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The Effect of Enteric Biochemical Modifications and Imbalances on the Progression of Parkinson's Disease

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Abstract

This paper analyzes various biochemical differences observed between patients afflicted with Parkinson's Disease and those without the diagnosis. Such disparities explored in this work include the accumulation of alpha-synuclein in the form of Lewy Bodies along with significant changes in the bacterial populations of Akkermansia, Streptococcaceae, Prevotella, and Lactococcus. Specifically, these distinct markers are examined in the context of the enteric system as a means of synthesizing connections between nuanced changes within the gut and the progression of PD. Essentially, this study supports an alternative attempt of better understanding the disease beyond a sole, isolated focus on the brain and central nervous system. Further confounding variables that have been linked to PD are also examined in a similar context; for instance for the variable age, it was indeed found that one of the potential reasons why age is a risk factor for PD can be tied back to the gut through an analysis into calprotectin. Ultimately, from a broad standpoint, the significance of this paper lies in the connections and pathways tying the aforementioned markers associated with PD to the gut, which are laid out in extensive detail. In turn, based on the findings of this paper, there is potential for driving future research relating to the origin and treatment of the disease, through possibly looking at systematic changes occurring within microbiomes as a promising starting point in addition to the extensive research on the brain already being carried out.

Introduction

Parkinson's Disease, commonly known as PD, is a neurologic disorder whose progression often results in the gradual deterioration of movement within the body, usually attributed to the depletion of substantia nigra pars compacta dopaminergic neurons. [1] The extent to which certain motor movements are affected ranges from tremors in the hands to, later on, overall stiffness and difficulty walking. At large, there remain a lot of unknowns that surround this condition given that researchers have not yet been able to definitively conclude (or identify) why this condition appears in certain people over others, which is necessary in order to develop a reliable cure. Another complication of PD is that the biochemical progression and symptoms differ across individuals, making it much more difficult to make larger claims about the disease. Given that it is a neurological issue, much of the research on PD has been centered around specific chemical pathways in the brain which help to understand the origins of the condition and its progression. Research focusing on the brain thus far has been fruitful in attributing distinct characteristics to PD, such as the discovery of Lewy Bodies: large groups of proteins in the brains of patients with PD found during postmortems. This insight then led researchers to focus on how this accumulation and misfolding of protein took place. Undoubtedly, neuroscientific research has proven to be vital in better understanding PD, however, there are also certain limitations that can arise when solely looking towards this isolated region of the body to understand the disease.

Indeed, in recent years there has been a greater degree of research invested in understanding Parkinson's from a systemic standpoint and studying how potentially other

[1] Travagli, R.A., Browning, K.N. & Camilleri, "M. Parkinson disease and the gut: new insights into pathogenesis and clinical relevance," *Nat Rev Gastroenterol Hepatol* 17, 673–685 (2020). https://doi.org/10.1038/s41575-020-0339-z

regions of the body can affect its progression. From a broad standpoint, it is not surprising that the gut, for instance, would be one such candidate of focus given that certain gastrointestinal symptoms such as dysphasia, constipation, and delayed gastric emptying often precede the onset of PD in many afflicted patients[2]. As such, this paper concerns itself with synthesizing and drawing new lines amongst the current research surrounding PD to ultimately better understand how the gut can inform us about the disease's progression.

The paper is broken into 2 main sections: the first focuses on understanding the biochemical connections between the gut and PD, and the second section is dedicated to tracing the origins of confounding variables related to PD to the gut as well. Ultimately, this research is important in the sense that focusing on meritable connections between the enteric system and Parkinson's may lead to a whole new and fruitful bank of information which can then be factored into the research that is focused on potential treatments and causes of the disease in the first place.

Alpha-Synuclein Aggregation

As aforementioned, in those with PD, Lewy Body accumulation is a common pathological occurrence, of which alpha-synuclein is thought to be a major component of. Alpha-synuclein is an acidic protein encoded by the SNCA gene and can be found in various regions of the body including the brain, heart, muscles, and the gut. Currently, there is substantial research underway attempting to better understand the function of alpha synuclein. Thus far, it is known that the protein affects synaptic vesicle functioning, as it interacts with dopaminergic neurons and influences the release of neurotransmitters such as dopamine, which, as will be explored later in the paper more extensively, has a connection to motor function. In terms of the

[2] Travagli, "M. Parkinson disease and the gut: new insights into pathogenesis and clinical relevance."

correlation between its function and structure, alpha-synuclein typically takes upon a helical conformation, and is harmless as a monomer in its unfolded state in stable environmental conditions. However, the protein may aggregate as a beta-sheet once it begins to fold irregularly if it, for instance, interacts with membranes or if the mitochondria functions irregularly.[3] Specifically, the abnormal folding proceeds with a toxic intermediate oligomer conformation and goes on to create a highly insoluble condensed form, which aggregates into fibrils as a Lewy body, as shown below.[4]

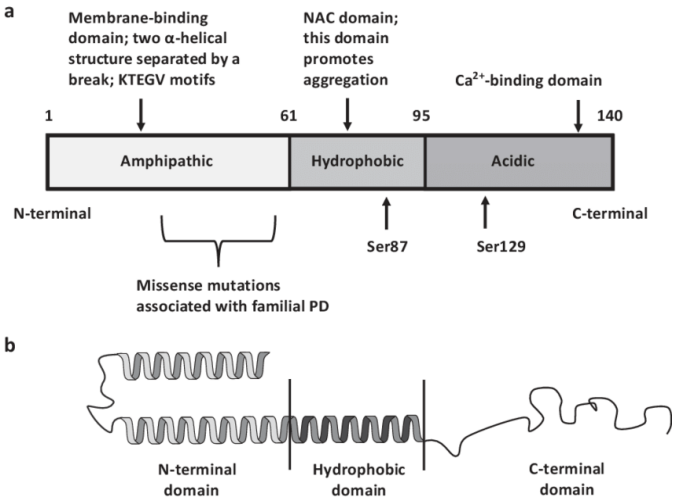

Figure 1: Alpha-synuclein structure and sites of potential aggregation stimulation[5]

Abnormal accumulations of these alpha-synuclein clumps contributing to Lewy Body deposits promote the degradation of dopamine-producing neurons, which suggests a direct link to PD, in which motor impairment is a common development.[6] Lewy Body formation entails a complex process involving the fibrillation and aggregation of alpha-synuclein as well as

[3] Bendor, Jacob T et al. "The function of α-synuclein." *Neuron* vol. 79,6 (2013): 1044-66. doi:10.1016/j.neuron.2013.09.004
[4] Delenclos, Marion, et al. "Cellular Models of Alpha‐Synuclein Toxicity and Aggregation." *Wiley Online Library*, John Wiley & Sons, Ltd, 30 July 2019, onlinelibrary.wiley.com/doi/10.1111/jnc.14806.
[5] Török, Nóra & Majlath, Zsofia & Szalárdy, Levente & Vecsei, László. (2016). Investigational ?-Synuclein Aggregation Inhibitors: Hope For Parkinson's Disease. Expert Opinion on Investigational Drugs. 25. 10.1080/13543784.2016.1237501.
[6] Mor, Danielle E, and Harry Ischiropoulos. "The Convergence of Dopamine and α-Synuclein: Implications for Parkinson's Disease." *Journal of experimental neuroscience* vol. 12 1179069518761360. 8 Mar. 2018, doi:10.1177/1179069518761360

posttranslational modifications that collectively influence how alpha-synuclein clumps interact with and harm membranes of critical organelles like the mitochondria. The alpha-synuclein fibrils, which can be considered to be precursors to the Lewy Bodies, are not as harmful as the Lewy Bodies themselves, which are said to have a negative impact on mitochondrial functioning as well as synaptic signaling involving dopaminergic neurons, which is a significant step in the advancement of neurodegenerative diseases such as PD.[7]

