlate to greater levels of IFNγ. The opposite is seen for Dorea species which negatively correlates to IFNγ response. Taxonomic associations are generally associated with bacterial-induced inflammatory cytokine response [38]. Such microbiota changes and cytokine immune responses are correlated with symptoms of Parkinson's Disease [39]. Changing gut microbiota in Parkinson's patients is associated with varying concentrations of IFNγ and TNFa [39]. Such changes in cytokine levels were thought to contribute to irregular immune responses and enhance inflammatory processes in Parkinson's disease patients [39].

Gut microbiotas produce metabolites that play critical roles in inflammatory signaling. When such changes occur, disrupting the balance of the gut, alterations to the immune response follow [40]. The reciprocal host-gut microbiota axis describes the relationship between gut microbiota and immune responses [41]. The axis' primary defense is innate immunity, which correlates to gut microbiota taxis [41]. Gut-associated Lymphoid Tissue (GALTs) is critical in establishing a connection between gut microbiota and innate immune homeostasis [42]. When gut microbiota is disturbed, GALTs structure is altered, interfering with the local immune responses. Although the mechanism is not entirely understood, one theory is based on the pro-inflammatory cytokines produced by GALTS derived from different gut microbiota. Such cytokines can lead to greater susceptibility to autoimmune disorders [42]. The microbiota-gut-brain axis connects gut, immune, and brain functions. Gut dysbiosis and increased pro-inflammatory cytokines are said to improve intestinal and BBB permeability leading to an accumulation of misfolded proteins and axonal damage [43–45]. It is through damaged protein buildup that the pathogenesis of neurodegenerative diseases occurs, including Parkinson's disease [43]. PD patients with high levels of *Enterobacteriaceae* displayed increased levels of lipopolysaccharides within the blood, which acts as a neurotoxin [43–45]. Serum LPS may induce the pro-inflammatory cytokine response, which disrupts the blood-brain barrier leading to the degradation of dopaminergic neurons and substantia nigra [43–45].

Studies show that changes in an individual's gut microbiota result in many symptoms. How these changes to the gut microbiome occur varies by patient. Alterations to the gut microbiota composition may be due to environmental factors, stress, and diet. Such changes result in changes in epileptiform activity [46]. Modifications within the gut microbiota associated with neuroinflammation are also common symptoms of aging. As one ages, gut microbiota decreases, affecting the gut-brain axis [47]. Such environmental factors would trigger T-cell infiltration and immune-mediated neural damages that coincide with symptoms of Parkinson's [48]. Aging supports a pro-inflammatory microenvironment and, when coupled with a decrease in gut microbiota diversity, can result in increased progression of neurodegeneration [49]. Both increase and decrease in specific genera of bacteria, including enriching *Lactobacillus*, *Akkermansia*, and *Bifidobacterium*, as well as depleting *Lacchnospiraceae* family, are some of the most commonly displayed alterations in Parkinson's disorder patients [50]. The GI tract is the primary communication site between the host immune system and the gut microbiota, highlighting the importance of the relationship [51]. Dysregulation of the relationship results in disorders like that of Parkinson's disease. Understanding the variations of gut microbiota and its relationship with the inflammatory immune response will help to advance the treatment of patients with Parkinson's disorder.

Parkinson's Treatments

Most clinically approved treatment modalities for PD center around treating PD symptoms and do not address underlying pathophysiology in the brain or gut. Current first-line pharmacological treatment for PD symptoms includes dopamine replacement therapies such as levodopa (L-Dopa) and combination levodopa-carbidopa [52–54]. L-Dopa is regarded as the standard gold treatment for early-stage PD, administered to replenish the loss of striatal dopamine [54–56]. It has a long history of use in alleviating the primary motor effects of PD, namely Parkinsonian tremors. However, these effects can be limited [57]. Prolonged high-dose L-Dopa treatment is complicated by the onset of characteristic involuntary motor movements referred to as levodopa-induced dyskinesia (LID) [54,58]. LID symptom onset occurs in approximately 40% of PD patients on L-Dopa therapy lasting four or more years [58,59]. Clinical data has established a positive correlation between L-Dopa dose

