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The role of gut microbiota dysbiosis on Parkinson's pathogenesis

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Thesis

**THE ROLE OF GUT MICROBIOTA DYSBIOSIS ON PARKINSON'S
PATHOGENESIS**

by

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**THE ROLE OF GUT MICROBIOTA DYSBIOSIS ON PARKINSON'S
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SALMA A. IBRAHIM

ABSTRACT

In this paper, the role of gut dysfunction and gut dysbiosis in Parkinson's disease (PD) was investigated. Using literature reviews and published data, many findings were reported. Those of importance to the scope of this paper are (1) Gastrointestinal (GI) dysfunction was reported in PD patients in statistically significant amounts, (2) pre-clinical data from mice findings showed changes in microbiota such as *Prevotellaceae* when mice were subjected to neurotoxins that mimic PD pathology, (3) fecal tests performed on PD patients showed reductions in short chain fatty acids (SCFAs) and other beneficial bacteria and (4) growing evidence for the presence of α -synuclein in the gut early in disease may suggest that PD pathology begins in the GI tract. Other findings such as prodromal constipation (PC) may indicate changes to underlying gut microbiota early in PD manifestation. While it remains unclear whether GI dysfunction causes PD pathogenesis or vice versa (gut-first or brain-first pathology), it is known that GI dysfunction is a clinical symptom present in disease. Prospective twin studies that follow the impact of diet on Parkinsonism may prove beneficial.

TABLE OF CONTENTS

ABSTRACT	iv
TABLE OF CONTENTS	v
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	ix
PART I: PARKINSON'S DISEASE	1
Scientific Interest Grows in the Study of Parkinson's Disease	1
A. PD and Genetics	2
1. Monogenic/inherited PD	4
2. Sporadic PD	8
B. Protein Aggregation, Neurodegeneration and Dopamine Loss in the Brain	10
C. Parkinson's and Parkinsonism; Symptoms of Neurodegeneration and Clinical Diagnostics	14
D. Therapeutics for Parkinson's	15
E. Risk Factors; A Focus on Intervention for Sporadic PD	16
F. Prodromal PD; Intervention via Early Diagnosis of PD	25
SPECIFIC AIMS	29
PART II: THE GUT MICROBIOME	30
A. What is the Gut Microbiome	30
B. Compositions of the Gut Microbiome in Healthy Individuals.....	31

C. Factors Affecting Gut Health	33
D. Alterations to the Gut Microbiome	35
E. α -synucleinopathy in the Gut: Clinical Marker of PD?	36
PART III: GUT-BRAIN AXIS AND PD	38
A. Braak's Hypothesis	38
B. Braak's Staging and Gut-Brain Progression in Parkinson's Disease	40
C. Scientific Discourse; Brain-First or Gut-First	41
D. Clinical Studies on the Gut-Microbiome-Brain Axis (GBA)	42
E. The Gut-Microbiome; Therapeutics	45
CONCLUSION	46
FUTURE SUGGESTIONS FOR PD PROGNOSIS	47
REFERENCES	48
CURRICULUM VITAE	67

LIST OF TABLES

Table 1. Monogenic Variants Known to be Associated with PD.....	5
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LIST OF FIGURES

Figure 1. Global Prevalence of Parkinson's Disease, 2016	2
Figure 2. A Distinction Mapped Between Normal Physiological Conformations of Alpha-Synuclein and those Associated with Disease.....	7
Figure 3. The Direct and Indirect Pathways	13
Figure 4. Anaerobic Exercise Promotes Formation and Modification of Synaptic Pathways, Neuroplasticity and Brain Health	19
Figure 5. Prevalence of Hypertension with Increasing Age in Men and Women	23
Figure 6. Incidence of PD and Use of Antihypertensive Medication Compared using ROR (Reporting Odds Ratio).....	24
Figure 7. Taxonomic Classifications of Bacterial Organisms and Examples of Species Comprising the Gut Microbiome	32
Figure 8. Braak's Gut-Brain Axis	39
Figure 9. A Framework for Pathogenesis in Sporadic PD.....	41

LIST OF ABBREVIATIONS

ACEI	Angiotensin Converting Enzyme
ARB	Angiotensin Receptor Blocker
BBB	Blood Brain Barrier
BDNF	Brain-Derived Neurotrophic Factor
CCB	Calcium Channel Blockers
CNS	Central Nervous System
COMT	Catechol-O-Methyltransferase
COPD	Chronic Obstructive Pulmonary Disease
CSF	Cerebral Spinal Fluid
DBS	Deep Brain Stimulation
DMV	Dorsal Motor Nucleus of the Vagus Nerve
ENS	Enteric Nervous System
EOPD	Early-Onset Parkinson's Disease
GI	Gastrointestinal Tract
GWAS	Genome Wide Association Studies
HDAC	Histone De-acetylase
IBS/IBD	Inflammatory Bowel Syndrome/Disease
iRBD	Idiopathic REM sleep behavior disorder
LB	Lewy Body
L-DOPA	Levodopa
LN	Lewy Neurites

LOC	Loss of Consciousness
MAO	Monoamine Oxidase
MPP ⁺	1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine
NBB	Non-specific β -adrenoreceptor antagonists/blockers
PC	Prodromal Constipation
PD	Parkinson's Disease
PET	Positron Emission Tomography
PNS	Peripheral Nervous System
RBD	REM sleep behavior disorder
ROS.....	Reactive Oxygen Species
SCFAs	Short Chain Fatty Acid
SCOPA-AUT	Scales for Outcomes in Parkinson's Disease- Autonomic Dysfuction
SNpc.....	Substantia Nigra Pars Compacta
SNPs	Single Nucleotide Polymorphism
TBI	Traumatic Brain Injury
UPDRS	Unified Parkinson's Disease Rating Scale
YOPD	Young-Onset Parkinson's Disease

THE ROLE OF GUT MICROBIOTA DYSBIOSIS ON PARKINSON'S PATHOGENESIS

INTRODUCTION:

Part I: Parkinson's Disease:

Parkinson's disease (PD) is a multi-system disorder¹ and is the second most common neurodegenerative disorder after Alzheimer's disease.² The exact cause of PD remains unclear; however, disease progression follows dopaminergic neuron degeneration.³

The age range for Parkinson's diagnosis is approximately 60 years or older (Figure 1).⁴ This is what is classified in the literature as Late-onset Parkinson's disease.⁵ Outliers are seen, usually a rare percentage of the total PD cases, where PD diagnosis occurs at a younger age (50 years or younger) known as Early-Onset Parkinson's Disease (EOPD).⁶ Not much is known about the mechanism of EOPD.

Scientific Interest Grows in the Study of Parkinson's Disease

With the advancement of healthcare, in part due to the industrial revolution, an increase in the aging population has increased the prevalence and incidence of PD.⁷ Prevalence is defined as the percentage of people in the population that are living with Parkinson's disease, whereas incidence is defined as the number of new cases of PD each year.⁸ In fact, estimate values generated by the Parkinson's Foundation highlight that as many as 1.2 million people will be living with PD in 2030 in the U.S alone, approximating more than a 20% increase

from the current ~500,000 - 1 million people reported to have PD in the U.S.⁹

Although the second most common cause of neurodegeneration, the increase in Parkinson's disease diagnosis yearly has led neurodegeneration to become one of the leading causes of disability in the world. Many have come to refer this as the Parkinson's pandemic.⁷

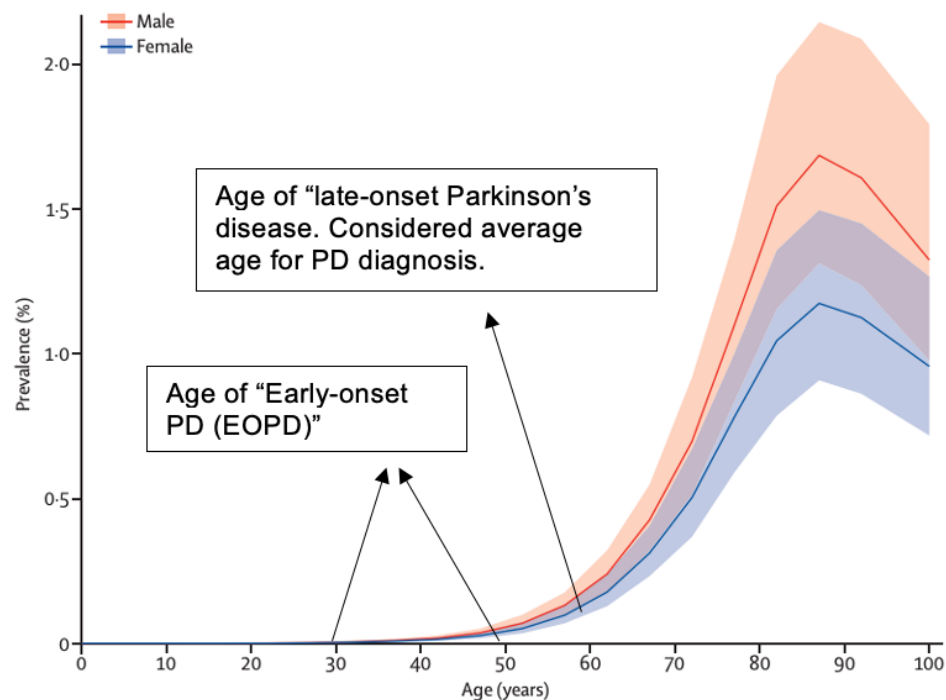


Figure 1: Global prevalence of Parkinson's Disease, 2016. The shaded region indicates 95% uncertainty intervals (Copied from Naghavi et al., 2016.)⁴

A. PD and Genetics

Early twin studies on the cause of Parkinson's disease were performed by Duvoisin et al., 1981.¹⁰ The investigation compared the brains of monozygotic twins to dizygotic twins with PD. They concluded that there was no significant difference in PD etiology, and that there were minimal genetic factors contributing

to the onset of the disease. The assumption here was that the cause of PD was sporadic, not inherited.

