Microbiota Dysbiosis and Parkinson's Disease: From the gut to the brain

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Abstract

In recent years, growing evidence has emerged indicating a link between gut microbiota and the progression of the pathology of Parkinson's Disease. While studies have established that the gut microbiome plays an essential role in neurodegenerative conditions like Parkinson's Disease, these studies often lack insight into the disease's mechanism and fail to indicate whether microbiota dysbiosis is a cause or effect of gut-induced inflammation observed in Parkinson's Disease patients. This review particularly examines the role of microbiota dysbiosis in relation to Parkinson's Disease. This paper aims to conduct a comprehensive survey of literature looking at results from animal models and clinical trials to be able to elucidate the potential mechanisms of how gut-microbiota could contribute to neuroinflammation, immune activation, and alpha-synuclein pathology in Parkinson's Disease patients opening the door to a whole new arena of therapeutics and preventive care for these patients. The significant findings in this paper consisted of the presence of an alteration in the relative abundances of various bacterial genera and gut metabolites including short-chain fatty acids causing an increase in the production of pro-inflammatory cytokines, increased intestinal barrier permeability, oxidative stress, microglial activation potentiated by toll-like receptors, enhanced alpha-synuclein pathology in both human trials and rodent models of Parkinson's Disease. The restoration of the gut microbiome to halt the progression of Parkinson's Disease can be accomplished by the use of probiotics, prebiotics, synbiotics, antibiotics, and Fecal microbiota transplants. These results help establish the role of the enteric nervous system as a contributing factor in the pathology of Parkinson's disease making it a novel remedial target or an early diagnostic tool for Parkinson's Disease patients.

Introduction

Parkinson's Disease (PD) is the second most common neurodegenerative condition after Alzheimer's Disease, and an estimated over 10 million people are living with this disorder currently with the projected number of people suffering from this disorder to double by 2030 (1)(2). The traditional clinical hallmarks of PD include but are not limited to motor symptoms like bradykinesia, tremors, instability, and freezing of gait. Non-motor symptoms such as anxiety, depression, and dementia are also quite common (3). The pathology of this disease involves the accumulation of alpha-synuclein and protein aggregates called Lewy bodies as well as dystrophic neurites like Lewy neurites in the substantia nigra (SNc) of the midbrain and the striatum leading to neuronal cell death and a selective loss of dopaminergic neurons in the SNc pars compacta resulting in dopamine deafferentation which is the root cause of motor disturbances observed in PD (4)(5)

The exact mechanisms leading to Lewy Bodies depositing and accumulating in the brain are unknown, however, several theories have been proposed which might be able to explain the mechanisms of neurodegeneration. Some of the cellular alterations reported include oxidative stress, mitochondrial dysfunction, protein aggregation, and neuroinflammation (5). Post-mortem analysis of PD patients indicates pro-inflammatory factors in the brain. A pro-inflammatory cytokine release by activated microglial cells and astrocytes leads to worsening of motor symptoms and loss of dopaminergic neurons in the SNc. The peripheral immune system also plays its role in the pathogenesis of PD; clusters of immune cells from the periphery of the body are present in and around the PD-affected brain regions (6).

Another striking feature observed in PD patients is the onset of gastrointestinal dysfunction particularly constipation, such manifestations are observed in 60-80 % of patients and early diagnosis of these symptoms might help in the long-term management of the disorder. Humans harbor a collective genome of about 100 trillion microbial organisms that colonize the human body outnumbering human cells by 10 to 1. Among these reside our gut bacteria lining our gastrointestinal tract which not only aids in digestive processes but also closely connects to the immune system and brain health. Any alterations in the gut microbiome could be associated with devastating outcomes including neurological disorders (7)(8). Baraak's hypothesis states that Lewy pathology seen in PD is caused by a pathogen that enters through the nasal cavity and makes its way to the intestinal tract suggesting that the pathology of PD first originates in the enteric nervous system (9)(10)(11). Intestinal hyperpermeability could initiate alpha-synuclein pathology in the enteric nervous system which could then spread in a prion-like fashion via the vagus nerve to the lower brainstem and ultimately the substantia nigra. Disruption in the blood-brain barrier could also lead to the gut-derived endotoxin products, Lipopolysaccharide (LPS) producing gram-negative bacteria, or peripheral immune signaling molecules being passed off into the systemic circulation leading to PD-related neurodegenerative symptoms (12). Scientists have also identified the role of toll-like receptors such as Toll Like Receptor-4 in initiating the pathogenesis of PD. Overstimulation of these receptors caused by gut-dysbiosis could trigger inflammatory cascades and lead to enteric microglial activation responsible for the observed alpha-synuclein pathology (13). Analysis of the colon and fecal samples of PD patients indicated a pro-inflammatory profile with a reduced count of anti-inflammatory bacteria. Studies have shown the deposition of alpha-synuclein Lewy neurites in the gastrointestinal system about decades before the onset of motor symptoms, strengthening the evidence for this link. Furthermore, reconstituting the gut microbiome of germ-free mice with the microbiota of PD patients was shown to exacerbate the motor symptoms and further contribute to the alpha-synuclein pathology in these mice (14). This bidirectional relationship between the gut and brain manifests itself through the gut- brain-microbiome axis, which includes the enteric nervous system and our gut microbiota.

Studying the gut-brain-microbiome presents an opportunity to understand a possible cause of neurodegeneration, investigate the interactions between multiple organ systems, and potentially develop drugs targeting gut-induced inflammation to alleviate the symptoms of PD. This review focuses on results and evidence from studies proving the link between the brain and the gut in PD with regards to microbiota and fecal sample analyses, the hunt for immune and inflammation markers as well as results from alpha-synuclein imaging. It will also discuss the contribution of toll-like receptors in the pathogenesis of gut-induced PD and the treatment options devised by novel medicine to reverse these anomalies in the intestinal tract by using techniques like fecal microbiota transplants (FMTs), probiotics, and synbiotics (15).

Detailed Explanation

Microbiota Analysis Reports

To understand the contribution of the gut microbiota in the pathogenesis of PD,there have been many studies in the past that have carried out a comparative analysis of the gut microbiota in PD patients as compared to control subjects. A study conducted by Burns and colleagues with a sample of 197 PD patients and 130 control subjects, 16S rRNA gene sequencing was carried out and the results suggested that the relative abundances of *Bifidobacteriaceae, Christensenellaceae, Tissierellaceae, Lachnospiraceae, Lactobacillaceae, Pasteurellaceae and Verrucomicrobiaceae* were significantly different in PD patients compared to control group participants. These results may have been linked to atrazine- the most common herbicide detected in potable water which is known to cause dopaminergic cell death and motor imbalance in animal models, it also increases the probability of developing PD. The microbiota dysbiosis in PD may be a result of the increased activation of pathways that degrade xenobiotics (substances foreign to a biological system) such as atrazine and this degradation takes place primarily within the gut which brings us to question whether xenobiotics play a role in initiating the altered gut bacteria profile or lead to exacerbated neurodegeneration. (16)(17).