Given that alpha synuclein aggregates are a dangerous, yet common sight in individuals with PD, the biochemistry behind what causes aggregation will now be explored through its interaction with membranes. The protein itself is composed of an alpha-helical N-terminal, a hydrophobic NAC domain, and a highly acidic C-terminal tail. When point mutations occur in the SNCA gene specifically in the N-terminal of the protein, which commonly stem from autosomal dominant inheritance patterns, the protein is unable to bind to membranes, resulting in the harmful aggregation of alpha-synuclein.[8] Additionally, the hydrophobic NAC domain of the protein plays an important role in membrane interactions. In the absence of mutations, when regular alpha-synuclein is embedded in the membrane, the NAC region can either face the exterior of the cell or the cytoplasm. When exposed to the cytoplasm, NAC attracts more alpha-synuclein monomers, creating aggregates which are then cleaved from the membrane. Thus, promoting certain pathways and mutations to allow NAC to face the cytoplasm may promote the further propagation of alpha-synuclein, which may deter proper motor functioning and create an environment that makes the body more susceptible to developing PD.[9] The acidic

[7] Mahul-Mellier, A., Burtscher, J., Maharjan, N., Weerens, L., Croisier, M., Kuttler, F., . . . Lashuel, H. (2020, March 03). The process of Lewy body formation, rather than simply α-synuclein Fibrillization, is one of the major drivers of neurodegeneration. Retrieved April 22, 2021, from https://www.pnas.org/content/117/9/4971

[8] Stefanis, Leonidas. "α-Synuclein in Parkinson's disease." *Cold Spring Harbor perspectives in medicine* vol. 2,2 (2012): a009399. doi:10.1101/cshperspect.a009399

[9] Hijaz, B.A., Volpicelli-Daley, L.A. Initiation and propagation of α-synuclein aggregation in the nervous system. *Mol Neurodegeneration* 15, 19 (2020). https://doi.org/10.1186/s13024-020-00368-6

C-terminal may also promote alpha-synuclein clustering depending on certain environmental conditions. The negatively charged C-terminal is not very hydrophobic and exists as a stray coil under normal circumstances. However, certain in vitro studies have shown that decreasing the pH of the protein's surroundings may neutralize the negatively charged end and create opportunities for misfolding to occur. In this case, interactions between the C-terminal and hydrophobic NAC domain can counteract progressive accumulation and help resolve the issue.[10]

Knowing the effects of the accumulation of alpha synuclein, beyond just the general protein misfolding caused by mutations and irregular interactions with membranes, a connection to the enteric system and gut can also be observed as early signs of PD.[11] Within the gut, alpha-synuclein aggregation may be stimulated by certain viral and bacterial GI infections, as intestinal nerves release high amounts of the protein. The aggregates may also be released from nerve cells by attaching to vesicles holding neurotransmitters in order to be transported to the exterior of the cell, where it can induce an immune response. The accumulation of alpha-synuclein as a response to gastrointestinal infections may cause inflammation, which is also a common instigator of PD.[12]

Purposefully altering the gut microbiota also appears to negatively impact motor functioning through an accumulation of alpha synuclein: an early sign of the potential development of PD. A study on intestinal pathology conducted in 2018 involved a weekly injection of MPTP into the membrane of the abdomen of adult male mice in order to observe changes in gastrointestinal functioning, motor functioning, and alpha-synuclein accumulation.

[10] Emamzadeh, Fatemeh Nouri. "Alpha-synuclein structure, functions, and interactions." *Journal of research in medical sciences : the official journal of Isfahan University of Medical Sciences* vol. 21 29. 9 May. 2016, doi:10.4103/1735-1995.181989
[11] Chandra, Rashmi et al. "α-Synuclein in gut endocrine cells and its implications for Parkinson's disease." *JCI insight* vol. 2,12 e92295. 15 Jun. 2017, doi:10.1172/jci.insight.92295
[12] Barbut, Denise et al. "Targeting Alpha-Synuclein in the Gut May Slow Down Parkinson's Disease." *Journal of Parkinson's Disease*, vol. 9, no. 2, 2019, https://www.journalofparkinsonsdisease.com/targeting-alpha-synuclein-gut-may-slow-down-parkinsons-disease.

MPTP is a drug that is commonly used to study PD and can be metabolized to form the neurotoxin MPP+, which can impair dopaminergic neurons. Throughout the study, GI function was determined by measuring stool frequency and its water content, while motor function was determined by pole tests, which observe the ability of a mouse to reach its home by moving down a pole. The results demonstrated that MPTP led to abnormal GI functioning before leading to problems in motor functioning, which suggests that changes in the gut microbiome can impact the brain and, thus, contribute to the development of PD, a neurodegenerative disease.[13]

The MPTP drug can inhibit Complex I in the inner mitochondrial membrane during cellular respiration. This can affect ATP production, which is the source of energy required to maintain electrochemical gradients across cell membranes in order to release signals and neurotransmitters. Problems in these pathways may ultimately deplete motor functioning due to the destruction of dopaminergic neurons. However, an immediate response to the presence of MPTP in the gut is the oxidation and nitration of alpha-synuclein, harming gastrointestinal functioning, which may increase the prospects of alpha-synuclein aggregation in the brain.[14]

Signaling from certain cells in the gut may also instigate alpha-synuclein propagation. Enteroendocrine cells (EECs) found in the gut are responsible for releasing particular hormones in response to stimulants and, thus, act similar to neurons in the brain and are said to attach to enteric nerves. Specific EECs hold alpha-synuclein and may create a response by connecting to nerves that also contain the protein. This communication bridges the gut and nervous system, through which alpha-synuclein may aggregate due to environmental or toxic stimulators, such as

[13] Lai F, Jiang R, Xie W, Liu X, Tang Y, Xiao H, Gao J, Jia Y, Bai Q. Intestinal Pathology and Gut Microbiota Alterations in a Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) Mouse Model of Parkinson's Disease. Neurochem Res. 2018 Oct;43(10):1986-1999. doi: 10.1007/s11064-018-2620-x. Epub 2018 Aug 31. PMID: 30171422.
[14] Hijaz, B.A., Volpicelli-Daley, L.A. Initiation and propagation of α-synuclein aggregation in the nervous system. *Mol Neurodegeneration* 15, 19 (2020). https://doi.org/10.1186/s13024-020-00368-6

MPP+. Alpha-synuclein clumps can then travel to the brain, negatively impacting dopamine release and motor function, which is a common indicator of PD.[15]