However, further studies by Polymeropoulos et al., 1997 led to the groundbreaking discovery of a familial form of Parkinson's disease.¹¹ They looked at a rare familial case concerning 60 individuals presenting with early/young onset PD (YOPD) in the Contursi Kindred of Salerno Italy.¹² The investigation had led to the discovery of an autosomal dominant mutation affecting the folding properties of a small neuronal protein of 140 amino acids called α -synuclein. A missense mutation in the SNCA gene encoding α -synuclein resulted in PD. The research established a causal relationship between α -synuclein's structural dysfunction and subsequent neuropathology in rare cases of EOPD.¹³

Over the years, it has become well-established that PD outcomes are caused by monogenic variants, associated with a single gene mutation as seen in the Contursi Kindred that accounts for ~10% of PD cases, as well as complex genetic and environmental components, contributing to idiopathic/sporadic, accounting for ~85% of PD.^{14,15}

Some scientists argue that it would be an oversimplification to categorize Parkinson's disease as either monogenic or sporadic. On the one hand, high penetrance mutations result in a PD phenotype and are most likely present in familial cases due to a high genetic risk factor. On the other hand, low penetrance mutations have a low-variable presentation of a PD phenotype and

the environment coupled with a genetic risk factor contribute to the manifestation of the disease.¹ The exact role of genetics in sporadic cases is unknown, but varying genetic susceptibility, whether the penetrance is high vs low, can affect the role environmental factors have on disease presentation.

Nonetheless, examining monogenic variants has helped tremendously in understanding the etiology of PD and its progression. More importantly, understanding the pathogenesis can enhance pharmacological targets and the development of new therapies. In this paper, inherited and sporadic PD will be characterized based on differences in onset of disease.

1. Monogenic/Inherited PD

In Table 1, a comprehensive list is shown of monogenic variants known to cause Parkinson's disease. The inheritance pattern is categorized into autosomal dominant, only need one defective copy of a gene to cause PD, and autosomal recessive, where two copies of a defective gene are required for pathogenicity.^{16,17,18} Interestingly, most of the monogenic variants lead to early onset Parkinson's disease although the exact cause remains unclear.

Table 1: Monogenic variants known to be associated with PD. (Adapted from Day et al., 2021.)¹⁹ AD = Autosomal Dominant, AR = Autosomal Recessive

Penetrance	Genes (HGNC Name)	Alternative Gene Names	Inheritance	Pathogenic	PD Phenotype	Function
High	SNCA	PARK1, PARK4, NCAP	AD	Pathogenic	Early-onset	Uncertain, encodes α -synuclein
High	PINK1 (PTEN-induced novel kinase 1)	PARK6	AR	Pathogenic	Early-onset	Mitochondrial
High	PARK7	DJ-1	AR	Pathogenic	Early-onset	Mitochondrial
High	PRKN	PARK2, PARKIN	AR	Pathogenic	Early-onset	Mitochondrial
Variable	LRRK2	PARK8, DARDARIN	AD	Pathogenic	Late-onset	Lysosomal, mitochondrial, microtubule

i. Autosomal Recessive:

PINK1 (causes 2-4% of sporadic EOPD) and PRKN (causes 50% of autosomal recessive EOPD and 15% of sporadic EOPD cases) genes are the most common causes of all autosomal recessive associated Early-onset PD.^{20,21} PINK1 encodes a serine/threonine protein kinase, PARK7 encodes the protein DJ-1 and PRKN/PARK2 encodes Parkin which is an E3 ubiquitin ligase.²² All three genes are involved in modulating mitochondrial function and homeostasis particularly in neurons. Studies have shown the role Parkin protein plays in regulating oxidative stress, polyubiquitination and in proteasomal degradation.^{23,24} Loss of function in the PRKN gene impairs mitochondrial activity. Mitochondrial role in PD is not clearly defined, although inhibition of complex I of the electron transport chain is thought to enhance neuronal death and dysfunction.^{25,26} Similarly, mutations in PINK1 affect dopamine release from

neurons resulting in clinical symptoms of PD, although Lewy body accumulation is not a common presentation in autopsy findings.²⁷

ii. Autosomal Dominant:

Leucine-rich repeat kinase 2 (LRRK2) gene mutations constitute most autosomal dominant cases of familial PD (5-13%). Mutations in LRRK2 account for 1%-5% of sporadic PD due to its high mutation frequency.²⁸ Mutations in LRRK2 have been linked to an increase in SNCA aggregation and subsequent α -synucleinopathy. Carballo-Carbajal et al., 2010 showed that LRRK2 may induce SNCA transcription via kinase-dependent activation of MAPK/ERK pathway.²⁹ Other evidence suggests that LRRK2 is expressed in microglia in the brain as a pathway in inducing neural inflammation and the recruitment of pathological α -synuclein.^{30,31} Although the mechanism of action largely remains unclear, the causative relationship between the LRRK2-PD and the SNCA gene is known to ultimately result in Lewy body accumulation in half of LRRK2-PD cases.³² In the remaining cases, LB pathology is different than that seen in sporadic PD. In fact, the pathology resembles that of Alzheimer's disease with pathogenic markers such as neurofibrillary tangles and/or amyloid plaques reported.³³

Mutations in the SNCA gene, albeit rare, are the second cause of EOPD in autosomal dominant inheritance.³⁴ The encoded α -synuclein protein is abundantly present in presynaptic axon terminals and its proper function is associated with SNARE complex assembly for vesicle trafficking in neurons and dopamine release via exocytosis.³⁵ Alpha synuclein is also expressed in

nonneuronal cell types including immune cells and glial cells, although its role in these cells is less investigated.³⁶ Mutation in the SNCA gene affects the structure of the protein and its function.

Structure Influences Function in PD Pathology

Whether the structure of alpha-synuclein in aqueous solution is defined or undefined remains unclear. The long-standing hypothesis is that as a functional protein, α -synuclein exists in equilibrium between its soluble natively unfolded monomer and its membrane-bound α -helical form (Figure 2).^{37, 38}

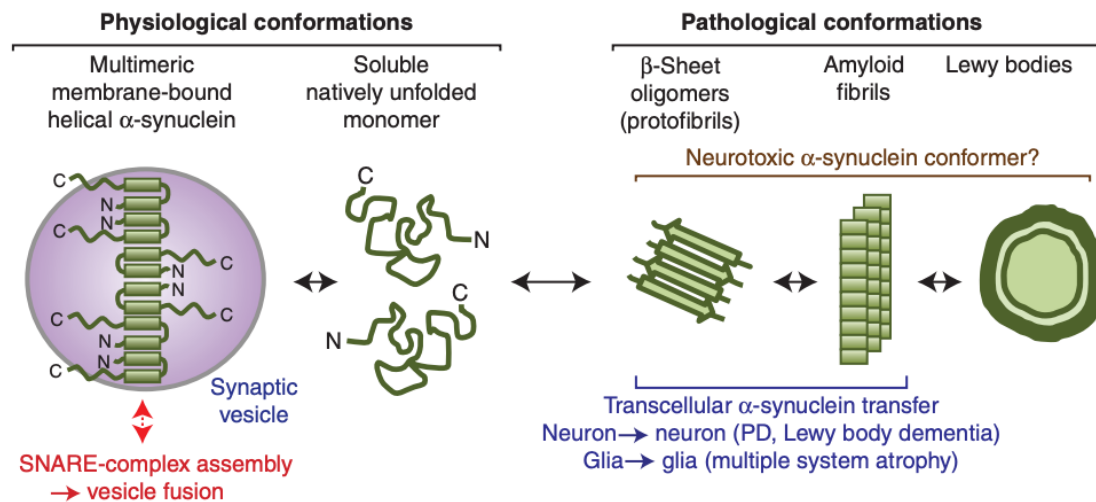


Figure 2: A distinction mapped between normal physiological conformations of alpha-synuclein and those associated with disease. (Copied from Jacqueline Burre et al., 2010.)³⁷

Soluble α -synuclein binds negatively charged headgroups, i.e. phosphatidylinositol, on the presynaptic cell membranes, prompting conformational changes that induce calcium dependent neurotransmitter release from synaptic vesicles.³⁹ In PD pathology, the soluble natively unfolded monomer

is misfolded, aggregates into amyloid fibrils that resist degradation, ultimately depositing as Lewy bodies.^{37,38}

Bartels et al., 2011, however, have suggested that the α -synuclein protein has a defined α -helical tetrameric structure despite the significant focus on the protein's natively unfolded monomeric structure when in the unbound state.⁴⁰ They argued that recombinant bacterial expression used in many laboratories for *in vitro* studies coupled with denaturing and overexpression protocols contribute to the equilibrium between the natively unfolded monomer and the multimeric helical α -synuclein observed in other studies.

In their competing model using native human tetramers, Bartels et al., 2011 showed that the existing helically folded tetramer is first destabilized, further promoting α -synuclein misfolding, aggregation into amyloid fibrils and downstream prion-like propagation. That is, the tetrameric state is a protective mechanism against α -synucleinopathy, and tetramer degradation is required for Lewy body formation.⁴¹ Despite the varied hypotheses, there is no discourse that misfolding and aggregation of the α -synuclein protein drives PD disease state and makes up a major component of Lewy bodies consistent with neurodegeneration and sporadic PD.⁴²

2. Sporadic PD:

There is no specific gene that is implicated in sporadic PD, and this complicates the understanding of the disease process. In fact, studies have focused on looking closely at variants of genes or single nucleotide

polymorphisms (SNPs) in a gene that could confer a risk of developing disease including LRRK2 and SNCA due to their association with familial cases, α -synuclein production and LB accumulation.^{43,44,45} This has propelled scientists to carryout genome wide association studies (GWAS) to understand what the gene variants are, which variants are seen often in sporadic patients, and if these variants are heritable in PD.⁴⁶

Longitudinal twin studies have assessed the role of genetics in sporadic PD. Wirdefelt et al., 2011 examined the concordance rates for PD between monozygotic and dizygotic twins to understand the heritability of the disorder in sporadic PD.⁴⁷ The results showed that the concordance for PD or parkinsonism in monozygotic and dizygotic twins was 13% and 5% respectively. They deduced the heritability estimate, a measure of the role of genetics in phenotype (for this purposes of this paper, a PD phenotype), to be 40%.

One of the earliest longitudinal studies on PD was performed by Piccine et al., 1999. His study highlighted a very important concept in PD pathology and progression, that heritability of the disorder may be difficult to identify especially due to its late progression.⁴⁸ In fact, manifestation of disease may happen over a long period of time, complicating the study of heritability in twin studies. Nonetheless, they used ¹⁸F] dopa and positron emission tomography (PET scan) to investigate dopaminergic loss in monozygotic and dizygotic twins without clinical PD symptoms. Over years of follow-up, they showed the

combined concordance for PD in 12 monozygotic and 9 dizygotic twins to be 75% and 22%, respectively.