Additionally, Petrov and colleagues carried out 16S rRNA gene amplicon sequencing with eighty-nine PD patients and sixty-six control subjects. Results illustrated a decreased microbiota flora in PD patients as compared to their counterparts. The abundances of fourteen bacterial genera (*Dorea, Bacteroides, Prevotella, Faecalibacterium, Bacteroides massiliensis, Stoquefichus massiliensis, Bacteroides coprocola, Blautia glucerasea, Dorea longicatena, Bacteroides dorei, Bacteroides plebeus, Prevotella copri, Coprococcus eutactus, and Ruminococcus callidus*) decreased while 10 bacterial genera (*Christensenella, Catabacter, Lactobacillus, Oscillospira, Bifidobacterium, Christensenella minuta, Catabacter hongkongensis, Lactobacillus mucosae, Ruminococcus bromii,* and *Papillibacter cinnamivorans)* count increased in PD population used in the study. The increase in *Lactobacillus* stems from the manner in which these bacteria play an important role in interacting with gut neurons regulating alpha-synuclein secretion which may contribute to PD pathology in numerous ways. The specific taxonomic diversity observed in PD patients is also linked with a pattern of microbiota which could trigger inflammation and lead to deposition of Lewy bodies and alpha-synuclein as seen in PD. PD is also associated with a lower body mass index and the carriage of microorganisms such as *Christensenella* is characterized with weight loss as observed in gnotobiotic mice infected with these species. (18)(19).

Hopfner and colleagues examined the gut microbiota of twenty-nine PD patients and twenty-nine controls. The *Lactobacillaceae, Barnesiellaceae,* and *Enterococcacea* bacteria were found to be higher in PD patients as opposed to the age-matched control group. Another important finding was that the beta diversity was significantly different in the two experimental groups whereas no difference was observed in the alpha diversity index. The study confirms the results obtained in previous research with regards to the increased concentration of *Lactobacillus*. The increased values of *Barnesiellaceae,* and *Enterococcacea* could have a predictive value for PD, however further independent studies replicating these results are required to strengthen the hypothesis (20).

By utilizing rRNA-targeted reverse transcription-quantitative PCR, Hasegawa and colleagues found and quantified the abundances of the gut bacteria in which it was revealed that Clostridium

coccoides,*Prevotella* and *Bacteroides fragilis* decreased and *Lactobacillus* increased in the gut microbiome of individuals with PD. The reduced count of *Prevotella* in PD patients indicates decreased mucin synthesis and increased intestinal hyperpermeability. The *C. coccoides* species is a member of an obligate anaerobe; the sum of these anaerobes was significantly decreased in PD patients. Past studies show that decreased values of these anaerobes are generally observed in people with conditions like irritable bowel syndrome, diabetes, or cancer. Hence we can conclude that the number of these bacteria are not associated specifically with PD but are a by-product of a myriad of other diseases. The correlation of the abundance of *C. coccoides* with inflammatory bowel disease may hint at another possible method by which the gut might influence the aggression of PD and will be dealt with more in detail in the latter half of this review. (21)(22)(23)(24).

Another study conducted with twenty-four PD patients and fourteen controls showed a decrease in the number of cellulose-degrading bacteria like *Blautia, Faecalibacterium and Ruminococcus* and an increase in pathobionts such as *Escherichia-Shigella, Streptococcus, Proteus, and Enterococcus* in PD patients compared to healthy controls. The reduced count of *Clostridiaceae* and *Lachnospiraceae* in PD patients is directly proportional to gut health because these bacteria are considered to be useful butyrate-producing bacteria which is an essential source of energy for the gut epithelium cells which also inhibits the activity of the NF-κB transcription factor. This reduces the intestinal mucosal inflammation and thereby mediates the impact of pro-inflammatory cytokines. The lower abundance of these bacteria results in a pro-inflammatory profile of the gut and plays a role in producing toxic substances like inflammatory cytokines and contributes in their travel to the brain advancing the progress of PD (25).

Another report has detected a decrease in putative anti-inflammatory short-chain fatty acids (SCFA) butyrate producing bacteria in PD patients as well as decreased abundances of the following genera- *Dorea, Roseburia* and *Anaerostipes*.The microbiota community in patients with PD showed an inflammatory dysbiotic nature which is what led the researchers to hypothesize that altered dysbiotic microbiome can trigger immune activation in the colon through TLR-4(toll like receptor) signaling pathways which are ultimately responsible for the neuroinflammation and degeneration observed in PD. This hypothesis drew evidence from the fact that PD patients showed a microbiota profile signaling abundance of endotoxin-producing bacteria and systemic endotoxin leak as serum lipoprotein binding protein increased in PD patients explaining the clinical phenotypes observed in people with PD. (26)

Vascellari and colleagues reported a decrease in bacterial groups associated with neuroprotective and anti-inflammatory effects particularly the members of the *Lachnospiraceae* family such as *Butyrivibrio, Pseudobutyrivibrio, Coprococcus, and Blautia.* Further evaluation of gut metabolites revealed changes in types of lipids, amino acids and other organic compounds. Most of this altered metabolite composition was in direct correlation with the abundance of the *Lachnospiraceae* family providing strong evidence for how these altered bacterial genera could be responsible for altered metabolism in PD patients. A reduction in short-chain fatty acids like acetate, butyrate, and propionate may decrease the anti-inflammatory nature of these molecules which also orchestrates the activities of the enteric nervous system and promotes gastric motility. A drop in these numbers leads to the development of gastrointestinal motility dysfunctions thereby elucidating their role in the pathogenesis of PD. Additionally, PD patients had a significant reduction in glutamic acid derivative pyroglutamic acid; however this is contradictory considering glutamic acid is important for the advancement of PD.These results could reflect another possibility- the increased oxidative

neuronal stress in the progression of the disease. The cyanobacteria members produce certain neurotoxins causing protein misfolding and aggregation which is seen in a myriad of neurodegenerative conditions. The role of the bacteria *Dolichospermum* is not yet clear and requires further investigation regarding its pathophysiological role (27).

Given Barak's hypothesis suggests the nasal cavity as an entry point for pathogens, Pereira and colleagues compared the nasal microbiota in PD patients and healthy control subjects. However, no significant difference was observed in the two groups. A comparative analysis of the oral microbiota in the two groups was carried out wherein it was noticed that there is an increased beta diversity index in PD microbiota as compared to healthy control subjects. This was accompanied by a marked rise in the numbers of *Prevotella, Prevotellaceae, Veillonella, Solobacterium, Veillonellaceae, Lactobacillaceae,* and *Coriobacteriaceae* and a decrease in the abundance of *Capnocytophaga, Rothia, Kingella, Leptotrichia, Actinomyces and Leptotrichiaceae* in PD patients oral microbiota. Oral microbiota communities were dominated by *streptococcus* and potential oral pathogens were observed in all subjects, including healthy subjects suggesting that the pathogenicity of these microorganisms is opportunistic in nature. Several taxa-containing bacteria analogous with oral pathologies were reduced in abundance in PD patients, which explains how oral hygiene may have a partial role in development of PD; however it alone cannot explain the underlying differences. All members of the *Prevotellaceae* family were found to have increased in PD, which is contradictory to the findings obtained when investigating the gut microbiota(wherein there was a reduction in this species). Yet this makes sense because there are often different strains of bacteria in the gut and oral cavities. Genus *Prevotella* consists of opportunistic oral pathogens; it may be a possibility that the increase of this genus in PD is due to the hygienic variability owing to motor/ non-motor symptoms leading to reduced efficiency of oral health care (28).

