GREEN TEA CATECHINS AS A PREBIOTIC INTERVENTION FOR THE MODULATION OF THE GUT MICROBIOME, METABOLOME, AND ALPHA-SYNUCLEIN IN PARKINSON'S DISEASE

by

ELIZABETH CHAPMAN RIEGELMAN

(Under the direction of Jia-Sheng Wang)

ABSTRACT

The correlation between lifestyle choices, dietary habits, and the onset of chronic illnesses has been well-established. With the increasing population and life expectancy in the United States, there's a heightened susceptibility to age-related neurodegenerative conditions. Parkinson's disease (PD) is one such condition, characterized by factors including dopaminergic neuron degeneration, increased inflammation, and abnormal α-synuclein protein accumulation. While the exact causes of PD remain elusive, both environmental and genetic susceptibility factors are believed to play a role. Emerging evidence suggests that disturbances in the gut microbiome and resulting metabolites may contribute to PD pathology, prompting intensified research into the mechanisms of gut-related inflammation and α-synuclein protein aggregation. Plant polyphenols, found abundantly in various medicinal plants, have shown promise in laboratory studies by disrupting aberrant protein aggregation, influencing cellular signaling, and regulating gut microbiome imbalances. Epidemiological evidence suggests that regular intake of plant polyphenols, namely tea catechins, could reduce the risk of PD by shielding neurons, although clinical trials are still limited. The biological effects of tea catechins can vary, prompting

interest in assessing their dietary significance. We hypothesized that tea catechin intake would modify the composition of the gut microbiota and gut-microbiota dependent metabolic pathways. Resulting microbes and the specific metabolites would then lead to the enhancement of the neuroprotective effects of tea catechins by attenuating α-synuclein aggregation and circulation. As such, we aimed to investigate the molecular mechanisms of tea catechins in a transgenic PD mouse model that overexpresses mutant human A53T α-synuclein protein; investigate whether tea catechin altered gut microbiome could contribute to lowering α-synuclein aggregation *in vivo* following a 90-day tea catechin treatment; and to combine metagenomics with metabolomics to investigate the impact of tea catechins on the composition and function of the gut microbiota in a transgenic PD mouse model.

INDEX WORDS: Polyphenols, green tea, tea catechins, α-synuclein, Parkinson's disease, gut-brain axis, gut microbiome

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As the saying goes, it takes a village.

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CHAPTER 1

INTRODUCTION

1. Background

Parkinson's Disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the *substantia nigra* portion of the midbrain. As the second most common progressive neurodegenerative disease, PD affects over 8.5 million individuals globally [1] and that number is expected to increase for the foreseeable future. The primary motor symptoms of PD include muscular rigidity, resting tremors, bradykinesia, and impaired postural reflexes [2]. PD is not limited to motor symptoms; antecedent non-motor symptoms such as mood and cognitive disorders, sleep disturbances, autonomic dysfunction, and constipation can significantly impact the overall well-being of individuals years before the onset of motor symptoms [3]. What sets PD apart from other forms of parkinsonism is the presence of Lewy bodies (LBs) and Lewy neurites (LNs), which are ubiquitinated protein aggregates predominantly composed of the misfolded form of the presynaptic protein α-synuclein. α-synuclein's normal function remains enigmatic due to the challenges of assessing normal functions of the protein. However, α-synuclein's presynaptic localization and affinity for interaction with highly curved membranes and other synaptic proteins suggests that α-synuclein may play a role in regulatory function associated with synaptic activity, plasticity, vesicle trafficking, neurotransmitter release, dopamine metabolism, and/or synaptic vesicle pool maintenance [4]. Misfolded α-synuclein, primarily in the form of oligomers and aggregates, is thought to be toxic, with recent evidence showing the protein's propagation between neurons. However, many aspects remain uncertain, such as the reasons for the selective vulnerability of dopaminergic neurons in PD, the factors that

trigger α-synuclein aggregation and pathology, and the influence of aging in the development of PD. Although the complexities behind the misfolding of α-synuclein are not fully understood, several factors are thought to contribute to this phenomenon in the context of PD, including genetic mutations in the SNCA gene, environmental factors, protein interactions, and post-translational modifications [5,6]. The majority of PD cases are idiopathic; therefore, it is believed that a combination of complex genetic and environmental factors contributes to the onset and progression of PD. Without a clearly defined disease mechanism, a cure for PD has proven difficult to develop. Available treatment strategies for PD aim at increasing or substituting dopamine or reducing PD symptoms via surgical procedures, gene and immunotherapy, and cell transplantation [7]. Both medications and surgical procedures have moderate side effects that can often produce lackluster results. Preventive measures that slow or halt neurodegeneration have been long sought after, especially given the absence of a cure for PD.