The vagus nerve is a possible means through which this transport of alpha-synuclein can occur from the enteric nervous system to the lower brainstem, specifically to "the dorsal motor nucleus of the vagus (dmX)". This brain-gut communication demonstrates how the gut microbiome can be a source of alpha-synuclein, as it accumulates to reach the brain, playing a significant role in causing PD. The accumulation of alpha-synuclein Lewy bodies has been said to affect the proper functioning of the dmX and other critical cells in the brain such as the substantia nigra, which contains dopamine.[16] A recent study found a correlation between vagus nerve transport and alpha-synuclein accumulation in the brain. Researchers began by injecting a sample of pre-formed protein fibrils into the gut, specifically into the pylorus (bridge between the stomach and small intestine) and the duodenum (beginning of small intestine), of mice who had PD. The pre-formed alpha-synuclein fibrils were stained to allow researchers to observe its transport from the gut to the brain over a three month period. After this time interval, the stain was observed in parts of the brain, such as the medulla (located at the base of the brain). By previously knowing that regular alpha-synuclein exposure to pre-formed protein fibrils can promote misfolding, the researchers deduced that alpha-synuclein aggregates sourced from the gut accumulate up the vagus nerve. Thus, the ultimate depletion of dopamine molecules in the brain may lead to impaired motor functioning in PD patients. It is important to note, however, that the importance of the vagus nerve in the transportation of alpha-synuclein is dictated by age,

[15] Chandra, Rashmi et al. "α-Synuclein in gut endocrine cells and its implications for Parkinson's disease." *JCI insight* vol. 2,12 e92295. 15 Jun. 2017, doi:10.1172/jci.insight.92295

[16] Walter, Uwe et al. "Atrophy of the Vagus Nerve in Parkinson's Disease Revealed by High-Resolution Ultrasonography." *Frontiers in neurology* vol. 9 805. 27 Sep. 2018, doi:10.3389/fneur.2018.00805

additional health conditions, possible shifts in gut microbiota due to bacterial behavior, and other factors that may determine the likelihood of developing PD through this gut-brain connection.[17]

Bacterial Imbalances: Akkermansia and Streptococcaceae increases in the presence of PD

Beyond analysis of alpha synuclein, in an attempt to explore the central question from a different angle now, it is hypothesized that changes in the bacterial composition in the gut microbiome may provide insight into the development of PD. Accompanying abnormal bacterial concentrations are significant systemic issues at large, one being exacerbated inflammation, a common symptom of PD, which can eventually lead to chronic constipation, fatigue, and weakened immune system, potentially contributing to disease development. This idea will be explored by looking at bacterial families that increase in the presence of PD, and how those may increase susceptibility to symptoms of PD, suggesting a new link between the gut and the disease progression.

A recent study investigated various bacterial population sizes across healthy and afflicted patients and concluded that Akkermansia is one of the major bacterial families seen to be more prevalent in patients with PD. Typically, Akkermansiaceae is a common mucin degrader and improves gut barrier function and anti‑inflammatory immune stimulation.[18] Essentially, the bacteria breaks down mucus and converts it into short chain fatty acids (SCFAs) and acetate, which can be energy sources of other bacteria that strengthen the immunity of the gut. In addition to production of SCFAs and acetate, Akkermansia also promotes the cell within the epithelial cell linings to produce more mucus, having an overall beneficial effect to the host in terms of

[17] Kim, Sangjune et al. “Transneuronal Propagation of Pathologic α-Synuclein from the Gut to the Brain Models Parkinson's Disease.” *Neuron* vol. 103,4 (2019): 627-641.e7. doi:10.1016/j.neuron.2019.05.035

[18] Bedarf, J R et al. “Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naïve Parkinson's disease patients.” *Genome medicine* vol. 9,1 39. 28 Apr. 2017, doi:10.1186/s13073-017-0428-y; Heintz-Buschart, Anna et al. “The nasal and gut microbiome in Parkinson's disease and idiopathic rapid eye movement sleep behavior disorder.” *Movement disorders : official journal of the Movement Disorder Society* vol. 33,1 (2018): 88-98. doi:10.1002/mds.27105

superficial protection and the bacteria as well as it eventually degrades some of the mucin it signals other cells to produce.[19] However, as the study suggests, higher concentrations of Akkermansia is observed in PD patients compared to healthy controls. Figure 2 below supports these findings with a higher number of Akkermansia in PD, represented by the red upward

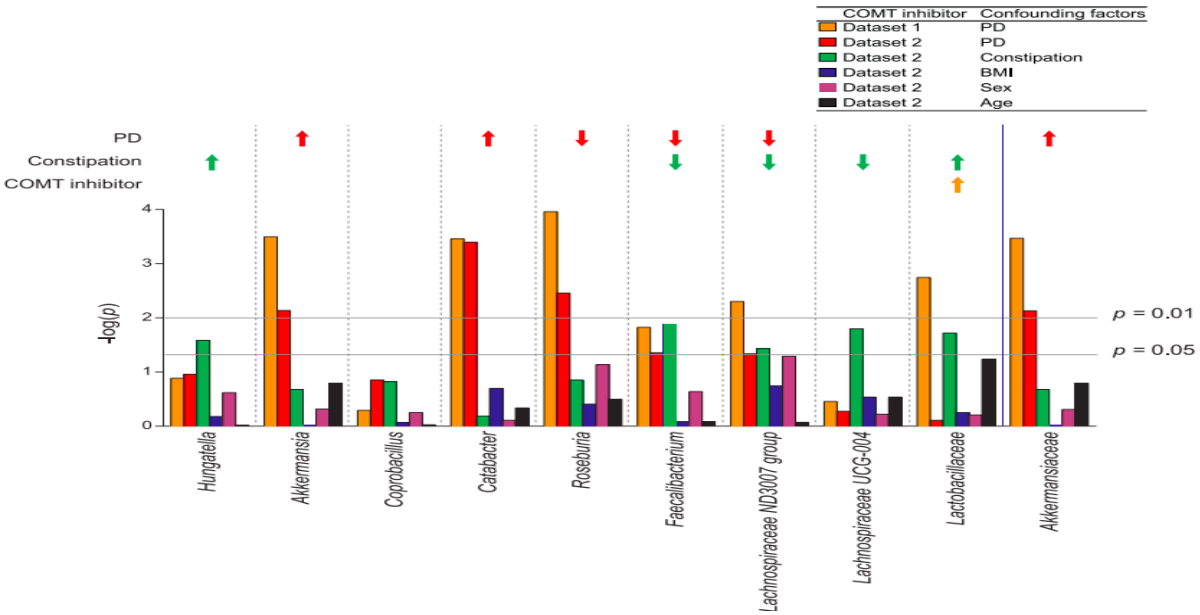

Figure 2: Result of bacteria composition concentration differences in PD patients[20]

arrow.[21] (Hiroshi et al., 2020). This leads to the question as to how this difference in Akkermansia levels may contribute to one's susceptibility to PD symptoms.

Essentially, the increase in this bacterial family in PD patients can be a potential contributor to inflammation, as it can lead to excessive mucus degradation (as more mucin is degraded than potentially generated) and allow for greater exposure to pathogens such as S.

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[19] Liu, Li et al. "*Akkermansia muciniphila* protects intestinal mucosa from damage caused by *S. pullorum* by initiating proliferation of intestinal epithelium." *Vet Res* 51, 34 (2020). https://doi.org/10.1186/s13567-020-00755-3

[20] Hiroshi, Nishiwaki et al. "Meta‐Analysis of Gut Dysbiosis in Parkinson's Disease" (2020). Movement Disorders. 35. 10.1002/mds.28119.

[21] Hiroshi, Nishiwaki et al. "Meta‐Analysis of Gut Dysbiosis in Parkinson's Disease" (2020). Movement Disorders. 35. 10.1002/mds.28119.