These longitudinal studies indicate that genetics plays a role in PD. Although, most of the longitudinal twin studies show a larger heritability estimate in monozygotic twins compared to dizygotic twins, heritability of PD phenotype seen in dizygotic twins suggests that genetics is also involved in sporadic PD. However, the genetic basis for sporadic PD is not completely clear and studies have examined how other risk factors (different environmental toxins and alterations to body systems such as the gastrointestinal tract) that could culminate in PD outcome can be mitigated.^{49,50.}

Since most PD cases are sporadic, any mention of PD henceforth will be referring to this subtype of the disorder unless otherwise specified.

B. Protein Aggregation, Neurodegeneration and Dopamine loss in the Brain

There is already evidence of neurodegeneration as age progresses with age considered the main risk factor for most sporadic neurological disorders.⁵¹ Autopsy reports performed on older individuals with no known neurological disease diagnosis have shown loss of brain volume in large areas of the brain, presence of neurofibrillary tangles, amyloid plaques, Lewy body aggregates, neural loss etc.⁵² This can be caused by (1) loss of protein homeostasis (proteasomal degradation) as a damage marker for an aging brain and (2) autophagy.^{53, 54}

Neuronal proteostasis is important for proper brain activity as it regulates the balance between protein production and function, such that if a misstep occurs in protein synthesis, i.e misfolding, the protein becomes tagged for degradation to prevent its accumulation and subsequent damage to neural tissue.⁵⁵ Likewise, autophagy removes accumulated protein structures and misfolded aggregates via autolysosome formation and when the mechanism is impaired protein aggregation is promoted.⁵⁶

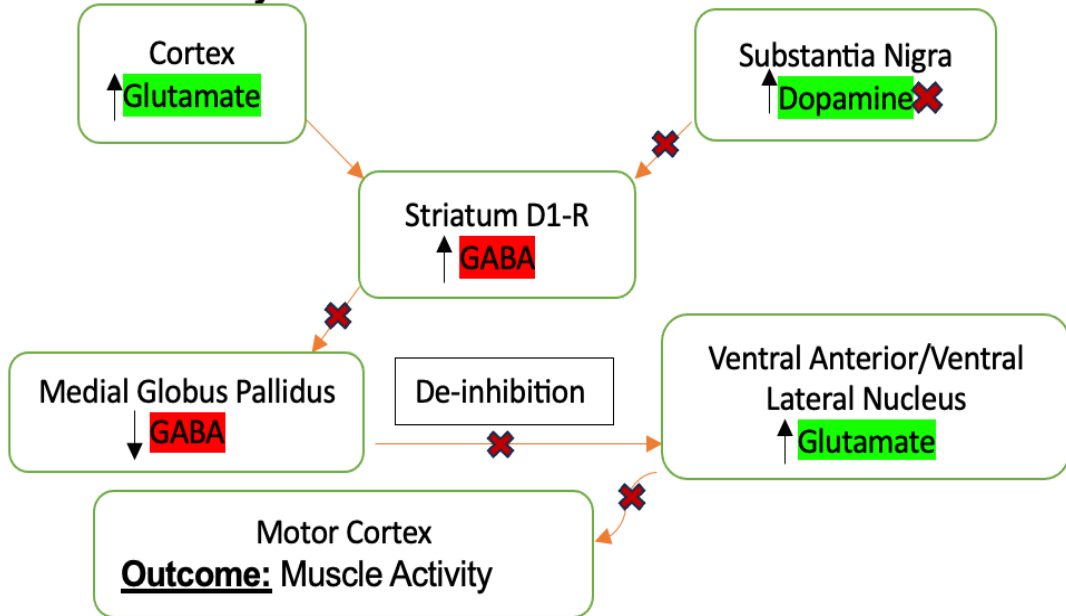
Many factors affect the process of protein homeostasis as the body ages including an increase in transcription errors and SNPs.⁵⁷ As a result, protein misfolding can overwhelm the proteostasis machinery and autophagy, impacting proteasomal degradation and the accumulation of misfolded proteins. Whether aging causes neurodegeneration or vice versa, it is known that α -synuclein aggregation in LBs promotes the loss of neurons in the brain resulting in sporadic PD.

PD neurodegeneration is associated with damage to dopaminergic neurons resulting in loss of the neurotransmitter (NT) dopamine in the substantia nigra pars compacta (SNpc) affecting the basal ganglia network.^{3, 58} Dopamine functions in conjunction with other neurotransmitters to regulate motor functions and mobility.⁵⁹ To better understand the implications of the disease on mobility and function, it is also best to look closely at how dopaminergic neuron loss propagates in Parkinson's disease. Once dopaminergic neuron loss begins via aggregation of misfolded α -synuclein proteins that trigger neuroinflammation, this

pathology is transmitted to other dopaminergic neurons across the SNpc damaging the basal ganglia network.^{60,61} In fact, for PD to present clinically, ~60% neurodegeneration in SNpc and ~70% of dopaminergic neuron death will have occurred.⁶² The progression of neural tissue inflammation and subsequent damage impacts different stages of disease.

Under normal conditions/normal dopamine release, motor function is regulated by two pathways of the basal ganglia acting in concert, namely the direct and indirect pathways.⁶³ These pathways impact the excitation and inhibition of motor functions (Figure 3). In the direct pathway, dopamine acts on the dopamine receptor (D1-R) in the striatum to release GABA, an inhibitory neurotransmitter, de-inhibiting downstream neurotransmitter release to excite muscle activity.⁶⁴ The indirect pathway indirectly inhibits the release of GABA from the medial Globus Pallidus to increase motor activity. Loss of dopamine affects both the direct and indirect pathways, cumulatively resulting in the motor symptoms of PD such as bradykinesia.^{64, 65}

Direct Pathway:



Indirect Pathway:

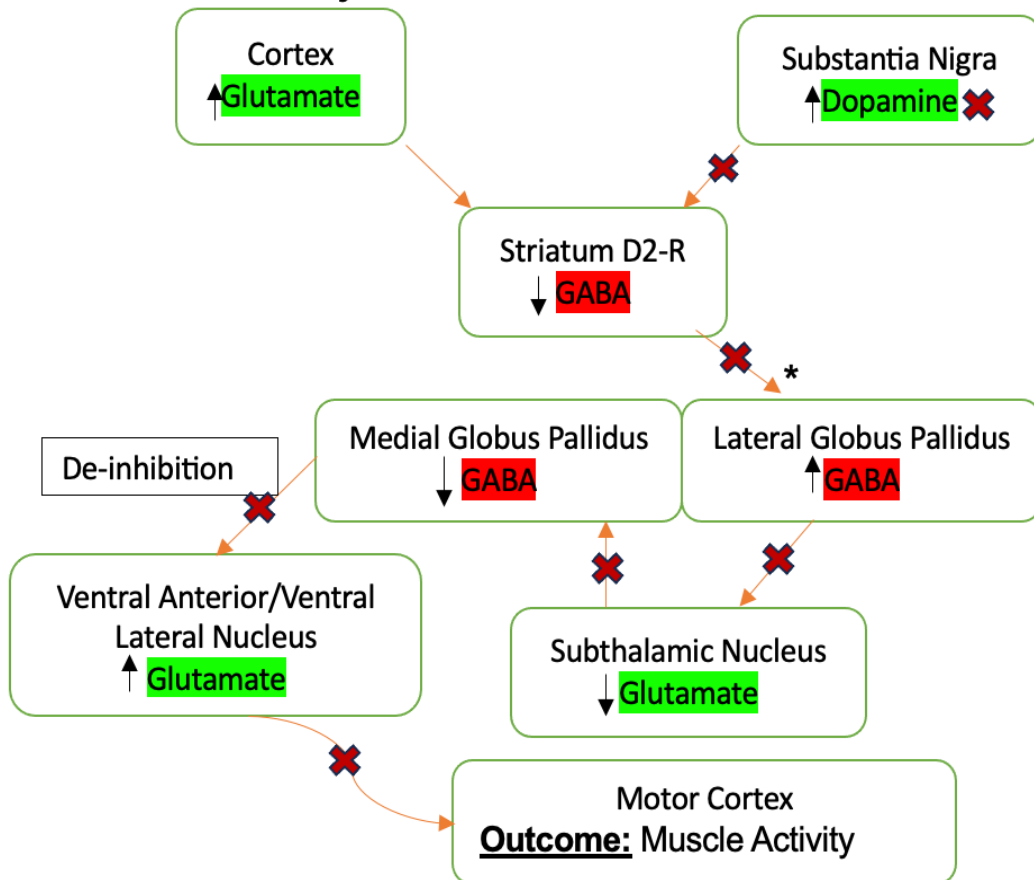


Figure 3: The direct and indirect pathways are shown above. The activation of D1-R pathway results in motor excitation by stimulating the direct pathway whereas dopamine binding the D2-R inhibits the indirect pathway, preventing the release of GABA from the striatum and further downstream cascade results in de-inhibition of Medial Globus Pallidus of the VA/VL nucleus ultimately resulting in motor activity (direct and indirect pathways converge at this point). * Indicates that there is no inhibition of GABA from the striatum on the Lateral Globus Pallidus, X indicates pathway functions affected due to the loss of dopamine from the SNpc. Inhibitory neurotransmitters (NTs) are highlighted red and excitatory NTs are highlighted in green. (Adapted from Roshan et al., 2016.)⁶⁶

C. Parkinson's and Parkinsonism; Symptoms of Neurodegeneration and

Clinical Diagnostics

Parkinsonism had existed prior to the neurodegenerative classification of Parkinson's disease which was performed by James Parkinson in 1817, in his monograph, *An Essay on the Shaking Palsy*.^{67,68}

Distinction Between Parkinson's and Parkinsonism:

Parkinsonism is a common term that describes clinical conditions and symptoms that other neurological disorders share with Parkinson's disease. Non-PD disorders are classified as atypical parkinsonism i.e., Dementia with Lewy Bodies and Multiple System Atrophy.⁶⁹ Although, it is known that Parkinson's is the common cause of parkinsonism, the shared symptoms can complicate diagnosis with PD.⁷⁰ There are four clinical symptoms presented that are considered positive indicators for parkinsonism. These are rest tremor, bradykinesia or slowed movement, rigidity or stiffness in muscles and unstable coordination/ posture.⁷¹

Since there are no definitive tests for diagnosis of Parkinson's, medical professionals need to assess a patient's family history of PD, medications taken that could be confounded with parkinsonism, dopaminergic neurons in the brain via DaTscan (a non-invasive procedure that looks at dopamine loss) etc. A comprehensive analysis of all these factors coupled with the four cardinal symptoms are used in diagnosis.