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and LID manifestation [60,61]. Alternatives to L-Dopa seek to achieve similar efficacy without LID but are still targeted toward symptom management. Preliminary clinical data suggest levodopa-carbidopa administered to the jejunum as a long-acting gel can increase LID-free periods associated with off-peak L-Dopa blood levels by approximately two h (95% CI -3.05 to -0.76]; p=0.0015) but requires surgical placement of jejunostomy, an effective and invasive procedure [62]. Research continues to seek methods to improve long-term L-Dopa efficacy, reduce LID-associated complications, or serve as less invasive, effective alternative interventions.

As interest in gut-brain axis involvement in PD pathophysiology has grown, so has the concurrent interest in gut-brain axis mediated prevention and intervention. Avenues of investigation that have made it to clinical trials include nutritional therapy via diet modification and direct modulation of the gut microbiome via pre, post, and probiotic means [16,63,64]. Thus far, these treatment avenues remain experimental and have not been widely adopted in the clinic.

A growing body of research has identified diet as a factor that can be protective against PD [64,65]. Specifically, the study finds the Mediterranean (MEDI) diet, rich in olive oil, fresh fruit and vegetables, fish, poultry, beans, and pulses, is protective [66–69]. Data from a 2019 Greek cohort study suggest adherence to the MEDI diet is associated with a modest 2% lower probability of developing prodromal PD symptoms, defined as gut motility issues, depression, and cognitive decline ($p < 0.001$) [66]. A 2021 analysis from the Rotterdam Cohort Study ($n = 9414$) finds adherence to a MEDI-like diet has a protective effect against PD with a hazard ratio of 0.89 (95% CI 0.74–1.07) [67]. Conversely, a 2009 study from Japan concludes diets high in consumption of animal fats, specifically cholesterol intake, were positively associated with PD risk finding an odds ratio (OR) of 2.09 (95% CI 1.21-3.64) --further suggesting a connection between diet and PD [70]. These data suggest dietary modifications, exceptionally minimal consumption of red meat, animal fats, and alcohol, and increased adherence to a MEDI-like diet can protect against PD.

Beyond preventative benefits, researchers have begun investigating diet as an intervention for those already diagnosed with PD, focusing on the MEDI diet and the related Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet [16,66,67]. The MIND diet differs from the MEDI diet in food prioritization, with the MIND diet emphasizing more leafy green vegetable consumption instead of MEDI's broader emphasis on fresh vegetable consumption [16,66]. Regardless, data suggests both diets may serve as nutritional therapy for PD patients. 2022 data from the ongoing Modifiable Variables in Parkinsonism study found patients adhering to a MIND diet reported 52.9 points lower on the patient-reported outcomes in Parkinson's disease (PRO-PD) tool--a self-report survey consisting of 32 sliding scale questions to assess PD symptom severity and frequency [17,68]. On average, patients adhering to a MEDI diet reported 29.6 points lower in the same metric [68]. Similarly, a 2017 cross-sectional analysis using PRO-PD metrics found patients adhering to diets like MEDI and MIND were associated with slower progression of PD symptoms ($p < 0.05$), while patients reported adhering to diets high in refined sugars, fried foods, and full-fat dairy products were associated with more rapid progression of PD ($p < 0.05$) [17]. Further differentiation in dietary factors also shows diet modification as a promising intervention. A small 2020 randomized control trial (RCT) of 47 PD patients concludes low fat and specifically ketogenic diets improve nonmotor and motor daily living experiences by 41% ($p < 0.001$) in patients already prescribed L-Dopa as reported using the Movement Disorder Society's Unified Parkinson's Disease Rating Scale MDS-UPDRS [71].