Typhimurium. (S.Typhimurium is a pathogen that induces inflammatory responses) In fact, a study on mice concluded that when there was a high concentration of Akkermansiaceae in mice, it exacerbated the S.Typhimurium-induced intestinal inflammation.[22] With more Akkermansiaceae, there was excessive mucin degradation, which the study hypothesized would lead to inflammatory bowel diseases, suggesting that this bacterial family may have an essential role in causing inflammatory responses in PD patients. Indeed, another study published in *Nutrients* discusses how increased concentrations of Akkermansiaceae may lead to "increased exposure of microbial antigens to immune cells and thus could have inflammatory potential".[23] All of the findings suggest overall that Akkermansiaceae plays a large role in PD, given that PD patients are more susceptible to gastric inflammation and weaker immune systems, that are often a result of the side effects seen due to the biochemical interactions and consequences of increased amounts of Akkermansiaceae in the gut, which further accredits the role of the gut at hand here.

In addition to susceptibility of inflammation, the increase in Akkermansiaceae may also be a factor to the aggregation of alpha synuclein in the intestine, which is said to be the cause of PD. A study confirms that an abundance in Akkermansiaceae may also lead to aggregation of alpha synuclein fibrils in the intestine. Intestinal environments low in dietary fibers may lead to the dysfunctioning of Akkermansiaceae, causing excessive mucin degeneration. In addition, the study adds that an increase in intestinal permeability is seen in PD patients.[24] With these two factors, an increase in Akkermansia and intestinal permeability, the barrier of the gut linings are

[22] Ganesh, Bhanu Priya et al. "Commensal Akkermansia muciniphila exacerbates gut inflammation in Salmonella Typhimurium-infected gnotobiotic mice." *PloS one* vol. 8,9 e74963. 10 Sep. 2013, doi:10.1371/journal.pone.0074963
[23] Gerhardt, Sara, and M Hasan Mohajeri. "Changes of Colonic Bacterial Composition in Parkinson's Disease and Other Neurodegenerative Diseases." *Nutrients* vol. 10,6 708. 1 June. 2018, doi:10.3390/nu10060708
[24] Hiroshi, Nishiwaki et al. "Meta‐Analysis of Gut Dysbiosis in Parkinson's Disease" (2020). Movement Disorders. 35. 10.1002/mds.28119.

exposed to oxidative stress, which is still in uncertainty as many researches are currently being done and may have a potential effect of leading to aggregation of alpha synuclein in the intestine. This leads to a probable explanation of how alpha synuclein may be found in the intestine, a large factor of the contribution to the development of PD.

Similar to Akkermansiaceae, Streptococcaceae is one of the bacterial families that is found to be commonly increased in patients with PD. Streptococcaceae is a gram-positive bacteria that has many functions depending on class; specifically, Streptococcaceae Clostridium tetani is a bacteria that produces neurotoxins, which leads to neurological disorders and heightened levels of inflammation.[25] A study discussed the difference in structure and composition of the gut microbiota in PD patients, which demonstrated changes in lipids, amino acids, and other organic compounds, along with higher relative abundance of bacteria, where Streptococcaceae is among the many other bacteria. Figure 3 below shows the results of the statistical difference of Streptococcaceae in PD patients compared to healthy controls, with 1.737% of Streptococcaceae in PD patients and 0.155% in healthy controls.[26]

Phylum	Family	Genus	↓/↑[b]	MD[c]	PD (%)[d]	HC (%)[d]	*P* value	FDR-adjusted *P* value[e]
	Streptococcaceae		↑	0.526	1.737	0.155	0.000	0.000

Figure 3 : Significant differences in bacteria composition between PD patients and healthy controls[27]

[25] Li, Wei et al. "Structural changes of gut microbiota in Parkinson's disease and its correlation with clinical features." Sci China Life Sci 60. (2017). doi: 10.1007/s11427-016-9001-4
[26] Vascellari, Sarah et al. "Gut microbiota and metabolome alterations associated with Parkinson's disease". (2020). mSystems 5:e00561-20. https://doi.org/10.1128/ mSystems.00561-20.
[27] Vascellari, Sarah et al. "Gut microbiota and metabolome alterations associated with Parkinson's disease". (2020). mSystems 5:e00561-20. https://doi.org/10.1128/ mSystems.00561-20.

Additionally, cadaverine, a polyamine responsible for inflammation, is found to be positively associated with Streptococcaceae, which produces cadaverine biosynthetic enzymes.[28] Thus, the increase in Streptococcaceae leads to an increased synthesis of enzymes that produce cadaverine, which leads to a higher production of cadaverine, as shown in PD patients. This leads to the question as to how does cadaverine participate in the inflammation of the gastrointestinal tract in PD?

Polyamines are di, tri, and tetra amines that are naturally found in our body. Cadaverine, one of the polyamines, regulates "neuronal cell biochemical activity in the brain, including interaction with neurotransmitter receptors such as the N methyl-D-aspartate receptor, regulation of substances in degenerating cells...and protection of neuronal cells from oxidative damage".[29] Cadaverine is oxidized by the enzyme spermine oxidase, whose concentration is up-regulated in PD patients, along with the concentration of cadaverine, creating many toxic metabolites such as ammonia and hydrogen peroxide that resulted from this oxidation process. [30] The increased levels of these toxic metabolites leads to an increased pro-inflammatory environment and motility dysfunctions of the gastrointestinal tract, a common symptom of PD.[31] The pro-inflammatory environment and motility dysfunction can lead to abdominal pain and impairment in immune functions, which yet again are observations that can be related back to PD. However, not only can observations about the increased bacterial levels be made, but certain

[28] Vascellari, Sarah et al. "Gut microbiota and metabolome alterations associated with Parkinson's disease". (2020). mSystems 5:e00561-20. https://doi.org/10.1128/ mSystems.00561-20.
[29] Paik, Man-Jeong, et al. "Polyamine patterns in the cerebrospinal fluid of patients with Parkinson's disease and multiple system atrophy". Clin Chim Acta. 2010 Oct 9;411(19-20):1532-5. doi: 10.1016/j.cca.2010.05.034. Epub 2010 Jun 1. PMID: 20515677.
[30] Makletsova, Marina G. et al. "Polyamines in Parkinson's Disease: Their Role in Oxidative Stress Induction and Protein Aggregation". Journal of Neurology Research, North America, 9, mar. 2019. Available at: <https://www.neurores.org/index.php/neurores/article/view/509/480>.
[31] Vascellari, Sarah et al. "Clinical Phenotypes of Parkinson's Disease Associate with Distinct Gut Microbiota and Metabolome Enterotypes". Biomolecules 2021, 11, 144. https://doi.org/10.3390/ biom11020144

bacteria that are seen to be in lower amounts in afflicted patients provide revealing insight as well in better understanding the connection between the gut and the disease itself.

Bacterial Imbalances: Prevotella and Lactococcus decrease in the presence of PD

Prevotella is a bacteria primarily found in the gut, as well as in other parts of the body such as the oral and vaginal microbiota. This bacteria has exhibited increased gut inflammation properties when found in decreased amounts. In normal, healthy patients, Prevotella has been found to reduce inflammation in the gut.[32] This can be especially noted in non-Western diets with high consumption of fiber as they tend to have increased amounts of Prevotella in their gut microbiota due to it being commonly found in fibrous plants.[33] In patients with PD, especially those with low fiber diets, it has been observed that low fiber intake can cause less diversity of the microbiome, leading to less Prevotella in the gut.