Patients with PD also present with non-motor symptoms, due to autonomic dysfunction, such as disruptions in proper gastrointestinal function, cognitive decline, impaired thermoregulation, among others.^{72,73} It is believed that non-motor symptoms (characterized as prodromal Parkinson's) may be present years before any clinical/motor symptoms are seen, further impacting when the disorder is diagnosed (discussed in depth later in this paper).⁷⁴

D. Therapeutics for Parkinson's

The best treatment currently available to manage PD is Levodopa/Carbidopa (L-DOPA). The drug, which was approved by the U.S Food and Drug Administration (FDA) in 1970, functions to lessen the motor complications of PD by increasing dopamine in the brain.⁷⁵ The mechanism of action of L-DOPA is as follows: (1) the drug crosses the blood-brain barrier (BBB), where (2) it is metabolized by a decarboxylase in the presynaptic terminal into (3) dopamine, the active agent, for dopaminergic neuronal excitation of motor function. Ultimately, the drug can be either cleared via catalysis by monoamine oxidase (MAO) or catechol-o-methyltransferase (COMT).^{75,76}

The efficacy of L-DOPA has been studied over the years. Studies have reported that Levodopa use improved motor symptoms in PD compared to other dopamine replacement therapies.^{77,78,79} However, research has shown that progression of disease may require changes to administered dose. Patients may experience Levodopa-induced dyskinesias (involuntary movement) that is associated with the short half-life of the drug and its intermittent use throughout the day that affect dopamine levels.^{79,80} Dyskinesias can be managed by using drug combinations that have a longer half-life or by lowering the dosage of orally administered Levodopa/Carbidopa.⁸¹ Benefits of PD drugs on clinical symptoms are measured using the Unified Parkinson's Disease Rating Scale (UPDRS) developed by the Movement Disorder Society (MDS) in 2008.⁸²

Advanced PD may require other treatment strategies in addition to Levodopa use. Deep brain stimulation (DBS) has been used to alleviate progressive symptoms of PD such as tremors and dyskinesias.⁸³ According to Groiss et al., 2009 DBS works to mitigate Levodopa-induced dyskinesias via surgical implantation of electrodes into the brain that reduce progressive motor symptoms. However, DBS is not widely used in PD patients due to its adverse side effects such as intracerebral hemorrhage (ICH).⁸⁴ For most PD patients, symptoms can be managed via physical, cognitive, and dietary modifications in addition to medication use.⁸⁵

E. Risk Factors; A Focus on Intervention for Sporadic PD

1. Exposure to Toxins:

Scientists have studied how certain toxins and chemicals can mimic symptoms of parkinsonism or lead to dopaminergic neuron death in the SNpc. One of the earliest examinations looked at the neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) that gained scientific attention in 1982 due to William Langston's "The Case of the Frozen Addicts."^{62,86} A clinical examination of a small group of heroin users presenting with Parkinson's disease found that they had accidentally taken MPTP, a by-product of synthetic heroin. Since the chemical structure of MPTP is like several pesticides used in agriculture such as rotenone and paraquat, understanding the neurological implications of MPTP became very important.^{86,87}

MPTP diffuses through the tight junctions of the blood-brain barrier (BBB) where it is metabolized by monoamine oxidase B (MAO-B) of glial cells to the neurotoxic MPP⁺ (1-methyl-4-phenylpyridinium).⁸⁸ MPP⁺ associated toxicity drives oxidative stress by disrupting mitochondrial function promoting the accumulation of reactive oxygen species (ROS) in the brain. Studies in mice and *Drosophila melanogaster* have shown that dopaminergic neurons are susceptible to uptake of MPP⁺ resulting in cellular damage and neurotoxic death of dopaminergic neurons in the SNpc.⁸⁹ Rotenone and paraquat are believed to result in similar outcomes.

Other inhaled toxins have also been examined. Data looking at the effects of smoking on PD outcome have shown interesting results. According to the American Parkinson's Disease Association, there is an inverse relationship

between smoking and PD diagnosis; however, it remains unclear if smoking is a protective mechanism against PD or if an underlying predisposition to PD causes an aversion to smoking.⁹⁰ Nonetheless, science is very clear on the hazards of smoking, and countless studies show its implications in many diseases such as lung cancer, heart disease and in COPD such as emphysema.^{91,92}

Similarly, ingestion of caffeine is associated with decreased PD risk in healthy individuals and may pose pharmacological benefits to individuals with PD. Caffeine is an adenosine A_{2A} receptor antagonist.⁹³ The A_{2A} receptors located in many dopaminergic areas of the brain, such as the basal ganglia, help to regulate the neurotransmitters dopamine and glutamate. As a result, caffeine dependent A_{2A} receptor antagonism is believed to modulate dopamine activity, mitigating neuroinflammation and protein aggregation and may confer a benefit.

2. Physical and Cognitive Activity:

Physical activity improves health outcome measures such as lowering risk of developing cardiovascular disease and increasing cognitive function.⁹⁴ Although it cannot be deduced if physical activity protects against developing PD due to the complexity of disease progression, multiple studies have looked closely at how physical activity may alleviate symptoms of PD. Dual-tasks, such as those that stimulate the mind and body simultaneously are considered beneficial in offsetting motor impairment.⁹⁵ It is important to note that these

benefits are varied based on age and gender. Ultimately, there may be an inverse association between physical activity (as well as stimulating exercises) and progressing symptoms of PD.

Animal models used to study PD have shown benefits of aerobic exercise and stimulating exercises (presence of toys) in increasing neuroplasticity (Figure 4), the ability of neurons in the brain to form and alter synaptic networks. Praag et al., 1999 showed hippocampus neurogenesis occurred in mice that were kept under these conditions.⁹⁷ Others indicate that increase in blood flow to the brain due to aerobic activity as well as increased neurotrophic factors such as brain-derived neurotrophic factor (BDNF) in humans, contribute to the increased neuroplasticity.⁹⁸

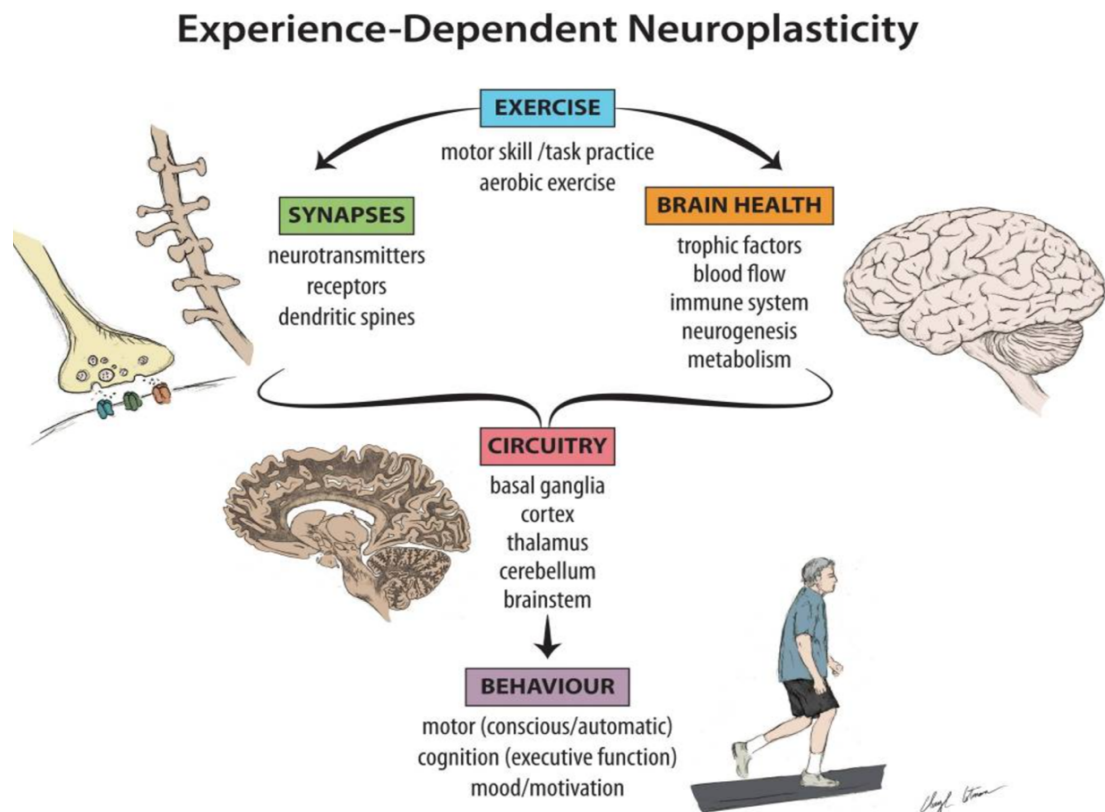


Figure 4: Anaerobic exercise promotes formation and modification of synaptic networks and neuroplasticity as well as overall brain health. Exercise dependent changes to the neurotransmitters dopamine and glutamate have also been reported. The overall findings suggest exercise induced changes to brain networking and health improve disease outcome in PD. (Copied from Petzinger et al., 2013.)⁹⁶

Further animal models showed that exercise/physical activity can alter the effects of neurotoxins on the brain. Varying results were reported in mice injected with MPTP to induce parkinsonism that were later subjected to anaerobic exercise. Findings ranged from lessened effect of MPTP-associated dopaminergic neuronal loss on the striatum in some cases while others showed no exercise-induced changes to dopamine markers after neural damage.⁹⁹ While clinical studies support the idea that physical activity improves PD, whether physical inactivity is a risk factor for this neurodegenerative disease is unknown.