Recent research has sought to move beyond associative measures and establish a causal relationship between diet interventions, gut microbiome changes, microbial metabolite changes, and disease states. This has led to experimental treatment avenues that directly modulate microbiome and microbial metabolites, such as short-chain fatty acid SCFA levels in PD patients [72,73]. An 86-subject 2020 case-control study not only confirmed gut microbial dysbiosis between healthy subjects and PD-diagnosed subjects but further concluded adherence to an ovo-lacto vegetarian diet and the addition of a bowel cleansing regimen in PD subjects currently treated with L-Dopa significantly improves MDS-UPDRS part III motor evaluation scores. It is associated with elevated levels of beneficial *Ruminococcaceae* microbial taxa known to metabolize dietary components to SFCAs (pooled

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$p < 0.01$) [72]. The benefit of such intervention may be ascribed to the dual effect introduction of SCFA precursors via an ovo-lacto vegetarian diet and a beneficial change in gut microbiota that generates more SCFAs via a bowel cleansing regimen [72]. Further, the 2021 open-label trial (RESIST_PD) of 87 subjects concluded that altering bowel SCFA levels via prebiotic dietary supplementation of resistant starches, i.e., starches that undergo digestion only by gut microbiota, results in less gut dysbiosis, increased colonic SCFA levels as measured by fecal butyrate levels ($p = 0.029$), and improvement of non-motor related PD symptoms on the Non-Motor Symptom Questionnaire (NMSQ) ($p = 0.001$) [73].

Future Research

Dietary intervention trials and RESIST-PD prebiotic trials have shown promise in the clinic as stand-alone and supplementary interventions to prevent PD onset and reduce symptoms associated with PD [73]. Research is ongoing to understand better how gut-brain axis-mediated interventions can treat or reverse PD symptoms.

Research into probiotic dietary supplementation has shown preliminary promise in clinical trials for PD-associated symptoms, mostly constipation [74,75]. Data from a 2016 120-subject RCT suggests probiotic dietary supplementation with fermented milk containing probiotic microbial strains and prebiotic fiber significantly increases reported complete bowel movements in PD suffering from PD-associated constipation with a mean difference of mean difference in movements of 1.1 (95% CI 0.4-1.8; $p = 0.002$) [74]. A smaller 46-subject 2022 RCT also concludes probiotic supplementation, including defined probiotic microbial strains, improves PD-associated constipation symptoms [75]. The study further concludes probiotics can partially restore beneficial microbial taxa that are often observed to be reduced or absent from PD patient gut microbial populations [75]. More research will be needed to connect probiotics and their modulatory effect on the gut microbiome to PD symptoms.

Another growing area of interest is using fecal microbial transplantation FMT to mitigate microbial dysbiosis and alleviate PD symptoms. Recent studies demonstrate that restoration of gut microbial diversity is linked to reducing pro-inflammatory signals and protective effects against PD symptoms [18,19]. A 2018 study concludes FMT reduces gut dysbiosis, suppresses pro-inflammatory TLR4 and TNFa signaling, and significantly PD associated motor symptoms in murine models of PD [18]. A similar 2021 study supports and expands on this model, concluding that FMT restores gut microbial diversity and barrier integrity, reduces pro-inflammatory TLR4 and NFkB signaling, and restores motor function in rotenone-induced PD mouse models [19]. This pre-clinical data shows promise and a roadmap for future human trials.

Conclusions

The importance of the gut-brain axis in neurodegenerative disorders is evident and should be further analyzed to uncover details regarding the pathophysiology of such diseases. Gut microbiome dysbiosis plays a crucial role in the dysregulation of inflammatory immune responses, which leads to neurodegeneration and is of great clinical importance. Understanding the connection between the gut microbiome and immune response can allow for specific targeted treatments to restore gut microbiome diversity and decrease inflammatory cytokines to prevent neurodegeneration. Particular increases and decreases of a specific genre of gut microbiota are associated with increased cytokines in patients with Parkinson's disease. An increase in inflammatory cytokines disrupts the blood-brain barrier resulting in neurodegeneration present in disorders like Parkinson's Disease. Homeostatic disruptions of the gut microbiome and innate immune response can result in motor and non-motor symptoms present in PD. Further studies uncovering the direct communication mechanism between the gut microbiota and the gut-brain axis's innate immune response could prevent or slow the pathophysiological symptoms displayed in neurodegenerative disorders.