Studies conducted in murine models of PD also show altered microbiota composition as observed in PD patients. The fecal microbiota of human PD patients transplanted into mice caused an alarming alteration in the microbiome of the recipient mice and what was even more noteworthy was that the disease status of the donor had a significant impact on the microbiota communities seen in these mice. Timothy and colleagues identified numerous genera differing in mice containing microbiota implanted from the microbiota of PD patients as compared to healthy controls. There was an increase in the relative abundances of *Proteus , Bilophila, and Roseburi*a in PD mice and a decrease in the abundances of *Lachnospiraceae, Rikenellaceae, and Peptostreptococcaceae, and Butyricicoccus*. The abundance of Short-chain fatty acids(SCFA) producing KEGG families like *K00929, butyrate kinase, K01034, K01035, acetate CoA transferase alpha, and beta* is increased in PD mice. Furthermore, mice receiving PD patient microbiota display a changed SCFA profile, which includes a lesser concentration of acetate and a higher concentration of propionate and butyrate compared to healthy control mice. The fact that mice who received microbiota transplants from PD patients showed worsened motor symptoms compared to those that received transplants from healthy individuals suggests that both alpha-synuclein overexpression (genetic factor) and environmental factors like dysbiosis come together to produce disease outcomes in mouse models(**Figure 1**).

If we extrapolate these discoveries to humans, it might bring to light a previously unacknowledged etiology for PD which elucidates the role of gene-environment(microbiome) interactions. The higher abundance of short-chain fatty acids in the gut microbiota could promote the maturation

and inflammatory properties of microglial cells. This subsequent increase in the activation state of microglia leads to the production of inflammation-inducing cytokines which could impact neuron function and potentially increase cell deaths in models of PD and other neurodegenerative conditions. Additionally, an inflammatory environment can further alpha-synuclein aggregation which will then enhance microglial activation promoting many feed-forward cascades giving way to the additional production of alpha-synuclein (29).

All of the above evidence demonstrates a direct link between PD and altered microbiota profiles, thus providing an insight into how gut and oral microbiota might contribute to the synucleinopathies seen in PD patients.

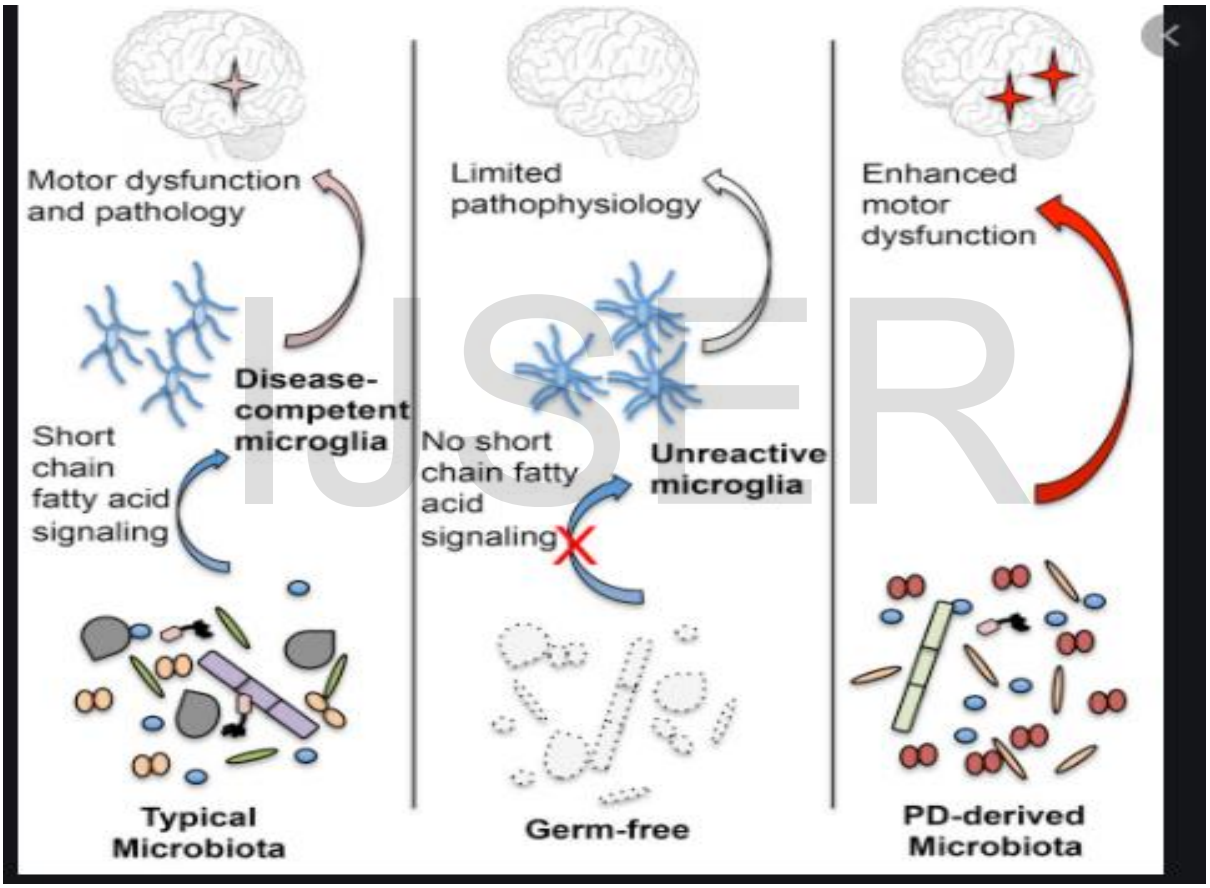

Figure 1- demonstrates the role of intestinal microbiota in the pathophysiology of neurodegenerative conditions like PD. Synucleinopathies seen in PD are marked by alpha-synuclein aggregation which often terminate motor dysfunction. The above 3 images depict the results obtained in mice models leading us to the conclusion that mice overexpressing alpha-synuclein results in microglial activation and motor deficits. Oral administration of certain metabolites in germ-free mice promotes motor defects and neuroinflammation. Furthermore, colonization of disease-free mice with the microbiota of PD patients exacerbates physical and mental impairment revealing the role of microbiome alteration as a risk factor for PD.

Adapted from Garrett, Wendy. "Faculty Opinions recommendation of Gut microbiota regulate motor deficits and neuroinflammation in a model of parkinson's disease." *Faculty Opinions – Post-Publication Peer Review of the Biomedical Literature*, 2017.

Markers of Pathology

Inflammation

In a Danish nationwide cohort study from 1977-2014, one of the longest population-based studies aimed at assessing the association between inflammatory bowel disease and PD. Patients suffering from inflammatory bowel disease (IBD) had an increased 22% risk of developing PD in the future as compared to non-IBD individuals. These numbers point towards the role of gut and inflammation as a possible mechanism of PD development. These results explicate the association between the intestinal environment and the central nervous system (CNS) . This gut-brain axis is often related to peripheral intestinal functions and any alteration in the functioning of the gut microbiome can have a direct impact on the CNS. A pro-inflammatory gut profile and other metabolic changes have been reported in PD patients. IBD pathogenesis is also influenced by dysbiosis. The enteric nervous system is home to enteric glial cells which are equivalent to the astrocytes of the CNS performing the same function of supporting and maintaining neural networks. IBD and PD patients are shown to have gut barrier dysfunction which partly might be a product of enteric glial dysfunction. Alpha-synuclein is a part of the enteric nervous system immune defense mechanisms so it has the ability to act as a chemoattractant for cells like monocytes and neutrophils which furthers mucosal inflammation contributing to a pro-inflammatory cytokine expression in the gut as observed in colon biopsies. The levels of interferon-gamma (IFN-γ), interleukin (IL)-6 and IL-1β in PD patients are fairly similar to those suffering from IBD. Alpha-synuclein could potentially induce the production of pro-inflammatory cytokines – drive helper, IL-5, IFN-y as well as cytotoxic T cell responses in PD patients by acting as an antigenic epitope as illustrated by the major histocompatibility complex. These processes of inflammation and disputed barrier integrity promote the assemblage of Alpha-synuclein aggregates but it is still in question whether alpha-synuclein is a cause or effect of the ongoing inflammation (30).