3. Brain Injury:

Delic et al., 2020 examined the link between brain injury and neurodegenerative disorders i.e PD, AD and ALS.¹⁰⁰ Growing evidence suggests that traumatic brain injury (TBI) poses a risk factor to Parkinson's disease in a dose-dependent manner, where risk of developing PD increases with increasing brain injury (using the Glasgow Coma Scale).¹⁰¹ In fact, individuals that have a mild-severe TBI are ~1.5 times more likely to have PD later in life. A loss of consciousness (LOC) that occurs in cases of severe traumatic brain injuries, especially LOC>1 hour, has been associated with increased risk of dementia,

parkinsonism and PD.^{101, 102,103} Head injuries post PD diagnosis can also affect disease progression and neuropathology.¹⁰⁴

Recovery from traumatic brain injuries can be difficult depending on damage to the brain that clinically presents as follows: (1) an acute phase that can last up to one week characterized by damage to the nerve cells directly affected by the injury and subsequent inflammation to neighboring tissue, (2) a post-acute phase that ranges from a week to a month, associated with reduced inflammation in areas affected and structural remodeling of neurons that can have pathological consequences, (3) a chronic phase that can take years and has implications in neurodegeneration and PD pathology for some patients.¹⁰⁰ In severe cases of TBI, neuroinflammation and α -synuclein aggregation in CSF is likely to promote pathogenesis and Parkinson's disease.¹⁰⁵

A lot remains unknown about the course of action of TBI in PD. Although meta-analysis data support an associated risk between TBI and PD, there are other non-genetic risk factors, such as substance use and depression, that can have an additive effect on TBI-related PD susceptibility. Whether these factors (a) lead to a higher risk of TBI occurrence, (b) are caused by sustained TBIs is still under research.¹⁰⁶ Albeit a risk factor, not everyone with a history of TBI develops Parkinson's, further outlining the complexity of the disease.

4. Drugs/Medications:

With age conferring the primary risk factor for PD, it is important to understand the underlying pathophysiology and/or medications that can affect

PD outcomes. Hypertension is considered a common finding as the population ages (Figure 5) with ~60% of cases occurring at the average age of PD onset (~65 years of age).¹⁰⁷ Risk of developing hypertension is race and gender specific.¹⁰⁸

Meta-analysis of prospective cohort studies has reported that individuals that had pre-existing cases of hypertension were more likely to develop PD later in life. That is, pre-existing hypertension may be a potential risk factor for PD diagnosis.¹⁰⁹ The mechanism by which hypertension may contribute to PD is unknown, but scientists have speculated on how this may occur. Since hypertension is associated with diminished vasodilation, the subsequent chronic elevation in blood pressure affects the cerebral vasculature and neurovasculature in the brain, impacts blood supply to the basal ganglia network (and the SNpc) and promotes PD-related neurodegeneration.^{110,111}

Another hypothesis incriminates inflammation as the connection between PD and hypertension. This hypothesis suggests hypertension promotes inflammation as a response to vascular injury, and changes to blood pressure may cause disruption to the blood-brain barrier (BBB). The disruption to the BBB likely makes the brain susceptible to neuroinflammation and neurodegeneration that can exacerbate PD.¹¹²

Several studies have looked at the association between antihypertensive medication and increasing risk for PD. These studies are important to understand if hypertension is the culprit or if antihypertensive medications are a confounding

variable in neuroinflammation and neurodegeneration.¹¹³ Several antihypertensive medications including Selective beta-1 receptor blockers (SBB) i.e metoprolol, angiotensin receptor blockers (ARB), angiotensin converting enzyme inhibitors (ACEI), calcium channel blockers (CCB) and thiazide diuretics were assessed with most showing little to no risk in developing PD.¹¹⁴ Non-specific β -adrenoreceptor antagonists/blockers (NBB) such as propranolol have been reported to have the most risk association with PD and is more likely in individuals that have chronic exposure to NBBs (Figure 6).

Beta-blockers/antagonists, particularly NBBs, acting on the β 2AR/ADRB2 (a regulator of the SNCA gene) are believed to increase the expression of α -synuclein.¹¹⁵ Upregulated expression of the protein may contribute to disease progression. Such data are important to consider when assessing the best antihypertensive medication to prescribe for long-term blood pressure regulation.

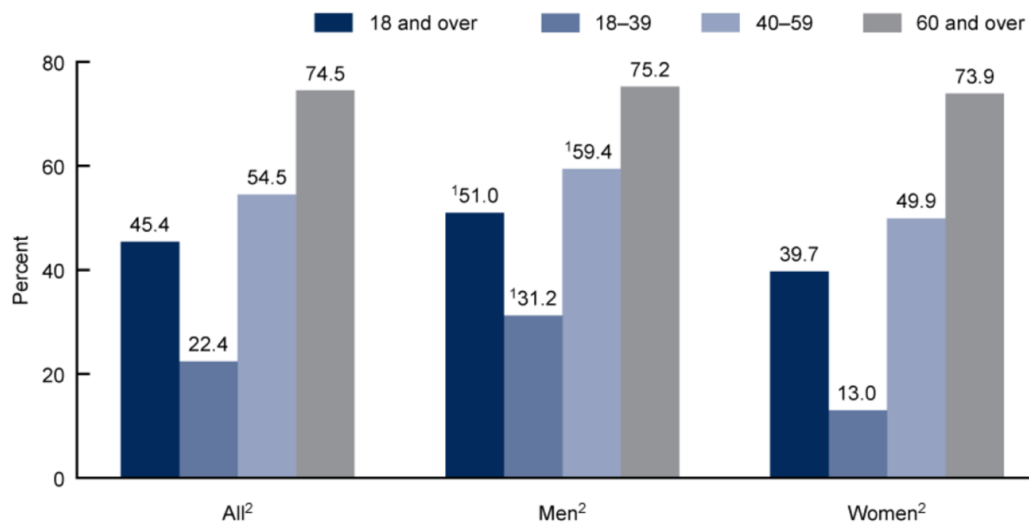


Figure 5: Prevalence of hypertension with increasing age in men and women. The increase in hypertension is gender-specific; but in both genders cases of hypertension increase with age. Copied from Centers for Disease Control and Prevention.¹¹⁶

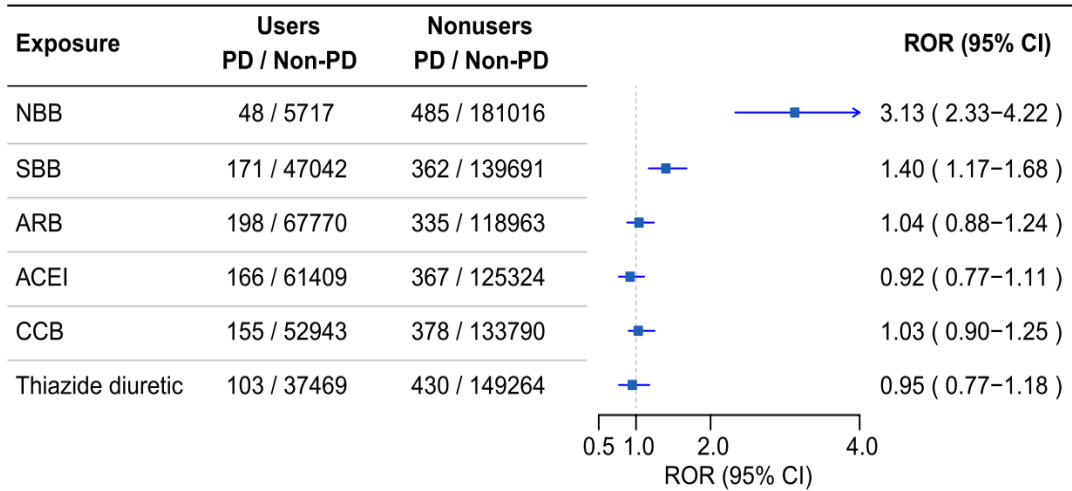


Figure 6: Incidence of PD and use of antihypertensive medication are compared using ROR (Reporting odds ratio) values. NBB has a significant associative risk in PD development compared to the other antihypertensive medications. CI; Confidence interval. (Copied from Feng et al., 2023.)¹¹⁴

5. Sex:

Much of the PD data has shown sex-related differences in the prevalence of Parkinson’s disease (Figures 1 and 5). Sex is considered an independent risk factor in PD and studies have focused on understanding the onset of disease, clinical presentation of symptoms and progression of disease in men vs women. Martinez-Martin et al., 2012 examined an important aspect of disease: how are early indicators of PD (non-motor symptoms) presented in men vs women for better therapeutic outcomes.¹¹⁷ The results found different non-motor symptoms to be prevalent in the different sexes. Although what causes this underlying predisposition is not known; it was shown that symptoms such as fatigue,

constipation and pain were more prevalent in women and other symptoms such as drooling and sexual problems were more prevalent in men.

It is important to note that sex-based differences in PD have not been studied fully and to what extent sex can complicate early disease phenotype and intervention remains unknown. However, epidemiological studies show that prevalence and mortality of PD progresses in a sex-dependent manner; prevalence is higher in men (~2x), whereas progression of PD and mortality is highly observed in women (maybe confounded by other unknown variables).^{118,119}

F. Prodromal PD; Intervention via Early Diagnosis of PD

Preventative medicine focuses on risk assessment, pathophysiology, and underlying clinical symptoms of disease to prevent disease outcomes. Understanding the early clinical symptoms of PD has become an important focus of science to mitigate progression of disease and mortality associated with late-onset PD. Since familial PD has a genetic basis, the clinical symptoms are secondary to the genetic mutation and clinicians focus solely on treatment strategy for lessening cognitive and physical complications of disease outcome (via drug therapies i.e Levodopa, exercise, and surgery). While the same therapeutics are used in care for sporadic PD, the lack of a known genetic mutation (albeit the culpability of genetics in PD) has shifted the focus to the clinical manifestation of the disorder.

1. Constipation:

As mentioned previously, non-motor symptoms present years (~10 or more years) before any clinical/motor presentation and diagnosis of PD occurs.¹²⁰ One such symptom that has been of interest is constipation. Autonomic dysfunction (dysautonomia) caused by progression of PD is known to affect different bodily functions regulated by the autonomic nervous system. Autopsy findings report the presence of Lewy body deposits in the ANS suggesting progressive pathogenesis. As a result, ~50-80% of people with PD report having constipation and reduced GI motility due to the impacted enteric activity.^{121,122} With increasing prevalence of constipation in PD, clinical research has examined if this non-motor symptom is an important risk factor in early diagnosis of Parkinson's.