References

1. Aho VTE, Pereira PAB, Voutilainen S, Paulin L, Pekkonen E, Auvinen P, et al. Gut microbiota in Parkinson's disease: Temporal stability and relations to disease progression. *EBioMedicine*. 2019;(44):691–707.

2. Houser MC, Tansey MG. The gut-brain axis: Is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? *Npj Parkinson's Disease.* 2017;(3):3.
3. Zhao Z, Ning J, Bao X, Shang M, Ma J, Li G, et al. Fecal microbiota transplantation protects rotenone-induced Parkinson's disease mice via suppressing inflammation mediated by the lipopolysaccharide-TLR4 signaling pathway through the microbiota-gut-brain axis. *Microbiome* 2021;(9):226.
4. Lin CH, Chen CC, Chiang HL, Liou JM, Chang CM, Lu TP, et al. Altered gut microbiota and inflammatory cytokine responses in patients with Parkinson's disease. *J Neuroinflammation*. 2019;16(1):129.
5. Challis C, Hori A, Sampson TR, Yoo BB, Challis RC, Hamilton AM, et al. Gut-seeded alpha-synuclein fibrils promote gut dysfunction and brain pathology specifically in aged mice. *Nat Neurosci*. 2020;23(3):327-36.
6. Shi J, Wang Y, Chen D, Xu X, Li W, Li K, et al. The alteration of intestinal mucosal α-synuclein expression and mucosal microbiota in Parkinson's disease. *Appl Microbiol Biotechnol*. 2023;107(5–6):1917–29.
7. Chandra R, Hiniker A, Kuo Y-M, Nussbaum RL, Liddle RA. α-Synuclein in gut endocrine cells and its implications for Parkinson's disease. *JCI Insight.* 2017;(2):92295.
8. Prasuhn J, Strautz R, Lemmer F, Dreischmeier S, Kasten M, Hanssen H, et al. Neuroimaging correlates of substantia nigra hyperechogenicity in Parkinson's disease. *J Parkinsons Dis*. 2022;12(4):1191-200.
9. Jin M, Li J, Liu F, Lyu N, Wang K, Wang L, et al. Analysis of the gut microflora in patients with Parkinson's disease. *Front Neurosci*. 2019;13:1184.
10. Zhu M, Liu X, Ye Y, Yan X, Cheng Y, Zhao L, et al. Gut microbiota: A novel therapeutic target for Parkinson's disease. *Front Immunol*. 2022; 13: 93755.
11. Li Z, Liang H, Hu Y, Lu L, Zheng C, Fan Y, et al. Gut bacterial profiles in Parkinson's disease: A systematic review. *CNS Neurosci Ther*. 2023;29(1):140–57.
12. Młynarska E, Gadzinowska J, Tokarek J, Forycka J, Szuman A, Franczyk B, et al. The role of the microbiome-brain-gut axis in the pathogenesis of depressive disorder. *Nutrients*. 2022;14(9):1921.
13. Aho VTE, Houser MC, Pereira PAB, Chang J, Rudi K, Paulin L, et al. Relationships of gut microbiota, short-chain fatty acids, inflammation, and the gut barrier in Parkinson's disease. *Mol Neurodegeneration*. 2021;16(1):6.
14. Nuzum ND, Loughman A, Szymlek-Gay EA, Teo W-P, Hendy AM, Macpherson H. To the gut microbiome and beyond: The brain-first or body-first hypothesis in Parkinson's disease. *Front Microbiol*. 2022;13:791213.