In a case-control study, a research group numerically analyzed the presence of inflammatory markers in the feces of PD patients to test for intestinal inflammation and permeability markers. Results obtained showed elevated calprotectin levels in PD patients, which is a fecal marker for intestinal inflammation and dysfunction of the intestinal barrier. Two fecal markers of intestinal permeability alpha-1-antitrypsin and zonulin also showed an increased profile. Another fecal marker of intestinal inflammation, Lactoferrin, showed a trend leaning towards elevated concentrations in the PD group, however, the difference was not enough to make it statistically significant. None of the aforementioned fecal markers had a correlation with disease severity, type of PD, dopaminergic therapy, or presence of constipation. The results obtained ratify the hypothesis of intestinal inflammation as a contributor to the pathology of PD. The lack of significance of lactoferrin might be elucidated by the lower specificity and sensitivity of the marker. Elevated fecal markers did not correlate with any other clinical features of PD, longitudinal studies are necessary to define whether elevated intestinal inflammatory markers are linked to early

stages of PD, are a recurrent feature in PD individuals and whether or not they are confined to a certain PD subpopulation. The noninvasive nature of fecal markers paves the way for it to be a promising tool of research. Future endeavors on the subject should seek to unravel the interaction between these markers and other parameters implicated in gut inflammation. (31)

Some scientists propose that leucine-rich repeat kinase 2 (LLRK2) might be the missing link between inflammatory bowel disease and PD. Expression of the Mutants of the LLRK2 gene has been identified in autosomal dominant PD causing shortening of the dendritic branches in cultured neurons. There have been extensive research studies trying to elucidate the role of LLRK2 and its effects on neurons. There is strong evidence showing LLRK2 is highly expressed in immune cells and affects neuronal outgrowth. Elevated levels of LLRK2 are found in the inflamed colon tissue of people with IBD. Higher levels of LLRK 2 are also detected in peripheral immune cells of PD patients which might hint towards LLRK2s involvement in PD-associated inflammatory responses(**Figure 2**). Both PD and IBD patients demonstrate increased intestinal permeability due to decreased expression of intestinal junction barrier proteins. In IBD, these junction proteins show upregulation of claudin 2 in inflamed epithelium and downregulation of claudin 3 and 4 in diseased epithelium cells. There is also a reduction in occludin and zona occludens-1 (ZO-1). PD studies have also yielded similar results and that might lead us to predict that intestinal barrier integrity could be correlated with disease pathology and severity and could be used as a potential biomarker for the onset of the motor deficits in PD. The common phenotypes of intestinal, gut and peripheral inflammation could be caused by the disrupted barrier integrity. PD patients also have increased levels of pro-inflammatory cytokines and a higher mRNA expression of inflammatory substances like IL-1β, TNF, IFNγ, IL-2, IL-6, and CXCL8 in their cerebrospinal fluid which are commonly associated with progression and initiation of IBD. These cytokine levels had a negative correlation with disease duration which could be due to the fact that cytokines drive the initiation of PD but do not retain their high levels throughout the period of disease. We need to look at prospective patients that run a risk of suffering from PD and investigate the role of inflammatory anomalies in the pathogenesis of PD. Further research might help us target LLRK2 specifically in the context of immune activation and inflammation making it a suitable therapeutic target for future medicinal interventions (32)(33).

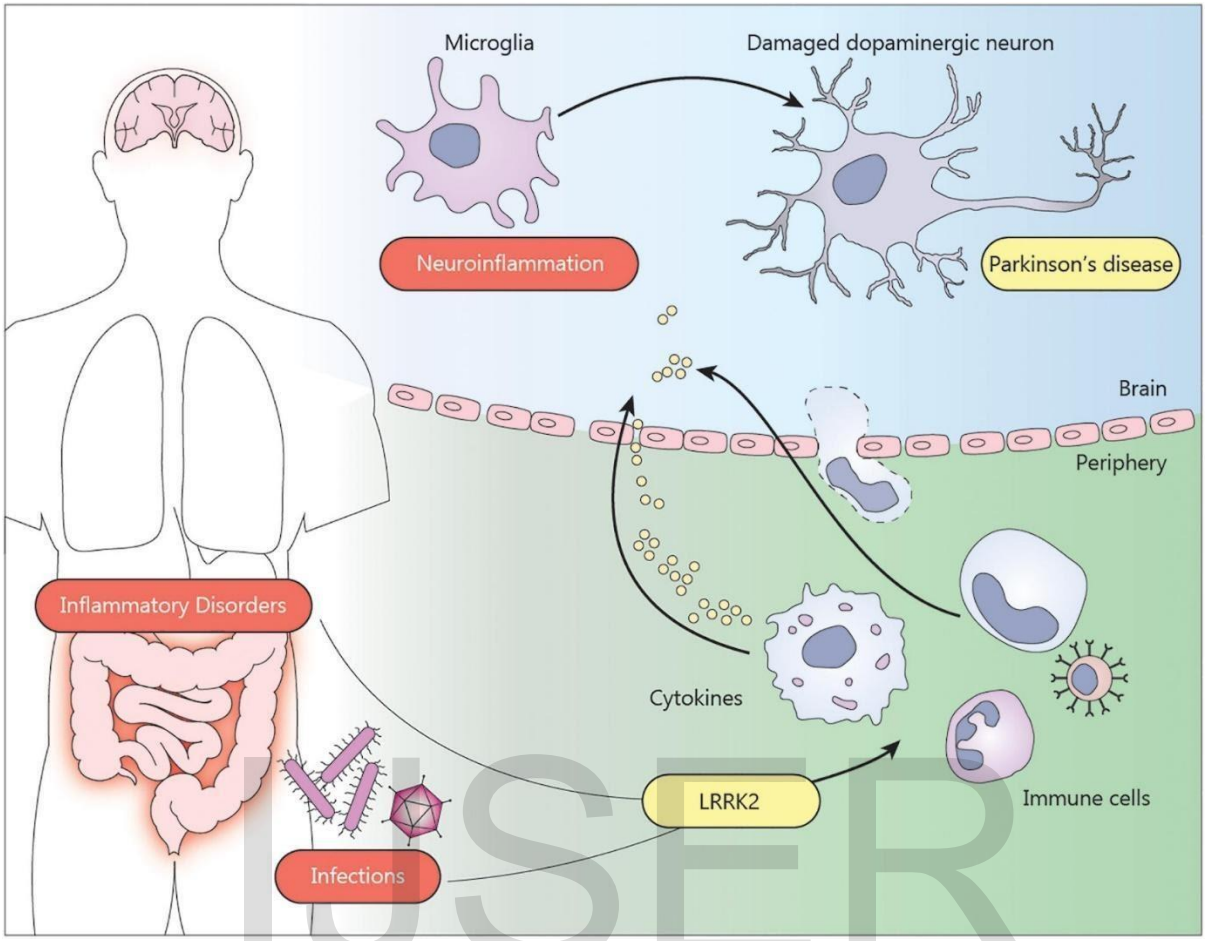

Figure 2 shows how apart from genetic causes, environmental variations in the form of gut microbiome alterations could be contributing factors in the pathogenesis of PD by triggering immune and pro-inflammatory responses. LLRK2 mutations specifically promote microglial and immune cells in the peripheral nervous system to produce inflammatory cytokines, T cells, and monocytes. This inflammatory environment of the gut can pass the blood-brain barrier and cause changes in the dopaminergic neurons in the substantia nigra exacerbating neurodegeneration.