According to the figure below, Prevotella was found higher in the controls (p (baseline) = 0.052, p (follow-up) = 0.011) and lower in the patients with Parkinson's Disease (PD).[34] Baseline indicates the first measurement evaluation of Prevotella amounts and the follow-up indicates the subsequent time they were measured for Prevotella amounts within this context. As seen in Figure 4, the legend depicts the family Prevotellaceae, which is composed of four genera including Prevotella, in light purple. The baseline for the control group clearly depicts a greater amount of Prevotellaceae when compared to the baseline for the PD group. As for the follow-up, Prevotella in PD is seen to be slightly increased whereas the Prevotellaceae in the control group is slightly decreased. However, despite this, Prevotellaceae in the baseline and

[32]Bedarf et al. " Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naïve Parkinson's disease patients."
[33]Bedarf et al. " Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naïve Parkinson's disease patients."
[34] Aho, Velma T.E., et al. "Gut Microbiota in Parkinson's Disease: Temporal Stability and Relations to Disease Progression." *EBioMedicine*, Elsevier, 18 June 2019,www.sciencedirect.com/science/article/pii/S235239641930372X.

follow-up in PD never surpasses the baseline and follow-up in the control group; ultimately the graph below clearly depicts supports the conclusion that lower amounts of Prevotella are associated with patients afflicted with PD and higher amounts in non-PD patients/control groups.

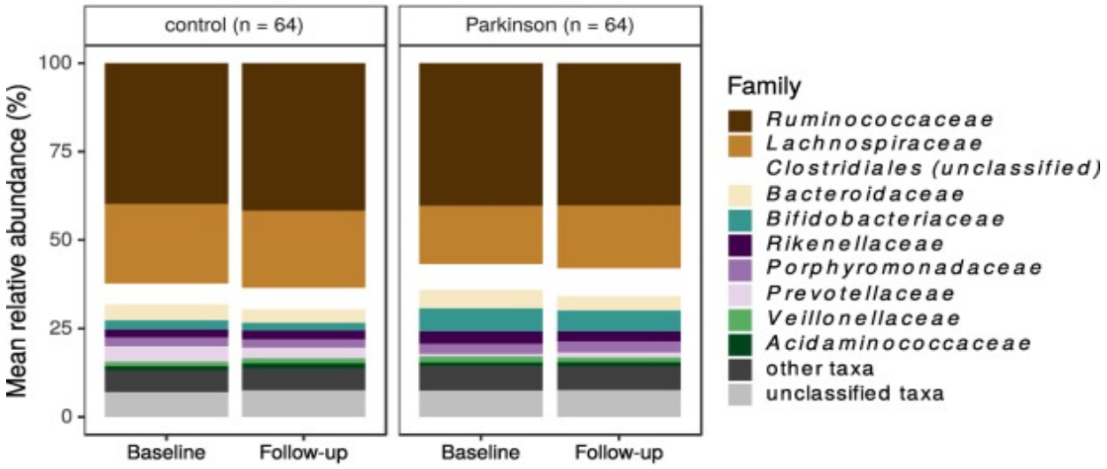

Figure 4: Baseline and follow-up comparisons between the control group and PD group[35]

In terms of the bridging connection now between this bacterial family and Parkinson's, inflammation will be the key link examined. Indeed, specific shifts in microbial species within a PD gut can obstruct the progression and production of short chain fatty acids, which is associated with gut inflammation.[36] It is hypothesized that these disruptions in the gut microbiome system acts as a biological consequence of Parkinson's Disease, although it may not necessarily directly cause the development of this disease.

Short chain fatty acids (SCFA's) are produced by the "good" gut bacteria fermenting fibers in the colon and may be linked to the reduced risk of inflammatory diseases. Additionally, SCFA's control inflammation to a certain degree by regulating immune cell cytokine production, which when produced in excessive amounts, can lead to systemic inflammation and pathological

[35] Aho, Velma T.E., et al. "Gut Microbiota in Parkinson's Disease: Temporal Stability and Relations to Disease Progression." *EBioMedicine*, Elsevier, 18 June 2019,www.sciencedirect.com/science/article/pii/S235239641930372X.

[36] Bedarf et al. " Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naïve Parkinson's disease patients."

diseases.[37] Prevotella is one of the gut bacteria that produces SCFA's, therefore, a decrease of Prevotella can lead to a disruption of the formation of gut bacteria.[38] In normal patients with a healthy gut microbiome system, Prevotella is found in abundance, meaning that they produce sufficient amounts of SCFA's to reap the bodily rewards of it, including it's anti-inflammatory properties. However, in PD patients, Prevotella is found in greatly decreased amounts, therefore, they would not be able to produce SCFA and will be prone to gut inflammation.

A decrease in Prevotella and, therefore, a decrease in SCFA's does not cause Parkinson's Disease, but, instead, it is merely a consequence that patients afflicted with this condition suffer from. In an attempt to test the anti-inflammatory properties provided by SCFA's as well as a potential therapeutic effect in the brain, researchers aim to put PD patients on a high fiber diet to boost production of SCFA by Prevotella.[39] Further investigation is required to determine the exact role of microbially-derived fatty acids within neuroinflammation in Parkinson's Disease.

Similar to Prevotella, the Lactococcus bacteria, a genus of lactic acid bacteria, was also observed in decreased levels in PD patients vs. non-PD control groups. To better understand the biochemistry behind this decrease in Lactococcus, researchers measured the lytic potential of the degree to which bacteriophages infect, and therefore, decrease the amount of the Lactococcus host bacteria. In Figure 5 below, representing data from a recent experiment, a value of 1 (log10°) indicates that the phage's genetic material was incorporated into the bacterial DNA and diminished the amount of regular Lactococcus. On the other hand, a ratio less than 1 demonstrates that the phage's DNA was not normally integrated into the host genome of

[37] Li, Meng, et al. "Pro- and Anti-Inflammatory Effects of Short Chain Fatty Acids on Immune and Endothelial Cells." *European Journal of Pharmacology*, Elsevier, 9 May 2018, www.sciencedirect.com/science/article/pii/S0014299918302607#:~:text=SCFAs%20might%20play%20an%20essential,are%20likely%20mediated%20by%20a.

[38] Bedarf et al. " Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naïve Parkinson's disease patients."

[39] Bedarf et al. " Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naïve Parkinson's disease patients."