15. Mekky J, Ahmad S, Metwali M, Farouk S, Monir S, ElSayed A, et al. Clinical phenotypes and constipation severity in Parkinson's disease: Relation to Prevotella species. *Microbes and Infectious Diseases*. 2022;0(0):0–0.
16. Fox DJ, Park SJ, Mischley LK. Comparison of associations between MIND and Mediterranean Diet scores with patient-reported outcomes in Parkinson's disease. *Nutrients*. 2022;14(23).
17. Mischley LK, Lau RC, Bennett RD. Role of diet and nutritional supplements in Parkinson's disease progression. *Oxid Med Cell Longev*. 2017;2017:6405278.
18. Sun MF, Zhu YL, Zhou ZL, Jia XB, Xu YD, Yang Q, et al. Neuroprotective effects of fecal microbiota transplantation on MPTP-induced Parkinson's disease mice: Gut microbiota, glial reaction and TLR4/TNF-alpha signaling pathway. *Brain Behav Immun*. 2018;70:48-60.
19. Zhao Z, Ning J, Bao XQ, Shang M, Ma J, Li G, et al. Fecal microbiota transplantation protects rotenone-induced Parkinson's disease mice via suppressing inflammation mediated by the lipopolysaccharide-TLR4 signaling pathway through the microbiota-gut-brain axis. *Microbiome.* 2021;9(1):226.
20. Sonne J, Reddy V, Beato MR. Neuroanatomy, substantia nigra. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2023.
21. Zahoor I, Shafi A, Haq E. Pharmacological treatment of parkinson's disease. In: Stoker TB, Greenland JC, editors. Parkinson's Disease: Pathogenesis and Clinical Aspects. Brisbane (AU): Codon Publications; 2018.
22. Cramb KML, Beccano-Kelly D, Cragg SJ, Wade-Martins R. Impaired dopamine release in Parkinson's disease. *Brain*. 2023; awad064.
23. Stefanis L. A-synuclein in Parkinson's disease. *Cold Spring Harb Perspect Med*. 2012;2(2):a009399.
24. Booth HDE, Hirst WD, Wade-Martins R. The role of astrocyte dysfunction in Parkinson's disease pathogenesis. *Trends in Neurosciences* 2017;40:358–70.
25. Sulzer D, Edwards RH. The physiological role of α-synuclein and its relationship to Parkinson's disease. *J Neurochem*. 2019;150(5):475–86.
26. Gómez-Benito M, Granado N, García-Sanz P, Michel A, Dumoulin M, Moratalla R. Modeling Parkinson's disease with the alpha-synuclein protein. *Front Pharmacol.* 2020;11:356.
27. Yamada K, Iwatsubo T. Extracellular α-synuclein levels are regulated by neuronal activity. *Mol Neurodegeneration*. 2018;13(1):9.
28. Kim S, Kwon SH, Kam TI, Panicker N, Karuppagounder SS, Lee S, et al. Transneuronal propagation of pathologic alpha-synuclein from the gut to the brain models Parkinson's disease. *Neuron.* 2019;103(4):627-41 e7.
29. Rietdijk CD, Perez-Pardo P, Garssen J, Van Wezel RJA, Kraneveld AD. Exploring braak's hypothesis of parkinson's disease. Front Neurol. 2017;8.