A sub-study of PRIAMO study, which investigated the effects of non-motor symptoms in PD outcome, was used to assess the role prodromal constipation (PC) has on developing PD and its severity later in life. Results showed that there was a positive risk association between constipation and PD.¹²³ The study also conferred a sex-based association with constipation, with a higher prevalence of PC in women vs men (45.6% and 34% respectively) that could affect severity and mortality of PD. The data also showed that age contributes to the increase in PC occurrence. Since PD is an age-related neurodegenerative disorder and aging accounts for increased disease risk such as hypertension, Alzheimer, etc., then aging may complicate the association between constipation and PD.

A case-control study by Savica et al., 2009 further reported on the impact of early prodromal constipation in patients with PD. The case study examined medical records using the Rochester Epidemiological Project of 196 subjects in Minnesota from 1976-1995.¹²⁴ The data records showed that symptoms of constipation reported as early as 20 years prior to the observation of motor symptoms of PD likely increased disease severity. Similarly, the Honolulu-Asia Aging Study (HAAS) found that men that reported reduced bowel movements had a higher risk of PD incidence.^{124,125} These findings may implicate GI in the early stages of PD that affect its progression. Constipation and reduced bowel motility may signal an underlying problem in the gut that could be culpable in sporadic PD.

2. REM Sleep Behavior Disorder (RBD):

Much like constipation, sleep problems are a complication of disease in people diagnosed with PD¹²⁶. As a result, REM sleep behavior disorder (RBD) is gaining scientific attention as an early predictor of PD. RBD is characterized by dream-enactment during REM sleep that sometimes results in violent manifestation of symptoms.^{127,128}

Studies have identified an increased risk association between diagnosed RBD (via video-polysomnography) and autonomic dysfunction. Assessing the autonomic dysfunction using the Parkinson's questionnaire SCOPA-AUT, the results reported significant differences in GI function between idiopathic RBD patients and the healthy controls.^{127,128} Damage to the ENS may result in severe

gastrointestinal outcomes such as constipation and α -synucleinopathy.¹²⁹

Clinically, this suggests that prior to the onset of the motor symptoms of PD and other α -synucleinopathies, some patients develop RBD.¹³⁰ Furthermore, the gut microbiome compositions of the two study groups, RBD and PD, against a healthy control group showed similar changes in abundance of gut microbiome taxonomy. Scientists have suggested that since idiopathic RBD (iRBD) results in the alterations of taxa of the gut microbiome, it is important to study isolated RBD as an early intervention for PD. Since RBD is prevalent in the PD population, 33%-46%, it would be a significant clinical marker in management of disease progression.¹³¹

The important prodromal symptoms of PD, RBD and constipation, identify the consequence of changes to the gut microbiome in PD outcomes. This indicates that the gut microbiome has a crucial role both in gut health and in disease throughout the body.

Main Question: What role does the gut microbiome play in Parkinson's pathogenesis?

Specific Aims of this Investigation

Research aimed at understanding the cause of Parkinson's disease has shown varying results. Since sporadic PD diagnosis occurs well into neurodegeneration, early detection of the disease has become of utmost importance. This involves examining the non-motor/prodromal symptoms of PD. This newer research has questioned the role the gut microbiome plays in the development of neurodegeneration specific to sporadic Parkinson's disease.

The following topics will be discussed in detail:

1. The gut-microbiome:
 - a. The composition of the gut microbiome to determine what is constituted as safe/healthy gut environment.
 - b. What disruptions are associated with the development of Parkinson's?
2. The gut-brain axis in PD pathogenesis.

Part II: The Gut Microbiome:

A. What is the Gut Microbiome?

The human gastrointestinal tract (GI) represents a large surface area of contact between the body and the environment (250-400 m²). The gut microbiome is important in health and disease.¹³² Understanding the proper function, composition, and dysfunction (“dysbiosis”) can help our understanding of pathologies associated with the gut and PD.

Much of our understanding of the associations made between the gut microbiota and PD include a comprehensive understanding of how the intestinal barrier is affected. The intestinal mucosal barrier (IMB) is selective for nutrient absorption, and it provides multiple layers of defense for the body against the external environment. The IMB comprises: (1) the outer mucus layer, which is made of mucins, highly glycosylated proteins that are a protective barrier for intestinal epithelium and provide lubrication to food, (2) outer commensal bacteria that are symbiotic and important for gut health, immunity and protection from pathogen infiltration, antimicrobial proteins (AMPs) and secretory immunoglobulin A (sIgA) that aid in fighting infection and regulating microbial properties and (3) inner immunity mediators.¹³³⁻¹³⁶ Thus, the intestinal mucosal barrier separates the immune cells from microbiota found along the digestive tract where communication between the two confers host physiological benefits including colonization resistant to pathogen infiltration.¹³⁷

Maintaining the integrity of the intestinal barrier is part and parcel in preventing infection and inflammation that can result in a host of diseases. In fact, changes to the gut microbiota affect the permeability of the intestinal barrier to external pathogens. Once the integrity is disturbed, increased intestinal permeability or a “leaky gut” exposes the bloodstream to invasion and chronic inflammation.¹³⁸ A host of GI issues also occur including constipation and nausea, symptoms seen in patients with Parkinson’s disease.

B. Compositions of the Gut Microbiome in Healthy Individuals

The gut microbiome consists of a wide array of organisms including bacteria, viruses, and yeast.¹³⁹ There are 6 phyla that comprise the gut microbiome namely, Actinobacteria, Firmicutes and Bacteroidetes (together make up 90% of GI microbiome), proteobacteria, Fusobacteria and Verrucomicrobia (Figure 7). Different parts of the intestinal tract vary in microbial composition due to environmental changes i.e., pH and O₂. However, the taxonomy of the gut microbiome is crucial in gut function. For example, Actinobacteria i.e., the Bifidobacterium genus (although it is said to constitute a minute percentage of the gut microbiome) functions to regulate gut function via homeostasis.¹⁴⁰ Others such as Firmicutes and Bacteroidetes partake in metabolic processes including carbohydrate metabolism.¹⁴¹

Species such as *Helicobacter pylori* (*H. pylori*) acquired early in life and *Escherichia coli* (*E. coli*) are considered normal gut flora and it is changes to the

gut environment or acquired strains of these species via the environment that can contribute to pathogenesis and gut dysbiosis.



Figure 7: Taxonomic classification of bacterial organisms and examples of species comprising the gut microbiome. The box shows the dominant phyla found in the gut. (Copied from Rinninella et al., 2019.)¹³⁹

Similarly, production of short-chain fatty acids (SCFAs) such as butyrate, result from saccharolytic fermentation by anaerobic bacteria. SCFAs regulate gut health by improving glucose levels, gut permeability, and immunity.^{142,143}

While the exact mechanism of gut microbiome function is complex, much of the microbial activity is attributed to metabolism, energy production, pathogenic resistance, intestinal epithelium integrity and immunity in the periphery and the brain.¹⁴⁴ Disruptions of the beneficial balance in the microbial community can shift the normal function in the gut to symptoms associated with gut disease and more. Although the exact compositions of the gut microbiome vary from one person to another, gut microbiome/bacteriome dysbiosis results in the manifestation of disease.

C. Factors Affecting Gut Health

Considering the significant role gut homeostasis plays in regulating normal bodily functions, the mechanisms that disrupt the microbial environment are examined below:

(1) Nutritional studies by Berding et al., 2021 examined the effects of diet on gut microbiota to understand what role the gut microbiome plays in brain health.¹⁴⁵

Although there are no human clinical studies to date that directly assess implications of the gut microbiota on the brain, human and animal models have shown the negative impact certain dietary components have on health (i.e.,

western foods are more likely to cause inflammation, obesity and dysbiosis).^{145,146} Beneficial foods such as fermented foods and polyphenols contribute to the gut microbiota's regulation of metabolism, inflammation and intestinal barrier integrity impacting neural pathways in the brain.¹⁴⁷

(2) Gut microbiome disruption may be a natural process of aging with some studies identifying a change in microbial diversity that affects pathogenic resistance.¹⁴⁸

(3) Medications taken throughout life have consequences on gut health and the gut microbiome. The most consequential of those are antibiotics. While the clinical use of antibiotics is important in fighting bacterial infections, their use can also disrupt the microbial environment to increase antibiotic resistance.¹⁴⁹ Such changes alter the diversity of the microbiome and increase susceptibility to pathogenic bacteria.

(4) Literature findings emphasize that the gut microbiome is acquired at birth.^{150,151} This indicates that changes to this environment occur throughout life and can be caused by or complicated by genetics. However, unlike the other factors that can be manipulated (diet, medications etc.), the complexity of genetics makes it difficult to understand how it interacts with the host environment and/ or alters the microbial environment to cause disease.

D. Alterations to the Gut Microbiome

Microbial diversity plays a significant role in GI with different bacterial organisms strengthening gut function and immunity. Gut dysbiosis is attributed to the loss of this diversity and/or gain in pathogenic bacteria as discussed below.

1. Changes to Normal Gut Flora and Beneficial Gut Bacteria:

Normal gut microbiota (Figure 7) maintain and support the intestinal epithelial barrier and immunity. Thus, it is understandable how loss or alterations of such bacteria could be detrimental to the gut and overall health. Investigation into which beneficial bacteria are impacted showed a strain-specific loss of SCFA producing bacteria as findings in PD patients shows a reduction in fecal SCFAs.¹⁵² SCFAs regulate key metabolic processes in the gut, but more importantly SCFAs regulate histone-deacetylase (HDAC) inhibition.¹⁵³ A loss of functional SCFAs such as butyrate can affect gene expression via HDAC and disease progression in the gut. Although it is unclear how SCFA inhibition of HDAC affects specific disease outcomes, some studies have looked at how HDAC (HDAC6) is involved in α -synuclein aggregation and Parkinson's.¹⁵⁴

Additionally, reduction of *Prevotellaceae*, SCFA-producing bacteria, has been suspected to affect the activity of ghrelin, a hormone produced by the enteroendocrine cells (EEC) of the stomach, that initiates hunger.¹⁵⁵ Bayliss et al., 2013 proposed that ghrelin provides neuroprotection by improving mitochondrial function and lowering oxidative stress that is associated with neurodegeneration and Parkinson's disease.¹⁵⁶ Similarly, previous findings suggest that the ghrelin-dopamine (*GHSR1a: DRD2*) receptor interaction may

regulate dopamine signaling and mitophagy.¹⁵⁷ Therefore, reduction in SCFA-producing bacteria may impact gut health and can lead to neuronal damage and PD.