Adapted from Cabezudo, Diego, et al. "Multiple-Hit Hypothesis in Parkinson's Disease: LRRK2 and Inflammation." *Frontiers in Neuroscience*, vol. 14, 2020.

It has also been hypothesized that gut bacteria are responsible for activating microglial cells during a viral infection via producing microbial metabolites, specifically SCFAs. It has been shown that there is a lower concentration of SCFAs, acetic acid, butyric acid, and propionic acid in germ-free mice(GF)as compared to specific pathogen-free mice(SPF) and gnotobiotic Altered Schaedler Flora mice(ASF). To determine the role of short-chain fatty acids on neuroinflammatory and neuroimmune activations, an experiment was performed using alpha-synuclein overexpressing mice and wild-type mice, wherein they were administered a mixture of SCFAs including propionate, acetate, and butyrate while the mice remained microbiologically sterile and showed significantly restored fecal SCFA concentrations upon administering the mixture. Microglial cells

in animals who were administered with SCFA display morphology signaling increased levels of activation as compared to untreated mice in the affected brain regions such as substantia nigra and cerebral cortex. Microglia from GF alpha-synuclein overexpressing mice that were administered SCFAs were larger diameter-wise than GF-Wild type mice who were fed SCFAs with decreased length and number of branches in cells. Abx-treated animals display similar microglial cell morphology to germ-free mice. There are changes in microglia diameter in the frontal cortex which are not seen in the other brain regions demonstrating the specificity of SCFA response in different brain regions. Hence, we can infer that through the production of SCFAs gut microbiota modulates the maturation and inflammatory actions of the microglial cells. A SCFA receptor called FFAR2 is necessary for microglia maturation however these cells do not express FAFR2 but these cells are known to express SCFA responsive genes like histone deacetylase which regulates gene expression. These SCFA can affect the pathophysiology of PD seen in cells of the CNS by crossing the blood-brain barrier or they could also have an impact on the cells of the peripheral nervous system through currently undetected mechanisms. Insoluble aggregates, as well as oligomeric forms of alpha-synuclein, can lead to microglial activation and its activated state, in turn, causes the production of inflammatory cytokines which impairs neuronal functioning and leads to increased cell deaths in models of PD and similar neurodegenerative conditions. A pro-inflammatory gut environment promotes aggregation of alpha-synuclein which further favors the activation of microglia upon contact with alpha-synuclein forming a feed-forward cascade leading to additional alpha-synuclein accumulation propagating the disease to progress into its next stage.(34) (35)

Immune activation

The accumulating evidence suggesting a link between gut dysbiosis, immune activation prompted researchers to study the change in immunoglobulin concentration in PD patients. To do this a total of 61 PD patients and 761 corresponding healthy subjects were sampled and serum levels were measured to decipher the quantity of T-cell subsets and immunoglobulin levels in order to calculate the varying degrees of peripheral immune activation across all subjects. Results suggested that PD patients had higher proportions of CD3+ T cells and CD4+ T lymphocytes in comparison to control subjects. CD 8+ T cell levels were approximately the same in both groups whereas the CD4/CD8 ratio was greater in the PD population. There were no statistically significant differences in quantities of IgA, IgG or IgM between both groups. There was an inverse correlation between CD4+ T cells and the disease stage as indicated by the Hoehn and Yahr scale. There was a positive association between IgG cells and disease duration. These correlations were confirmed in female patients however this association was not observed in male patients. The magnified immune activations in the peripheral nervous system in PD patients as suggested by the alterations in CD3+ T cells and CD4+ T lymphocytes, as well as IgG levels confirming the role of peripheral immune activation especially in the female PD subgroup. The CD3+ T cells are the total cells which are lymphocytes, CD4+ T cells are the helper T cells which are often correlated with anti-inflammatory properties and CD8+ T cells are cytotoxically associated with inflammation and the increased CD4+/CD8+ ratio in PD patients suggests enhanced immune peripheral nervous system activation which is also seen in other neurodegenerative conditions such as multiple system atrophy, amyotrophic lateral sclerosis, and Alzheimer's disease. CD4+ T cells play the role of modifying potentially catastrophic neuroinflammation and neurodegeneration. The increase in CD4+ T cells could highlight the need to compensate for the ongoing neuroinflammation. The numbers of lymphocytes in the PD population could have been influenced by dopaminergic

antiparkinsonian medications such as levodopa treatment which can inhibit the quantity of peripheral IFN γproducing T cells. Progression of PD into the next stages, the increase in IgG could occur simultaneously with the accumulation of alpha-synuclein aggregates leading to consequent dopaminergic cell loss. Further stages of the disease might lead to a decrease in CD4+ T cells which signals the central nervous system's need to engage CD4+ T lymphocytes from the peripheral nervous system to regulate immune responses by the brain. The decrease in phagocytic microglia activity could also cause the permeation of these CD4+ T lymphocytes. Gender has also become increasingly important to judge an individual's susceptibility and symptoms in response to PD and could have important implications for therapeutic approaches to managing PD. Sex hormones especially estrogen play an essential role in the pathogenesis of PD and could be responsible for the difference tween the two sexes. Estrogen can alter CD4+ T lymphocyte function and development, specifically their capacity of production of certain cytokine profiles. (36)

Toll-like receptors (TLRs) are responsible for generating immune responses by identifying conserved motifs in microorganisms and dysregulation in their functioning is indicated by an alpha-synucleinopathy like PD. Overstimulation of the immune system caused by gut dysbiosis, abnormal bacterial overgrowth in the small intestine coupled with increased gut barrier permeability could lead to local and/or systemic inflammatory responses also triggering enteric neuroglial activation ultimately causing the development of alpha-synuclein pathology. In order to define the role, TLR-4 plays in microglial and astroglia activation fostered by the following forms of alpha-synuclein: fibrillized, soluble full length and C-terminally truncated, an experiment was performed. Wild-type mice, some of which possessed TLR-4 while the others were TLR-4 deficient. These murine microglial and astroglial cell cultures were treated with recombinant alpha-synuclein. Following this, phagocytic activity, cytokine release, reactive oxygen species, and NFκB nuclear translocation were measured. The results of the study suggest that the C-terminally truncated form of alpha-synuclein was the most influential inductor of TLR-4 dependent microglial activation which shows us how TLR-4 modulates the extent of inflammatory responses produced by the glial cells. Additionally, it also modulates the production of reactive oxygen species provoked by alpha-synuclein aggregation. As opposed to microglial cells, astroglia cells do not require TLR-4 for the uptake of alpha-synuclein. This study provides insight as to the intricate mechanisms of the activation of microglia and astroglia triggered by alpha-synuclein. This might greatly inform our understanding of the pathology involved in alpha-synucleinopathies. Cytokine release by microglial cells depends on the form of alpha-synuclein and often presents with a different temporal profile. After sensing full-length soluble(sAS)and C-terminally truncated(tAS) alpha-synuclein in the medium, microglia react by the release of tumor necrosis factor- α (TNF-α) but the response generated by sAS by releasing tumor necrosis factor- α was rather shortlived in comparison to tAS. When treated with fibrillar alpha-synuclein, there was only a transient TNF-α release within 12 hours of the treatment. Additionally, tAS was the only alpha-synuclein form that caused the release of IL-6 within a 24-hour period and it also continues to increase the release of CXCL 1 which is already detectable after 2 hours of treatment with tAS. This release continues to release as time progresses. Microglia treated with alpha-synuclein were generally found to release cytokines commonly associated with NF-κB nuclear translocation. Microglia which were lacking TLR-4 did not show any NF-κB nuclear translocation from the cytoplasm to the nucleus which was accompanied by a decrease in cytokine levels, reactive oxygen species production as well as phagocytic activity, all of which is triggered by exposure to alpha-synuclein aggregates. So, we can conclude that TLR-4 plays a major role in regulating immune responses by the microglial cells and oxidative stress of microglia upon subjecting them to alpha-

synuclein. This resembles the role of TLR-4 on microglial activation when treated with amyloid-beta, the major component of amyloid-beta plaques found in patients with Alzheimer's Disease. (37)(38)