 doi:10.20944/preprints202410.0016.v1

30. Travagli RA, Browning KN, Camilleri M. Parkinson disease and the gut: new insights into pathogenesis and clinical relevance. *Nat Rev Gastroenterol Hepatol* 2020;17:673–85.
31. Böttner M, Zorenkov D, Hellwig I, Barrenschee M, Harde J, Fricke T, et al. Expression pattern and localization of alpha-synuclein in the human enteric nervous system. *Neurobiology of Disease* 2012;48:474–80.
32. Ong J-S, Lew L-C, Hor Y-Y, Liong M-T. Probiotics: The next dietary strategy against brain aging. *Prev Nutr Food Sci*. 2022;27(1):1–13.
33. Zhang H, Chen Y, Wang Z, Xie G, Liu M, Yuan B, et al. Implications of gut microbiota in neurodegenerative diseases. *Front. Immunol*. 2022; 13:785644.
34. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell*. 2016;167(6):1469-1480.e12.
35. Bonnechère B, Amin N, van Duijn C. What are the key gut microbiota involved in neurological diseases? A systematic review. *Int J Mol Sci*. 2022;23(22):13665.
36. Wu HJ, Wu E. The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes.* 2012; 3(1) 4-14.
37. Schrimer M, Smeekens SP, Vlamakis H, Jaeger M, Oosting M, Franzosa EA, et al. Linking the human gut microbiome to inflammatory cytokine production capacity. *Cell*. 2016; 167(4):1125-1136.
38. Lin CH, Chen CC, Chiang CL, Liou JM, Chang CM, Lu TP, et al. Altered gut microbiota and inflammatory cytokine response in patients with Parkinson's disease. *J Neuroinflammation*. 2019;16(1):129.
39. Cantanzaro R, Anzalone M, Calabrese F, Milazzo M, Capuana M, Itaia A, et al. The gut microbiota and its correlations with central nervous system disorders. *Panminerva Med*. 2015; 57(3): 127-43.
40. Yoo JY, Groer M, Dutra SVO, Sarkar A, McSkimming DI. Gut microbiota and immune system interactions. *Microorganisms*.2020;8(10):1587.
41. Jiao Y, Huntington ND, Zhang X. Crosstalk between gut microbiota and innate immunity and its implication in autoimmune diseases. *Front Immunol*.2020; (11).
42. Sarkar SR, Banerjee S. Gut microbiota in neurodegenerative disorders. *J Neuroimmunol*.2019;(328):98-104.
43. Block M, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci*. 2007;8(1):57-69
44. Chen H, O'Reilly E, Schwarzschild MA, Ascherio A. Peripheral inflammatory biomarkers and risk of Parkinson's disease. *AM J Epidemiol*. 2008;167(1):90-5.
45. Dobbs RJ, Charlett A, Purkiss AG, Dobbs SM, Weller C, Peterson W. Association of circulating TNF-alpha and IL-6 with ageing and parkinsonism. *Acta Neurol Scand*. 1999:100(1):34-41.
46. Reale M, Larlori C, Thomas A, Gambi D, Perfetti B, Nicola MD, Onofrj M. Peripheral cytokines profile in Parkinson's Disease. *Brain Behav Immun*. 2009;23(1):55-63.
47. Henion C, Khaw PT. The interplay between inflammation, immunity and commensal microflora in glaucomatous neurodegeneration. *AES*.2019;(4).
48. Russo R, Varano GP, Adornetto A, et al. Retinal ganglion cell death in glaucoma: exploring the role of neuroinflammation. *Eur J Pharmacol*. 2016;(787):134-42.
49. Frank MG, Barrientos RM, Biedenkapp JC, Rudy JW, Watkings LR, Maier SF. mRNA up-regulation of MHC II and pivotal pro-inflammatory genes in normal brain aging. *Neurobiol Aging*. 2006;27(5):717-722.
50. Romano S, Savva GM, Bedarf J, Charles IG, Hildebrand F, Narbad A. Meta-analysis of the Parkinson's disease gut microbiome suggests alterations linked to intestinal inflammation. *NPJ Parkinsons Dis*. 2021:(7).
51. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol*. 2009:(9);313-323.
52. Armstrong MJ, Okun MS. Diagnosis and treatment of Parkinson disease: a review. *JAMA*. 2020;323(6):548-60.
53. Church FC. Treatment options for motor and non-motor symptoms of Parkinson's disease. *Biomolecules*. 2021;11(4):612
54. Haddad F, Sawalha M, Khawaja Y, Najjar A, Karaman R. Dopamine and levodopa prodrugs for the treatment of Parkinson's disease. *Molecules*. 2017;23(1):40
55. Tanaka H, Kannari K, Maeda T, Tomiyama M, Suda T, Matsunaga M. Role of serotonergic neurons in L-DOPA-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. *Neuroreport*. 1999;10(3):631-4.
56. LeWitt PA. Levodopa therapy for Parkinson's disease: Pharmacokinetics and pharmacodynamics. *Mov Disord*. 2015;30(1):64-72.
57. Negrotti A, Secchi C, Gentilucci M. Effects of disease progression and L-dopa therapy on the control of reaching-grasping in Parkinson's disease. *Neuropsychologia*. 2005;43(3):450-9.
58. Fabbrini G, Brotchie JM, Grandas F, Nomoto M, Goetz CG. Levodopa-induced dyskinesias. *Mov Disord*. 2007;22(10):1379-89.
59. Ahlskog JE, Muenter MD. Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. *Mov Disord*. 2001;16(3):448-58.