Other changes to beneficial bacteria such as *Akkermansia* and *Bifidobacterium* have also been reported to be present in PD. However, further studies are needed to understand their effects in the disease.^{158,159}

2. Increase in Pathobionts:

Pathobionts are resident gut bacteria that can be pathogenic due to changes in gut environment/genetic factors.¹⁶⁰ These pathobionts include *H. pylori*, *Enterococcus faecalis* and *Desulfovibrio*.^{161,162} Interestingly, some of these pathobionts co-occur with reduced fecal SCFA, denoting an association between loss/reduction of beneficial bacteria and an increase of pathobionts in gut dysbiosis.

Pathobionts can lead to a host of diseases including colon cancer and inflammatory bowel disease (IBD)¹⁶⁰. In addition to inflammation, *Desulfovibrio* (*DSV*) is positively associated with constipation, a prodromal symptom of Parkinson's.

E. α -synucleinopathy in the Gut: Clinical Marker of PD?

Analysis of the gut-microbiome in disease is important because of clinical models that have shown an interplay between the gut-microbiome and Parkinson's disease. Much of our understanding of Parkinson's suggest that it impacts gut homeostasis. Similarly, gut dysbiosis may cause PD pathology in

several ways including altering (1) gene expression that can cause α -synuclein misfolding, (2) an immune response leading to inflammation and neuroinflammation and (3) increased intestinal permeability that further drive pathology of disease. That is, there is so much unknown about how these disease processes influence one another.

There is evidence for the presence of α -synuclein in the gut early in disease and this may suggest that PD pathology begins in the GI tract. A study by Hilton et al., 2014 examined the gastric, duodenal, and colonic biopsies of patients with Parkinson's disease prior to the observation of clinical/motor symptoms.¹⁶³ In most of those patients, they found an accumulation of α -synuclein in the upper and lower regions of the GI tract and persistence of α -synuclein until the onset of motor symptoms. Similarly, a mouse model studied by Sampson et al., 2016 showed that transplanting a PD gut-microbiome in mice overexpressing α -synuclein resulted in increased microglial activation (resulting in progressive neurodegeneration) and α -synucleinopathy when compared to healthy controls.¹⁶⁴ This suggests that α -synuclein is not removed from the gut and accumulates, ultimately reaching the central nervous system (CNS).

These findings are important in establishing a clinical marker for PD. In fact, much of the complications of the disorder are caused by delayed onset that reduce the efficacy of treatment. Understanding how PD progresses, identifying where the disease begins and what risk factors increase its incidence are crucial for developing therapeutic targets. Additionally, gut dysbiosis can be managed

early with dietary interventions prior to the diagnosis of PD. Early intervention markers can mitigate the cumulative effect certain factors (i.e., age, gender) have on disease progression.

However, to understand the propagation of α -synuclein in pathology, it is important to understand the connection between the gut and the brain. α -synucleinopathy beginning in the gut follows a certain pathway to the brain to result in neuronal damage. Therefore, how the disease progresses to the CNS as well as how α -synuclein aggregation due to gut dysbiosis is associated with Parkinson's disease need to be identified.

Part III: Gut-Brain Axis and PD:

A. Braak's Hypothesis

The gut-first hypothesis of Parkinson's disease was derived by Heiko Braak, a German neuroanatomist. He hypothesized that Parkinson's pathology (1) originates in the peripheral nervous system (PNS) and the ENS, where (2) α -synucleinopathy reaches the brain and propagates in stages and (3) ultimately results in neuroinflammation and degeneration of dopaminergic neurons in the substantia nigra pars compacta (Figure 8).¹⁶⁵

The pathological implications begin by pathogenic entry into the body that affects the enteric nervous system and the olfactory bulb, resulting in a dual-hit.¹⁶⁶ Once the pathogen is internalized, it reaches the gut and interacts with gut microbiology, as mentioned in the previous section, to generate an inflammatory cascade via glial cells and α -synucleinopathy that travels via the vagus nerve and

crosses the BBB to affect CNS function.¹⁶⁷ Although α -synuclein aggregates are found in the olfactory neurons in early PD, Braak et al., 2007 do not believe olfactory pathology results in CNS neurodegeneration. Braak et al., 2007 claim that propagation from the gut is seen as the primary contributor to Lewy body pathology in the brain.

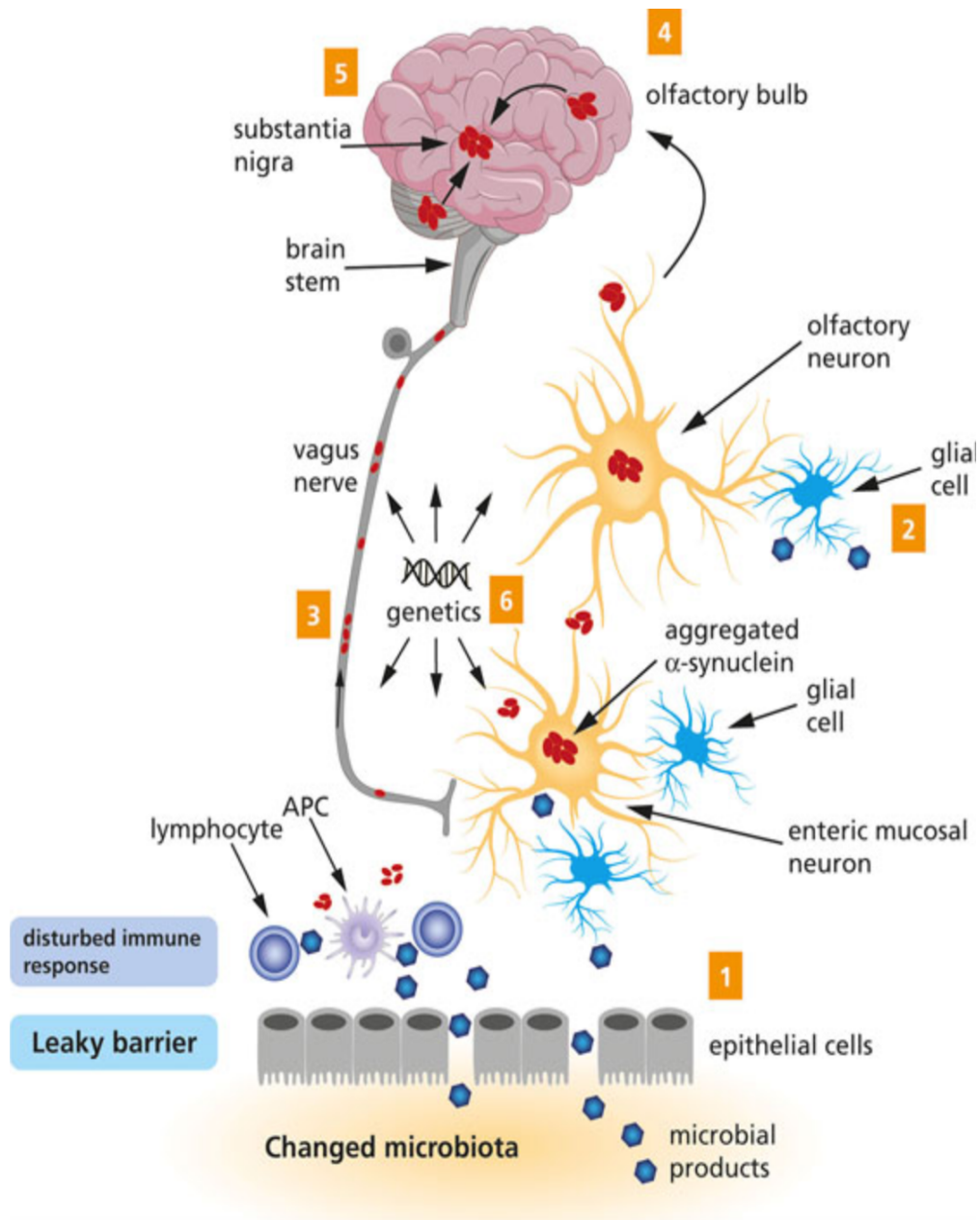


Figure 8: Braaks Gut-Brain Axis is depicted. The numbers show pathological progression of disease namely, (1, 2) increase intestinal permeability and immune response in the gut due to altered microbial diversity and α -synucleinopathy and propagation of α -synucleinopathy via (3) the vagus nerve and (4) the olfactory bulb resulting in (5) neurodegeneration in the substantia nigra and other areas of the brain. (Copied from Rietdijk et al., 2017.)¹⁶⁸

A lot is unclear in Braak's hypothesis and in Parkinson's pathology. For example, it is known that the gut-microbiome is implicated in PD; however, the exact pathogens that alter gut-microbiome function and culminate in disease are unknown. There is also evidence of α -synucleinopathy in the gut, olfactory neurons and in the substantia nigra; however, there are different LB pathologies in PD patients. All of this complicates the understanding of PD and the implication of the gut-brain axis in progression of pathology.

B. Braak's Staging and Gut-Brain Progression in Parkinson's Disease

Braak et al., 2003 proposed that once in the brain, pathology follows a specific route. He assessed the brains of 41 individuals with PD and 69 individuals without PD symptoms (referred to as incidental) against 58 healthy controls.^{165,169} Evidence was generated using immunoreactive staining for α -synuclein to follow pathological inclusions throughout the CNS in both study groups.

He proposed that Lewy neurites (LNs) spread to different regions of the brainstem, the midbrain and the neocortex while progressing into aggregates, neurodestructive Lewy bodies, that impact severity of disease at later stages.