Lactococcus, suggesting a higher amount of host genome, and a ratio of more than 1 means only a partial integration.[40]

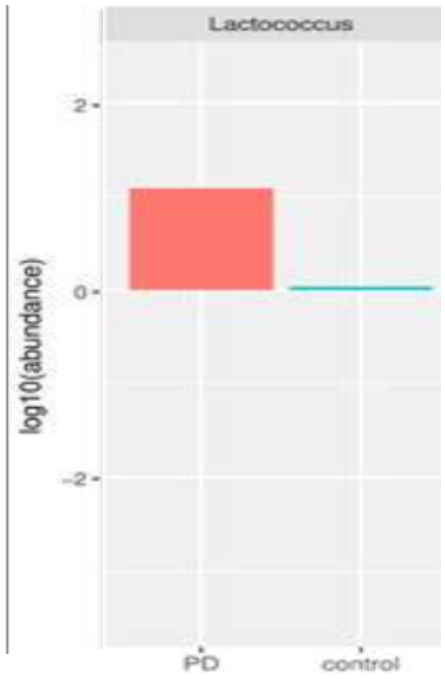

Figure 5: The phage/Lactococcus bacteria ratio in PD patients and control group members[41]

This data in the graph above suggests the bacteriophage's genetic material was partially included during the lytic phase, which is the cycle in which the bacteriophage infects and replicates the host Lactococcus. As such, as seen in the graph above, the Lactococcus spp. was seen in decreased amounts in PD patients when compared to the control group.[42]

[40] Tetz, George, et al. "Parkinson's Disease and Bacteriophages as Its Overlooked Contributors." *Scientific Reports*, Nature Publishing Group UK, 17 July 2018, www.ncbi.nlm.nih.gov/pmc/articles/PMC6050259/.
[41] Tetz, "Parkinson's Disease and Bacteriophages as Its Overlooked Contributors."
[42]Tetz, "Parkinson's Disease and Bacteriophages as Its Overlooked Contributors."

In the previous experiment, it was established that bacteriophages at large are responsible for this decrease in bacteria, however, another study goes to specify what subtype of phage is playing a more direct role. Researchers focused on two groups of phages to observe in PD patients and health individuals: lytic phages vs temperate phages. Lytic phages are the bacteriophages that only replicate through the lytic cycle, while temperate phages undergo replication through the lytic and lysogenic cycles. During the lysogenic cycle, the phage has the chance to integrate its DNA into the bacterial genome. It was found that although the total amount of lytic and temperate phages combined were nearly identical in PD and normal patients, there was almost double the amount of lytic phages in patients with PD, as seen in the graph below.

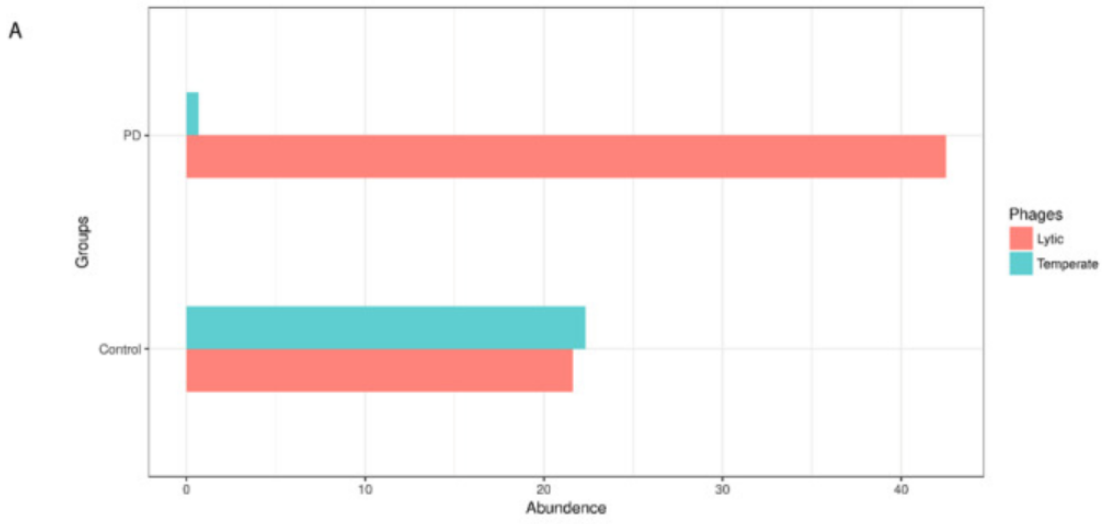

Figure 6: Abundance of virulent phages vs temperate phages in PD patients vs control group[43]

Having conducted the quantitative analysis of the bacteria between the experimental and control groups, the consequences and connection of Lactococcus to PD progression will now be examined. Lactococcus plays a role in managing dopamine levels, as a decreased amount of the

[43] Tetz, George, et al. "Parkinson's Disease and Bacteriophages as Its Overlooked Contributors." *Scientific Reports*, Nature Publishing Group UK, 17 July 2018, www.ncbi.nlm.nih.gov/pmc/articles/PMC6050259/.

bacteria leads to decreased levels of the neurotransmitter, a common issue leading to the development of PD. Thus, it is reasonable to conclude that less Lactococcus may have negative effects on dopamine levels, motor functioning, and be a possible sign of potential PD development.[44]

The findings derived from the research and the charts above brings to light the importance of Lactococcus in the metabolism of neurotransmitters. More specifically, the neurotransmitter that Lactococcus metabolizes includes dopamine, which is generally found to be deficient in patients suffering from PD.[45] Non-PD patients have a greater bacterial diversity in the gut, whereas, patients with PD are associated with the opposite. Therefore, Lactococcus is commonly found to be in decreased amounts in PD patients, who are lacking the microbiota-derived neurochemicals that are associated with the bacteria. Dopamine, one of the lacking neurochemicals in PD patients, is associated with early gastrointestinal symptoms of PD, as well as activating neurodegeneration associated with PD. This relationship between Lactococcus and PD suggests that common symptoms of PD such as constipation may be due to the decrease in dopamine production from low levels of this bacteria. In fact, this connection to this gut microbiota provides reasoning as to why PD patients experience more gastrointestinal symptoms.

In addition to the previous biochemical connections that were made to link the progression of PD with the enteric system, there also exist confounding variables, including, but not limited to age, lifestyle choices, health conditions etc. that can be analyzed as well. Upon examination of certain such variables in the context of PD, a direct path to the gut was able to be traced, further bolstering the argument of the paper at large.

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[44] Tetz, “Parkinson's Disease and Bacteriophages as Its Overlooked Contributors.”
[45] Tetz, “Parkinson's Disease and Bacteriophages as Its Overlooked Contributors.”

Confounding Variable: Age

Age is among the many confounding factors that may lead to different results in symptoms and susceptibility between those with PD in patients and the healthy controls. According to a study investigating the characteristics of PD in young and old patients, older people experienced greater intensity of motor and nonmotor symptoms of PD, and a greater dysfunction of dopaminergic function (motor symptoms include bradykinesia, and resting tremor, while nonmotor symptoms include autonomic, olfactory, and cognitive dysfunctions).[46] The reason why higher severity of motor signs is observed with increased age can be tied back to the gut as a precursor, specifically to calprotectin, a protein biomarker for gastric inflammation.

A recent study investigated the possibility of calprotectin as a biomarker for gut inflammation and intestinal barrier dysfunction in PD patients. Its results showed that there is a high amount of calprotectin in PD patients compared to healthy controls, claiming that the heightened level of calprotectin correlated with inflammations or infections of the gut barrier. In turn, this disrupted the immunity of the gastrointestinal tract, leading to the conclusion that calprotectin is a useful biomarker for PD.[47] Interestingly, higher levels of calprotectin were found to be positively associated with age, as shown in the figure below, which may help explain why there are more severe symptoms and inflammation seen in older patients of PD as well as serves as a potential avenue to explore why older people are more susceptible to PD in the first place.[48] It is of significance yet again that a biochemical connection to the gut, in this case through

[46] Pagano, Gennaro et al. "Age at onset and Parkinson disease phenotype." *Neurology* vol. 86,15 (2016): 1400-1407. doi:10.1212/WNL.0000000000002461

[47] Mulak, Agata et al. "Fecal Calprotectin as a Marker of the Gut Immune System Activation Is Elevated in Parkinson's Disease." *Frontiers in neuroscience* vol. 13 992. 27 Sep. 2019, doi:10.3389/fnins.2019.00992

[48] Zhernakova, Alexandra et al. "Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity." *Science (New York, N.Y.)* vol. 352,6285 (2016): 565-9. doi:10.1126/science.aad3369

calprotectin, is able to provide insight and correlations to provide potential explanations for limited observed patterns that accompany PD, specifically in this section, with age.