 doi:10.20944/preprints202410.0016.v1

60. Schrag A, Quinn N. Dyskinesias and motor fluctuations in Parkinson's disease. A community-based study. *Brain*. 2000;123 (Pt 11):2297-305.
61. Warren Olanow C, Kieburtz K, Rascol O, Poewe W, Schapira AH, Emre M, et al. Factors predictive of the development of Levodopa-induced dyskinesia and wearing-off in Parkinson's disease. *Mov Disord*. 2013;28(8):1064-71.
62. Olanow CW, Kieburtz K, Odin P, Espay AJ, Standaert DG, Fernandez HH, et al. Continuous intrajejunal infusion of levodopa-carbidopa intestinal gel for patients with advanced Parkinson's disease: a randomised, controlled, double-blind, double-dummy study. *Lancet Neurol*. 2014;13(2):141-9.
63. Tan AH, Lim SY, Lang AE. The microbiome-gut-brain axis in Parkinson disease - from basic research to the clinic. *Nat Rev Neurol*. 2022;18(8):476-95.
64. Vascellari S, Palmas V, Melis M, Pisanu S, Cusano R, Uva P, et al. Gut Microbiota and Metabolome Alterations Associated with Parkinson's Disease. *mSystems*. 2020;5(5):e00561-20
65. Jackson A, Forsyth CB, Shaikh M, Voigt RM, Engen PA, Ramirez V, et al. Diet in Parkinson's disease: Critical role for the microbiome. *Front Neurol*. 2019;10:1245.
66. Maraki MI, Yannakoulia M, Stamelou M, Stefanis L, Xiromerisiou G, Kosmidis MH, et al. Mediterranean diet adherence is related to reduced probability of prodromal Parkinson's disease. *Mov Disord*. 2019;34(1):48-57.
67. Strikwerda AJ, Dommershuijsen LJ, Ikram MK, Voortman T. Diet quality and risk of Parkinson's disease: The Rotterdam Study. *Nutrients*. 2021;13(11):3970
68. Mischley LK, Lau RC, Weiss NS. Use of a self-rating scale of the nature and severity of symptoms in Parkinson's Disease (PRO-PD): Correlation with quality of life and existing scales of disease severity. *NPJ Parkinsons* Dis. 2017;3:20.
69. Solch RJ, Aigbogun JO, Voyiadjis AG, Talkington GM, Darensbourg RM, O'Connell S, et al. Mediterranean diet adherence, gut microbiota, and Alzheimer's or Parkinson's disease risk: A systematic review. *J Neurol Sci*. 2022;(434):120166.
70. Miyake Y, Sasaki S, Tanaka K, Fukushima W, Kiyohara C, Tsuboi Y, et al. Dietary fat intake and risk of Parkinson's disease: a case-control study in Japan. *J Neurol Sci*. 2010;288(1-2):117-22.
71. Phillips MCL, Murtagh DKJ, Gilbertson LJ, Asztely FJS, Lynch CDP. Low-fat versus ketogenic diet in Parkinson's disease: A pilot randomized controlled trial. *Mov Disord*. 2018;33(8):1306-14.
72. Hegelmaier T, Lebbing M, Duscha A, Tomaske L, Tonges L, Holm JB, et al. Interventional influence of the intestinal microbiome through dietary intervention and bowel cleansing might improve motor symptoms in Parkinson's disease. *Cells*. 2020;9(2).
73. Becker A, Schmartz GP, Groger L, Grammes N, Galata V, Philippeit H, et al. Effects of resistant starch on symptoms, fecal markers, and gut microbiota in Parkinson's disease - The RESISTA-PD Trial. *GPB*. 2022;20(2):274-87.
74. Barichella M, Pacchetti C, Bolliri C, Cassani E, Iorio L, Pusani C, et al. Probiotics and prebiotic fiber for constipation associated with Parkinson disease: An RCT. *Neurology*. 2016;87(12):1274-80.
75. Du Y, Li Y, Xu X, Li R, Zhang M, Cui Y, et al. Probiotics for constipation and gut microbiota in Parkinson's disease. *Parkinsonism Relat Disord*. 2022;(103):92-7.