Alpha-synuclein accumulation

The best biomarker for Parkinson's Disease is alpha-synuclein and due to this researchers have directed numerous attempts to potentially detect peripheral alpha-synuclein in biopsies, tissue imaging techniques, and biofluids. The systemic synuclein sampling study, currently in clinical pre-development (S4) was designed in order to act as accurate and reliable biomarkers for PD. S4 will correlate with systemic alpha-synuclein levels and provide a quantitative measure of striatal dopaminergic function to enhance our understanding of the severity and stage of pathophysiological progress in P.D. patients. In order to implement this technique, biofluids obtained will be analyzed using alpha-synuclein transcript isoform sequencing to quantify the presence of alpha-synuclein in P.D. patients and healthy controls. Tissues will be analyzed using immunocytochemistry to detect alpha-synuclein and tissue-specific templates will be curated to be able to semi-quantitatively classify the density of alpha-synuclein fibers. After these steps, there will be a comparison of alpha-synuclein load in the biofluids and biopsies in the P.D. population using the dopamine transporter single-photon emission tomography (DATSPECT) method as well as a generalized linear model. This technique provides the exciting opportunity of validating the popular hypothesis suggesting that the pathology of PD originates in the enteric nervous system and spreads to the substantia nigra via the vagus nerve in a prion-like fashion however this hypothesis yet remains to be proven. S4 might provide some insight as to how this centripetal spread of pathology occurs, knowledge of this phenomenon currently is limited to data from cardiac autonomic nerves. (39)

To test the extent to which enteric dysfunction can influence the intensity of motor symptoms in PD and judge how alpha-synuclein pathology in the peripheral nervous system spreads to the central nervous system via associated neural mechanisms, a study was conducted with rodents and non-human primates. There were two separate models of mice used- the first being the alpha-synuclein overexpressing model and the second was the preformed fibril model. The rodents and non-human primates both of these were treated with enteric injections of adeno associated virus overexpressing the A53T alpha-synuclein mutant frequently observed in PD. After this treatment, rats were assessed for colonic motility on a monthly basis after which they were sacrificed at 1,6 and 12 months. The non-human primates on the other hand were sacrificed after a time duration of 12 months following the injection. After sacrifice, spread of PD pathology was examined across all animals used for the study. The rodents exhibited a temporary GI phenotype that subsided after 4 months. Minor alpha-synuclein accumulation was seen in the dorsal motor nucleus of the vagus and locus coeruleus of the brainstem one month post the preformed fibril injection. As time progressed no indications of pathology were observed neither in the preformed fibril model nor in the monomer treated/saline-treated animals. When analyzed using histopathological measures, the brain of the non-human primates showed no pathology in spite of the fact that there was a presence of robust alpha-synuclein pathology throughout the enteric nervous system which lasted for the entire duration of the study i.e. 12 months. This study illustrates how any alpha-synuclein build-up in the enteric nervous system is more than enough to induce gastrointestinal dysfunction. This leads us to believe that spread of alpha-synuclein pathology for extended time frames from the peripheral nervous system to the central nervous system and subsequent propagation is fairly

uncommon and presence of enteric alpha-synuclein pathology and gastrointestinal dysfunction represents a secondary symptom and by-product of suffering from PD. (40)

Motor impairments in PD are often associated with the aggregation of alpha-synuclein. By using antibodies that specifically recognized alpha-synuclein fibrils and aggregates, immunofluorescence microscopy was performed to detect alpha-synuclein aggregates in the brain of mice. In specific pathogen-free mice, there is noteworthy accumulation of alpha-synuclein in the substantia nigra and caudoputamen in alpha-synuclein overexpressing mice. These affected brain regions in the brain of mouse models formed a part of the nigrostriatal pathway which is generally affected in PD. In contrast, germ-free alpha-synuclein overexpressing mice demonstrated fewer counts of alpha-synuclein aggregates. In order to develop a numerical measure for the alpha-synuclein deposition, extracts from brain region were taken and western blots were performed which yielded the following results- there was notably less insoluble alpha-synuclein in the brain of germ-free alpha-synuclein overexpressing mice. To validate these results, dot blot analysis was carried out for alpha-synuclein aggregates in the inferior midbrain as well as the caudoputamen where the substantia nigra is positioned. The dot blot analysis gave similar results and showed a decreased accumulation of alpha-synuclein in germ-free alpha-synuclein overexpressing mice. Additionally, the alpha-synuclein aggregation followed a region-specific pattern. In the frontal cortex, germ-free alpha-synuclein overexpressing mice had lower alpha-synuclein aggregates compared to specific pathogen-free mice whereas in the cerebellum there was an equal amount of alpha-synuclein aggregates in both the mice. There is another possibility for obtaining these findings. Transgene expression variations could be responsible for causing these differences however that was not the case since there were similar levels of alpha-synuclein transcription and similar protein levels in the caudoputamen and inferior midbrain in germ-free alpha-synuclein overexpressing mice and specific pathogen-free mice. In the absence of such differences, we can conclude that it was the microbiota of mice that promote deposition of alpha-synuclein and/or prevent the clearing of non-soluble protein deposits. (41)

Drugs for gut-induced Parkinson's Disease

The presence of microbiota dysbiosis in PD is an indication of how the enteric nervous system might be involved in the pathology of PD making gut microbiota a potential therapeutic target. Several clinical trials have been organized to illustrate the role of probiotics in gastrointestinal processes. However, there haven't been any trials for patients with idiopathic constipation in patients with PD. A study was carried out to test the efficacy of prebiotics and probiotics in PD patients suffering from constipation. A randomized, double-blind, placebo-controlled tertiary environment trial was conducted with PD patients who were tested for clinical constipation using a 2-week stool diary data. 120 subjects were used for the study and were randomly assigned in a 2:1 ratio to the fermented milk group or the placebo group which was administered once daily for 28 days. In the fermented milk group, participants were given milk containing multiple probiotic bacteria strains as well as prebiotic fibers. To be able to quantify the efficacy of these treatments, the increase in the number of complete bowel movements was noted on a weekly basis which served as the primary efficacy end-point. Secondary efficacy end-point was 3 or more complete bowel movements/ an increase by 1 or more complete bowel movements in the later stages of the study(week 3 and 4). It was observed that for the primary endpoint, subjects who consumed fermented milk contained probiotic strains of bacteria and prebiotics caused an increase in the number of complete bowel movements compared to the placebo group. Similar results were

obtained for the secondary efficacy end-point- the group who consumed the probiotic-prebiotic mix reported 3 or more complete bowel movements or an increase by 1 or more during weeks 3 and 4. These results are fairly positive for a non-pharmacological intervention to improve constipation in PD patients by preventing loss of ganglia and associated dysfunction, these results support the idea that manipulating gut microbiota may help alleviate gastrointestinal distress. Prebiotics function as important components for bacterial metabolism and are also involved in the growth of microbiota. Also, a mixture of probiotic strains was used which is generally shown to be more successful in comparison to a single strain so it raises the question of whether these findings were a result of the synergistic effect or higher doses of probiotics. Apart from increasing fecal bulk, there are a myriad of bacteria-host interactions that activate the immune system, cause local inflammatory substance release, and affect gut barrier epithelial integrity/permeability. It also regulates neuromotor function or in this case dysfunction by bacterial metabolites. (42)