Study findings indicate neuropathology in sporadic PD progresses in a caudo-rostral manner as follows (Figure 9).¹⁷⁰

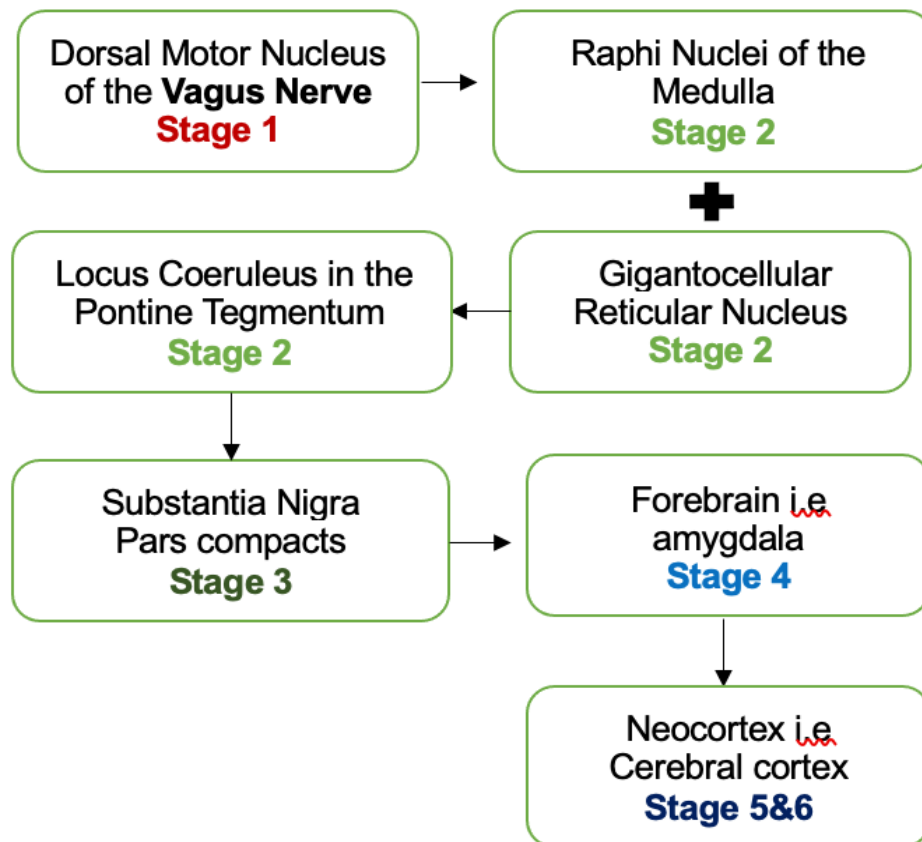


Figure 9: A framework for pathogenesis in sporadic PD. Stages Indicate introduction of Lewy neurites and Lewy Bodies to the CNS and severity of disease. (Copied from Visanji et al., 2013.)¹⁷⁰

C. Scientific Discourse; Brain-First or Gut-First?

Scientists have debated over the pathogenicity of Parkinson's disease for many years with much still unclear. Animal models and autopsy studies have attempted to find evidence to support one of two hypotheses namely, the brain-first hypothesis or the gut-first hypothesis of PD, with the latter gaining more attention in recent years. However, growing research investigating

neuropathology have found that LB pathology varies among patients. In fact, the Braak hypothesis does not apply to all PD patients and to some scientists is a controversial hypothesis.¹³⁰

Borghammer et al., 2021 suggests a third hypothesis; PD pathology can have either a PNS type (as determined by Braak) or a CNS type.¹⁷¹ The CNS type has been widely studied and assumes that damage to the SNpc happens prior to any dysautonomia. Borghammer et al., 2021 and Horsager et al., 2020 suggest that RBD might provide the best clinical marker for which PD subtype occurs in patients.^{171,172} That is, PD patients who are RBD-positive may have a PNS-first pathology and PD patients who are RBD-negative likely have a CNS-first pathology.

As a result, PD studies have moved away from limiting the disorder to neurological and consider it multi-systemic.¹⁶⁷ There is cross-talk between the ENS and the CNS and the bidirectional communication between the two has been studied in disease progression.^{173,174} CNS pathology can disrupt proper GI function and alterations to GI function can affect the brain. Regardless of the discourse over disease progression, it is very clear that GI dysfunction is implicated in PD, and it may be a marker for disease progression.

D. Clinical Studies on the Gut-Microbiome-Brain Axis (GBA)

Several preclinical and clinical studies have investigated the gut-brain axis in neurological disease. Here the findings from these studies will be discussed in detail.

- (1) Braniste et al., 2014 studied how gut-microbiota affect the blood brain barrier (BBB) and the CNS.¹⁷⁵ They reported that germ-free mice, lacking normal gut microbiome, had increased BBB permeability and lowered expression of tight junction proteins when compared against pathogen-free mice with a normal gut microbiome. They suggested that fecal transplantation from the pathogen-free microbiome into the germ-free mice or treating the germ-free mice with fecal SCFAs such as butyrate reduced BBB permeability. This indicates that alterations to the gut-microbiome and gut dysbiosis affect CNS integrity.
- (2) Kim et al., 2019 studied how alpha synucleinopathy that begins in the gut propagates to the brain in a mouse model.¹⁷⁶ They injected alpha synuclein fibrils, phosphorylated at serine 129, into the duodenum and pylorus of the mice. They found that α -synucleinopathy spread to the DMV and through the brain in a manner consistent with Braak's hypothesis and staging (Figure 9). Vagotomy hindered α -synucleinopathy of the gut from reaching the brain.
- (3) Lai et al., 2018 investigated the effects of neurotoxins on gut microbiology and Parkinson's disease.¹⁷⁷ They administered low doses of MPTP to mice intraperitoneally to observe if GI dysfunction and gut dysbiosis occur in the mice. They reported that changes in gut microbiota were found in these mice including changes in *Prevotellaceae* and other species. They also found that GI dysfunction and gut dysbiosis preceded motor symptoms of PD in the mice.
- (4) Aho et al., 2019 investigated gut-microbiota profiles in 64 PD patients and 64 healthy controls sampled over a 2 ½ year period.¹⁷⁸ Stool samples collected from

these subjects' showed variation in some bacterial taxa such as *Prevotella* and *Bifidobacterium*. Although a longer longitudinal study would be needed to make careful associations, it appears that loss of GI microbiome heterogeneity may progress disease.

Contradictory findings:

(5) Perez-Pardo et al., 2018 examined the bidirectionality of the gut-brain axis in PD. They created mouse models to assess the PNS-first vs CNS-first hypotheses of disease.¹⁷⁹ Using rotenone administered orally or injected directly into the striatum, they found that the neurotoxin induced PD-like motor deficits, neurodegeneration and GI dysfunction in a similar manner supporting the third hypothesis from Borghammer et al., 2021 and Horsager et al., 2020. Dietary modifications improved PD symptoms and GI dysfunction.

(6) Ulusoy et al., 2017 investigated the brain to gut axis of Parkinson's disease in rat models.¹⁸⁰ They reported that exogenous α -synuclein aggregates in the central nervous systems traveled to the periphery/the stomach via DMV and efferent vagal nerve fibers. As Braak et al., 2003 suggests that the DMV is responsible for the gut-brain pathology of Parkinson's, Ulusoy et al., 2017 argue that the DMV plays a key role in brain-gut transmission of α -synuclein pathology.

As the pathogenesis of PD continues to be debated, clinical and preclinical evidence in this review conclude that: (1) alterations to gut microbiology and gut dysbiosis in research can be reported before clinical symptoms of PD present and/or after (former may be evidence of the cause of

pathology and the latter progression of pathology) (2) direction of pathology may be towards the CNS (caudo-rostral), towards the PNS (rostro-caudal) or bidirectional (via the DMV). Scientific evidence for all these processes exists; however, *in vivo* protocols, age of PD-onset, sex etc. may affect results and findings.

E. The Gut-Microbiome; Therapeutics

Replenishing the gut microbiota via dietary modification and supplements has shown promising results in mitigating GI symptoms of PD. These include therapeutics such as probiotics.¹⁸¹

Several randomized control studies exploring the efficacy of probiotics in improving GI dysfunction and motility have shown that there are benefits in administering probiotics such as reduced constipation and alleviation of some motor symptoms of PD. The use of probiotics is believed to promote stability of the microbiota, protect against pathogen infiltration, and modulate inflammation that progresses symptoms.^{182,183} However, the exact benefits of probiotics on pathology are unknown.

Dietary modifications are recommended to benefit people with GI conditions such as IBS. Many studies have investigated the benefits of Mediterranean diet (high fiber, high plant) in decreasing PD risk in comparison to other diets such as the Western diet.^{184,185} Much of this may be due to increased microbial diversity. Plant-based diet improve *Prevotella* abundance in the microbiome and may confer important benefits to people with PD.¹⁸⁷ Functionally,

consuming the Mediterranean diet may be beneficial in increasing polyphenols and antioxidants that reduce oxidative stress and inflammation.¹⁸⁸ Other potential avenues have also been investigated including fecal microbiota transplant (FMT) to reintroduce normal gut flora into the gut microbiome of PD patients. This can help restore microbial diversity.

CONCLUSION:

In this literature review, it was concluded that the etiology of Parkinson's disease remains unknown. In sporadic PD, the complexity of genetics and environmental interactions make it difficult to identify the exact cause of disease. Genome-wide association studies (GWAS) have identified the alpha synuclein gene (SNCA) as a risk locus, yet a lot remains unclear about the specific genetic component of PD. There have been significant findings over the years about pathology, and there is scientific agreement that alpha-synuclein constitutes a major component of Lewy Neurites (LNs) and Lewy Bodies (LBs) that aggregate in dopaminergic neurons of the SNpc.

Aside from genetics, the study of PD has become a study of body-systems such as the CNS and the PNS. Research attempting to understand how dysfunction in the gut and the brain results have shown inconsistent findings. It is unknown whether PNS pathology occurs first, but GI dysfunction and gut microbiome dysbiosis are components of PD pathology. Dysautonomia impacting the GI results in constipation, the widely observed non-motor symptom.

As a result, many findings in PD have been supported by scientific evidence, (1) that neurodegeneration and synucleinopathy is progressive, (2) gut microbiota is disrupted and less homogeneity is linked to PD, (3) alpha-synuclein aggregates are found in the ENS and affect GI motility, and (4) the gut-brain axis or brain-gut axis may progress disease. Although gut dysbiosis is pathological, how exactly it is contributory to or is a cause of Parkinson's must be studied further.

Future Suggestions For PD Prognosis:

1. Find a way to separate GI dysfunction caused by Parkinson's from GI dysfunction that may cause PD and parkinsonism.
2. Monitor and regulate symptoms of RBD and constipation. These may serve as future indicators of Parkinson's onset.
3. Assess whether symptoms of GI dysfunction and gut dysbioisis are progressive in PD patients. Progression of dysbiosis may suggest a CNS first pathology.

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CURRICULUM VITAE