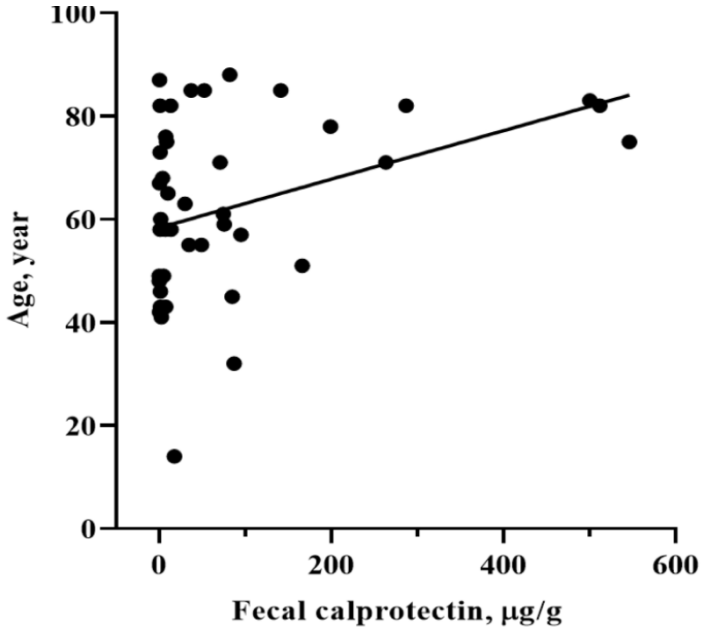

Figure 7: Positive correlation between fecal calprotectin and age[49]

In addition to the calprotectin connection, with age, mitochondrial DNA deletions accumulate, shortening the DNA, which causes the mitochondria to become dysfunctional ultimately. It is suggested that mitochondrial dysfunction is related to cell loss in PD, as well as creating oxidative stress in substantia nigra neurons; this increase in oxidative stress is then correlated to the misfolding and accumulation of alpha synuclein.[50] The accumulation of alpha synuclein in the gut, as previously discussed, is directly related to PD, solidifying another potential enteric connection to PD in relation to age

[49] Park, Shin Young. "Age-Related Fecal Calprotectin Concentrations in Healthy Adults." *Korean Journal of Clinical Laboratory Science*, Korean Society for Clinical Laboratory Science, 30 Sept. 2020, www.kjcls.org/journal/view.html?volume=52&number=3&spage=181.
[50] Reeve, Amy et al. "Ageing and Parkinson's disease: why is advancing age the biggest risk factor?." *Ageing research reviews* vol. 14,100 (2014): 19-30. doi:10.1016/j.arr.2014.01.004

Confounding Variable: Cigarette Smoking

In addition to age, there is much discussion around the extent of the association that tobacco and nicotine have with Parkinson's Disease in the context of their association and effects on the gut. Specifically, debate as to whether tobacco/nicotine increases the risk of PD or decreases/causes no effect is in question as research is being conducted to discover cures for PD through the chemicals of tobacco. From the research currently shown, there is no association between tobacco/nicotine usage and a large increase in PD risk. Instead, one of the more recent hypotheses claims that smoking can produce anti-inflammatory effects within the gut, leading to the decrease of PD development.[51]

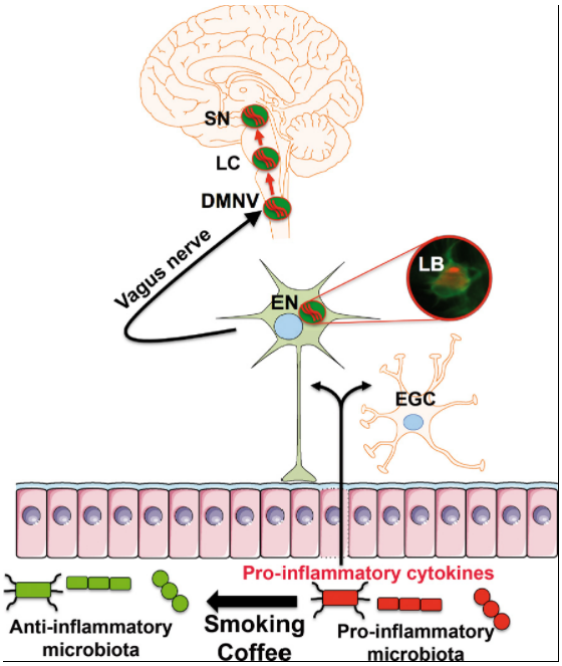

Figure 8: Possible role of smoking on microbiota-gut-brain-axis and PD development[52]

[51] Ma, C., Liu, Y., Neumann, S. *et al.* Nicotine from cigarette smoking and diet and Parkinson disease: a review. *Transl Neurodegener* 6, 18 (2017). https://doi.org/10.1186/s40035-017-0090-8

[52] Derkinderen, P., et al. "Gut Feelings about Smoking and Coffee in Parkinson's Disease." *Movement Disorders : Official Journal of the Movement Disorder Society*, U.S. National Library of Medicine, pubmed.ncbi.nlm.nih.gov/24753353/#&gid=article-figures&pid=figure-1-uid-0.

Figure 8 demonstrates the role of pro-inflammatory microbiota in the context of PD development. In the absence of cigarette smoking, enteric glial cells (EGC) in the gut produce inflammatory cytokines; an increase in these cytokines then promotes alpha-synuclein aggregation in Lewy Bodies (LB) within enteric nerves.[53] This could then cause the Lewy Bodies to spread further into the central nervous system through the vagus nerve, ultimately contributing to the development of PD. This biological process occurs when cigarette smoking is excluded from a patient's lifestyle choices, therefore, indicating a potential inverse relationship between smoking and PD development.

Another hypothesis states that cigarettes have the ability to alter the composition of the microbiome system in the human gut.[54] This is a positive association as the compositional change within the gut caused by nicotine promotes the mitigation of intestinal inflammation by lessening the misfolding of alpha-synuclein in enteric nerves.[55] As the decrease in alpha-synuclein misfolding occurs, it simultaneously reduces the propagation of alpha-synuclein aggregates to the Central Nervous System, which in turn lowers the risk of Parkinson's Disease. If the misfolding of alpha-synuclein in enteric nerves were to increase, it would induce neurodegeneration, leading to PD. [56] This biological process indicates that nicotine may provide anti-inflammatory properties that allow for physical relief and slow down the development and progression of PD. The effect that nicotine provides supports a previous hypothesis mentioned in a prior subsection - Prevotella produces short chain fatty acids (SCFA's) and has

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[53] Derkinderen, P., et al. "Gut Feelings about Smoking and Coffee in Parkinson's Disease." *Movement Disorders : Official Journal of the Movement Disorder Society*, U.S. National Library of Medicine, pubmed.ncbi.nlm.nih.gov/24753353/#&gid=article-figures&pid=figure-1-uid-0.