Synbiotic intake may also help restore gut microbiota and improve gastrointestinal health. A study aimed at assessing the efficacy of synbiotics was conducted with patients who reported slow transit time accompanied by constipation. 100 patients were randomly split into two groups. One group received a synbiotic and the other group received a placebo twice daily for a period of 12 weeks. The primary efficacy endpoint was until clinical remission or a marked improvement rate from before administering the synbiotics. These parameters were measured at weeks 4 and 12. After administering the drug or placebo, parameters like stool frequency, stool consistency, evacuation, colon transit time, other abdominal symptoms, input from patients about their constipation-related symptoms, gastrointestinal quality of life index scores, satisfaction scores as well as unfavorable events were constantly monitored. At week 4 and week 12, the rates for remission reached 37.5% and 45.8% respectively in the treatment group as compared to the following statistics in the placebo group- 13.3% at week 4 and 16.7% at week 12. Over a duration of 12 weeks, 64.6% of patients receiving the synbiotics experienced an improvement in clinical symptoms. After the same time period, only 29.2% of subjects in the placebo group experienced an improvement. The group receiving the treatment demonstrated an increase in stool frequency, had better stool consistency, ameliorated colon transit time, and a decrease in unfavorable symptoms pertaining to constipation. Given these positive results on gastrointestinal functions, synbiotics can be used to effectively modulate the metabolic activity of intestinal bacteria, help regulate strains of bacteria having beneficial properties and act as an inhibitor for pathogenic bacterial genera. All of the above features make synbiotics an attractive treatment option(**Figure 3)** to alleviate gut dysbiosis in neurodegenerative conditions like PD. (43) (44)

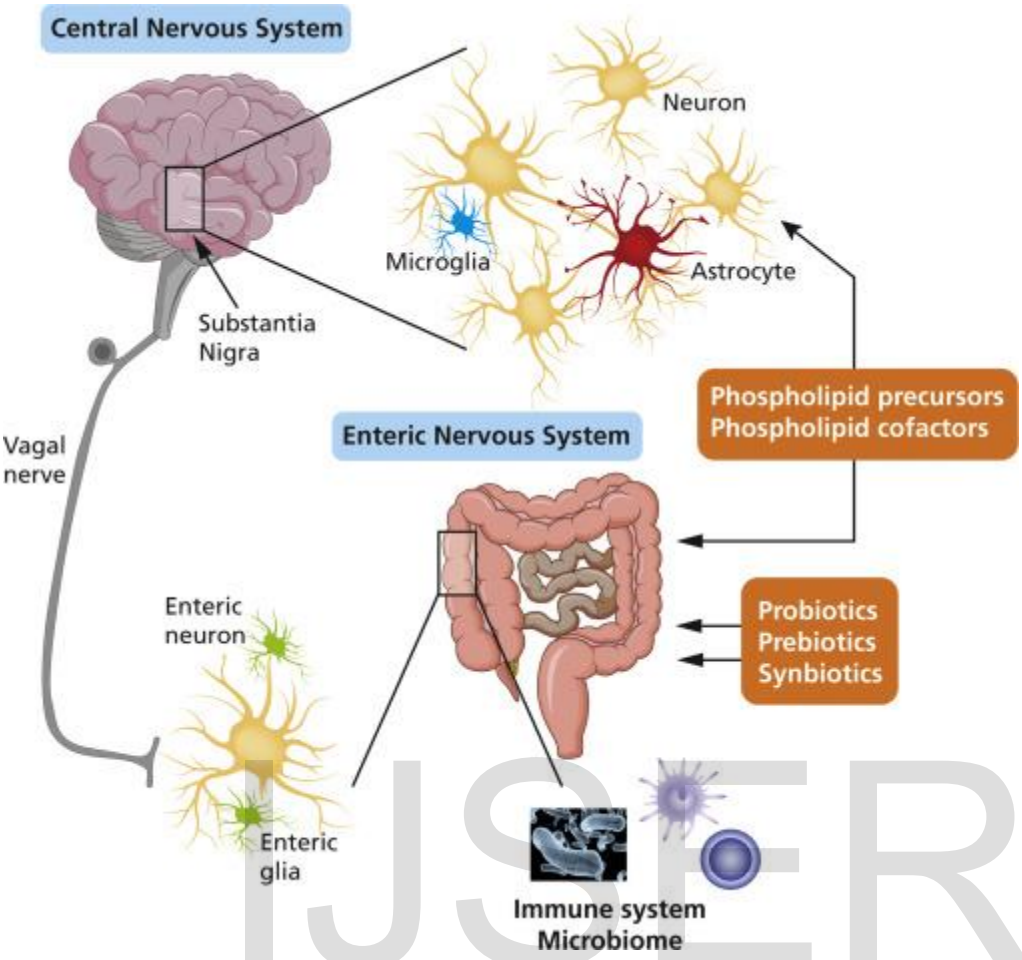

Figure 3- prebiotics, probiotics, and synbiotics have the ability to influence gut microbiome composition, maintain intestinal epithelial barrier integrity. It also increases beneficial bacterial strains causing a reduction in the production of pro-inflammatory cytokines by glial cells and immune activation of astrocytes impacting neuronal degeneration and the progression of PD. Dietary supplements can have an impact on both the enteric and central nervous systems helping alleviate motor and non-motor dysfunctions accompanying PD. In addition, it can reduce inflammation along with magnifying neuronal membrane formation and activity.

Adapted from Perez-Pardo, Paula, et al. "The gut-brain axis in Parkinson's disease: Possibilities for food-based therapies." *European Journal of Pharmacology*, vol. 817, 2017, pp. 86-95.

Alterations in the composition of the gut microbiome could have neurological implications as observed in PD. To test this connection, researchers observed the impact of alterations in the gut microbiome on the phenotypes exhibited by rodent models of PD. After being climatized to their setting, rats were administered with a mixture of non-absorbable broad-spectrum antibiotics in their drinking water which was replaced every 48 hours. This treatment was continued for 14 days before being injected with a neurotoxin named 6-hydroxydopamine (6 OHDA). These antibiotics were recontinued 12 weeks after the lesion. At the start of the study before the administration of antibiotics, before the surgical procedure wherein 6 OHDA was delivered into selected sites in the striatum and post-antibiotic treatment, fecal pellets were collected to analyze bacterial groups

present. When cecal contents were examined using RT-qPCR analysis using 16S primers it was noticed that the antibiotics caused a 74% reduction of bacteria (90% reduction in alpha diversity) in the gut of mice who had been administered the antibiotics. This was observed throughout the entire duration of the study. The toxicity of 6 OHDA depends on whether or not there is uptake by the dopaminergic transporter. The antibiotics had no significance in either the dopaminergic uptake in the striatum or the expression of dopaminergic proteins. Antibiotics diminished the effect of 6 OHDA and improved motor deficits 12 weeks post-surgery. The 6 OHDA rat model showed attenuation of TH+ immunoreactivity loss in the substantia nigra which shows how the damage done by 6 OHDA is decreased in the presence of antibiotics. In antibiotic-treated animals, there was no mRNA expression of any pro-inflammatory cytokines. By studying the gut microbiota, the researchers identified different factors influencing the loss of dopaminergic neurons through a mechanism involving diminished inflammatory markers. The intricate mechanisms behind these effects are yet to be detected but this data suggests that gut microbiome composition can be manipulated so as to modify the progression of PD. (45)

Fecal microbiota transplants are also being considered as a possible therapeutic intervention for neurodegenerative conditions such as PD. To test the efficacy and safety of FMT on PD, a study was conducted with fifteen PD patients- ten of these people received an FMT through a colonoscopy whereas the rest received an FMT via a nasojejunal tube. During the three months follow-up, motor and non-motor symptoms were assessed using scale scores. The Pittsburgh sleep quality index (PSQI), Hamilton depression scale (HAMD), Hamilton anxiety scale (HAMA), a questionnaire evaluating the quality of life (PDQ-39), and unified PD rating scale (UPDRS-III). The ratings from the above questionnaire significantly decreased one-month post-FMT with a significant improvement in quality of sleep and life and a reduction in anxiety and depressive symptoms. After performing the FMT, those who received the colonic FMT had a significant reduction in HAMD, PSQI, HAMA, NMSQ, PDQ-39, and UPDRS-III after a duration of one month. Patients receiving the nasointestinal FMT did not show significant improvement in scores other than a slight reduction in UPDRS-III scores. In the extended follow-up sessions, colonic FMT had a median gray appearance time was six months whereas the time was one month in the group that received the nasointestinal FMT. Colonic FMT had a higher efficacy for a long period(two patients demonstrated a satisfactory result for twenty-four months). There were no patients in the nasointestinal FMT group who were satisfied with the results for longer than three months. There was no long-term adverse effects however five patients one week within getting the FMT experienced mild, self-limiting side effects ranging from abdominal pain, diarrhea to flatulence. Gut microbiota and its associated metabolites have the ability to modulate the pathology of PD by regulating processes like neuroinflammation, immune activation, alpha-synuclein aggregation so a proposed mechanism is that FMT helps to change the composition of the gut microbiota and metabolites to restore beneficial strains of bacteria and optimize the structure of the gut microbiota which in turn alleviates the non-motor symptoms observed in PD. Efficacy might be affected by the colonization of microbiota in the gut. Nasointestinal FMT transplants the microbiota into the patient's jejunum. The pH of the small intestine interferes with microbiota colonization up to some extent causing reduced numbers of functional microbiota which might explain the reduced efficacy. (46)

Ketogenic diets have been hypothesized to have therapeutic effects on neurodegenerative diseases. Ketone diets are essentially a fat-rich and low-carbohydrate combination that has an effect resembling when fasting which helps guide ketosis in the body to suffice for metabolic

requirements. Its use as an anti-epilepsy treatment alludes to the neuroprotective effects it might possess. A study evaluating the efficacy of ketone diets on motor functions and comparing it to the effect achieved by pramipexole was conducted with 56 male Wistar rats who weighed 200-240 grams and were about 12-14 weeks in age. This population was randomly divided into 8 groups- ketone diet group, control group, sham-operated group, PD control group, ketone diet PD group, ketone diet pramipexole PD group, and pramipexole PD group. The efficacy of treatments was gauged using tests like cylinder task test, bar test, and beam traversal task test to assess motor symptoms in mice. Results suggested that the mean ketone count was significantly higher in the blood of rats after the ketogenic diet. As for the control and sham-operated group, there were no significant differences between the two groups. The mice had better motor phenotypes when they consumed the ketogenic diet as compared to the non- ketone diet group. Furthermore, the ketogenic diet enhanced the effect of pramipexole but not up to such an extent to make it statistically significant. An increase in ketone bodies is associated with fatty acid oxidation. Some fatty acids are responsible for modulating stimulatory properties of neural sheaths. It does so by decreasing inflammation and producing mitochondrial uncoupling proteins further causing the production of reactive oxygen species by inhibiting calcium/sodium channels diminishing oxidative injury. Apart from this, ketone bodies alone could also possess neuroprotective properties caused by reduced brain-derived neurotrophic factor and its primary receptor tyrosine kinase B, improved mitochondrial performance, attenuation of inflammatory mediators such as interleukins and tumor necrosis factor alpha. (47)(48)

Concluding Remarks

Parkinson's Disease, one of the most common neurodegenerative conditions, is often associated with the accumulation of protein aggregates(Lewy bodies) in the substantia nigra, leading to a loss of dopaminergic neurons. This review examines the available evidence to justify Braak's hypothesis of how the pathology of PD begins in the enteric nervous system. This literature survey looks at the role of microbiota dysbiosis in the pathogenesis of Parkinson's Disease by examining how altered gut metabolites/bacterial strain abundances could give rise to motor and non-motor symptoms seen in PD. The first step to validate this hypothesis is to conduct fecal microbiome analysis using rRNA sequencing. The results of this gene amplicon sequencing illustrated a change in relative abundances of certain bacterial genera. These changes were synonymous with an increase in opportunistic pathogens, neurotoxins, metabolic products which exacerbated neurodegeneration by promoting dopaminergic cell death, motor deficits, increase in inflammatory nature of the gut which could then spread to the brain in a prion-like fashion via the vagus nerve. There was a decrease in neuroprotective bacterial groups causing alpha-synuclein aggregation, neuronal oxidative stress activation of microglial cells causing an increase in immune activation. There was an increase in the concentration of bacteria advancing gastrointestinal barrier permeability/impacting Gastrointestinal motility. The correlation between IBD and PD could be due to disrupted barrier integrity which in part could be a product of enteric glial dysfunction. Elevated levels of calprotectin in PD patients could be a sign of intestinal inflammation and impaired barrier integrity/permeability.LRRK2 has also been shown to affect neuronal outgrowth by promoting inflammatory properties and immune cell activation. Rats who were administered with short-chain fatty acids demonstrated increased activation in the substantia nigra and a decrease in the length and branches of microglial cells. Immune markers such as CD3+ T cells as well as CD4+ T lymphocytes were upregulated in PD patients. TLR-4 has also been proven to play a major role in regulating immune and oxidative stress responses by microglia when subjected to

alpha-synuclein aggregates. Using mice models of PD, we can deduce how the microbiota of mice might be responsible for the clearance of protein aggregates. The mechanisms of how enteric dysfunction leads to the spread of alpha-synuclein in the CNS and brain were also discussed and the possibility of systemic synuclein sampling was explored to track the spread of PD pathology. In order to target this gut-induced pathology, treatment with probiotics and prebiotics has shown great promise as an effective therapeutic option. Antibiotics help attenuate the effects of neurotoxins and help improve motor symptoms. Colonic FMT is in preliminary stages of clinical trials; it has been shown to restore beneficial bacterial groups and modulate gut microbiome composition. Ketogenic diets have also shown efficacy against neurodegenerative diseases by attenuating inflammatory mediators and improving mitochondrial functions. Understanding the interactions within the gut-brain axis may help unravel the pathophysiological mechanisms of the progression of PD and present new avenues for the discovery and treatment of PD.

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