[54] Derkinderen, P., et al. "Gut Feelings about Smoking and Coffee in Parkinson's Disease." *Movement Disorders : Official Journal of the Movement Disorder Society*, U.S. National Library of Medicine, pubmed.ncbi.nlm.nih.gov/24753353/#&gid=article-figures&pid=figure-1-uid-0.

[55] Ma, C., Liu, Y., Neumann, S. *et al.* Nicotine from cigarette smoking and diet and Parkinson disease: a review. *Transl Neurodegener* 6, 18 (2017). https://doi.org/10.1186/s40035-017-0090-8

[56] Derkinderen, P., et al. "Gut Feelings about Smoking and Coffee in Parkinson's Disease." *Movement Disorders : Official Journal of the Movement Disorder Society*, U.S. National Library of Medicine, pubmed.ncbi.nlm.nih.gov/24753353/#&gid=article-figures&pid=figure-1-uid-0.

anti-inflammatory properties within the gut, which then produces a therapeutic effect on the brain.[57]

Overall, this recent finding has provided evidence that implies an inverse relationship between smoking cigarettes and PD, as well as a positive compositional change in the human gut; this directly again ties into showing how confounding variables such as smoking tie into the gut which then ties into the effecting the progression of PD. Cigarette smoking results in the compositional change in the human gut allowing for anti-inflammatory properties to flourish and delay the progression of PD. Although this compelling piece of research provides evidence that nicotine can be a potential source of prevention and mitigation of PD, there are discrepancies between individuals and methodologies. The specific cases mentioned above and any supporting arguments may have confounding variables, poor research methods, and high dropout rates that can influence the clinical outcomes. To better understand how nicotine affects PD, ultimately, there needs to be more research properly conducted in order to reach an irrefutable conclusion.

Complications and Further Discussion

Indeed, all of the arguments and pieces of evidence put forth in this paper thus far revolve around nuanced matters and as such it is important to recognize certain limitations within the paper. One qualifying factor of the merit of the conclusions drawn within this paper could be the fact that the various studies and experiments that were cited in this paper were inconsistent regarding factors such as age of participants, race, diet, gender, lifestyle, and the timing of data collected. For instance, the data presented in Figure 6 represents participants in certain given age groups while participants whose data are presented in Figure 4 are unknown. This is an important distinction to point out because as mentioned previously, age can have an effect on the

[57] Bedarf et al. " Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naïve Parkinson's disease patients."

progression of PD, and thus, the physiological characteristics of participants. Moreover, inconsistencies in the classification of the subjects themselves are another limiting factor to consider as certain studies, such as one that led to the conclusion that alpha-synuclein aggregates are from the gut and go up the vagus nerve, primarily used mice instead of humans.[58] Ultimately, other critical variables such as race, diet, lifestyle, and gender of participants were among other factors that were not analyzed in the studies that were referenced, which could have contributed to certain discrepancies and may have affected the conclusions drawn.

Beyond qualifying potential limitations of the paper, another key point of discussion is assessing how the treatments that are being used for PD currently speak to the relationship that can be drawn between the disease and the gut. As previously established, the gut microbiome plays a significant role in determining a person's susceptibility to PD. Specifically, a highly diverse gut microbiome indicates a healthier gut, which led researchers to make important connections and potentially develop effective treatments for PD. Certain inflammatory processes in the gut stimulated by a response to foreign bacteria or viruses have also been linked to alpha-synuclein aggregation, which, as discussed, may lead to the development of neurodegenerative diseases such as PD. In recent years, researchers have found innovative methods to circumvent neurological disorders as PD by targeting these processes through dietary interventions, fecal microbiota transplants, bowel cleansing, and managed use of specific probiotics. These methods have proven effective in cases where they were done simultaneously; improvements in gut microbiome diversity were seen when both dietary interventions and bowel cleansing were performed together. In terms of modifying diet, a vegetarian diet as well as a fiber-rich diet are said to help create a more diverse microbiome and also have anti-inflammatory

[58] Kim, Sangjune et al. "Transneuronal Propagation of Pathologic α-Synuclein from the Gut to the Brain Models Parkinson's Disease." *Neuron* vol. 103,4 (2019): 627-641.e7. doi:10.1016/j.neuron.2019.05.035

effects, which are beneficial to overall health, reducing the risk of developing PD. Thus, decreased levels of short-chain fatty acids (SCFA), critical for regulated brain-gut communication and general homeostasis, increase the chances of developing PD. SCFA can be administered orally, which can help in the development of microglial cells, which play an important role in maintaining homeostasis in brain tissue. Additionally, rectal enemas also pose as potential treatment methods, particularly for patients who have gastrointestinal inflammation.[59]

Probiotics have also been proven to have a positive correlation with gut microbiome diversity in addition to helping with constipation, and ultimately decreasing the likelihood of developing PD. Constipation is quite common in patients with PD, as it does not properly respond to laxative medications. Bowel functioning and stool consistency have been observed to improve upon treatment through probiotics distributed through fermented milk as well as daily capsules. However, research on the full potential of probiotics as an effective and long term treatment for neurodegenerative diseases such as PD are yet to be conducted. The arena for discovery remains wide, as there appear to be a variety of possible avenues that may lead towards a long-term cure for neurological disorders associated with the gut microbiome, such as PD.[60]

Conclusion

Holistically, given the data and subsequent analysis presented within this paper, there is strong evidence supporting the idea that changes within the enteric system have a direct role in affecting the progression of Parkinson's Disease. In summary, the paper started off by discussing the misfolding of alpha-synuclein (within the gut), which then traveled to the brain and helped

[59] Hegelmaier, Tobias et al. "Interventional Influence of the Intestinal Microbiome Through Dietary Intervention and Bowel Cleansing Might Improve Motor Symptoms in Parkinson's Disease." *Cells* vol. 9,2 376. 6 Feb. 2020, doi:10.3390/cells9020376
[60] Tan, Ai Huey et al. "Probiotics for Parkinson's disease: Current evidence and future directions." *JGH open : an open access journal of gastroenterology and hepatology* vol. 5,4 414-419. 20 Nov. 2020, doi:10.1002/jgh3.12450

contribute to the mass clumps of protein already accumulated there, leading to Lewy Body formation. Then there was discussion surrounding the biochemistry behind various bacterial populations, those that appeared in both increased and decreased amounts in relative correlation to the presence of PD, which further demonstrated how sensitivity within the gut led to imbalances that were seen in afflicted patients. The final portion of the paper delved into analyzing how confounding variables such as age and smoking further established a link between the nervous and enteric in the context of PD.

Ultimately, the significance of establishing this connection can prove to be vital for further research surrounding the development of treatments and drugs for PD, taking into account the biochemical effects that the gut can have on the disease. Moreover, there is potential to delve deeper into analyzing further markers of the changes in the enteric microbiome as a means of potentially predicting if someone is at higher risk of developing PD or not: this would be a monumental achievement considering that Parkinson's is a disease of which so much remains unknown. However, having established specific proteins and bacterial biochemical markers within the gut throughout this paper, there remains merit for further exploration beyond the scope of the brain, which hopefully can provide more answers for this elusive and complicated disease, in the hopes of one day improving the lives and having a means of potentially tracing a cause for the thousands affected.

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