Celebrated for its myriad health benefits, green tea holds promise as a potential therapeutic approach for preventing PD. Leaves from the evergreen shrub *Camellia sinesis* contain a variety of compounds including polyphenols, purine alkaloids, caffeine, theaflavins, and a mixture of natural flavonoids called tea catechins (TCs). Emerging research indicates a potential link between green tea consumption and a reduced risk of developing PD [8-11]. TCs are the most common plant-derived bioactive components, which have been recognized as health-promoting natural compounds for decades [12]. Owing to their multiple biological properties, TCs have been used as a dietary supplement for a variety of health benefit purposes [13]. Growing evidence has demonstrated that TCs can exert neuroprotective effects against neurodegenerative disorders, such as PD [14,15]. While the mechanisms underlying the beneficial potential of green tea are not yet clearly defined, several have been proposed, including (i) antioxidant properties that combat oxidative stress and alleviate mitochondrial dysfunction, (ii) anti-inflammatory effects that may

inhibit brain inflammation, and (iii) the inhibition of amyloid-beta protein aggregation, all of which may play a protective role against PD.

More than a trillion microbes inhabit the human GI tract. Collectively the hundreds to thousands of bacterial taxa are called the gut microbiome. The gut microbiome encodes more than 3 million genes, covering many metabolic functional genes, which impact host health status [16,17]. Recent studies have emphasized the critical impact of gut microbiota on progression of PD, and the alterations in gut microbiome composition and resulting metabolites may be related to the initiation or prevention of PD [18-20]. Recent studies of PD patients matched with healthy controls found that bacteria more commonly associated with anti-inflammatory properties, such as the genera *Blautia* [21-23,25], *Coprococcus* [21,22,24], *Roseburia* [21-28], and *Faecalibacterium* [23,24,26-28] are significantly reduced in fecal samples of PD patients. These studies indicated gut microbial dysbiosis and microbial metabolite alterations in PD pathogenesis and progression, however, clear roles and mechanisms have yet to be explored thoroughly, especially for understanding of the early interaction between the gut microbiota and the occurrence of PD.

A multitude of studies have demonstrated that TCs can affect the biodiversity of human gut microbiota which improves host health [29,30]. Our lab's previous studies found that long-term supplement of TCs significantly affected the composition and functions of gut microbiota and modified gut-microbiota- dependent metabolisms in time- and dose-dependent patterns in SD rats [31]. The beneficial microbe families *Bacteroidetes* and *Oscillospira* were significantly enriched whereas the *Peptostreptococcaceae* family was almost depleted in the gut. Research has demonstrated where the gut microbiome plays a critical role in regulating the formation of various colonic microbial ring-fission metabolites of TCs [32]. These TC metabolites are more easily absorbed into circulation and are attributed to the mode of action of TCs bioactivity [33,34]. However, little is known about these two-way interactions between TCs and gut

microbes. Based on our previous study results [31,35], we hypothesize that TCs intake will modify the composition of gut microbiota and gut-microbiota-dependent metabolic pathways, and consequently, the alternative microbes and their specific metabolites may lead to enhancement of neuroprotective effect of TCs via attenuating α-synuclein aggregation and pro-inflammatory response in PD pathogenesis and progression.

2. Purpose of This Study

In this dissertation, we combine metagenomics with metabolomics to investigate the effect of TCs on behavioral characteristics and the composition and function of the gut microbiota in the A53T transgenic PD mouse model that overexpresses human α-synuclein.

The animal model we have selected, hemizygous B6.Cg-2310039L15Rik$^{Tg(Prnp\text{-}SNCA*A53T)23Mkle}$/J (Strain #006823, common name: Hualpha-Syn (A53T transgenic line G2-G3)), express an A53T missense mutant form of human α-synuclein under the control of the murine prion promoter. These A53T mice express the familial PD associated A53T missense mutant form of human α-synuclein at approximately six times the level of endogenous α-synuclein. Hemizygous mice spontaneously develop neurodegenerative disease between 9-16 months of age, with a progressive motor dysfunction leading to death within 2-3 weeks of onset. Affected mice exhibit neuronal abnormalities, including pathological accumulation of α-synuclein and ubiquitin. Hemizygous mice also have adult-onset hyperactivity that is associated with D1 receptor and dopamine-transporter-mediated alterations. The A53T mutant α-synuclein mice display significantly greater *in vivo* neurotoxic effects compared to the A30P or wildtype α-synuclein expressing mice on the same genetic background. The simplest interpretation of the pathology in this α-synuclein transgenic model is that α-synuclein overexpression is generally toxic regardless of its location and is associated with α-synuclein self-assembly that is accelerated by PD-linked mutations [36]. Over-expression of α-synuclein, a hallmark of PD pathogenesis, also results in mitochondrial dysfunction, oxidative stress, and activation of cell death pathways, all of which

are consistent with the genetic linkage of human familial PD to duplications and triplications of *SNCA* gene that leads to elevated α-synuclein expression [37]. The A53T transgenic mice, with rapid onset of pathology, are well positioned as platforms to test therapeutic strategies aimed at limiting α-synuclein aggregation.

While we seek to explore the impact of TCs on the gut microbiome and behavioral characteristics in this transgenic PD mouse model, identifying specific microbes and metabolites that contribute to the neuroprotective effect of TCs is critical for determining which therapeutic mechanisms are at play. We will compare TC-treated mice with control mice to observe potential effects on behavioral characteristics, gut microbiome compositions, and pathological features through the analysis of neurobehavioral data, urine and fecal samples, blood plasma samples, and organs collected from necropsy. Understanding how the gut microbiome interacts with food bioactive components and identifying specific microbes, and their metabolic pathways involved with the efficacy of TC metabolization will lead to more accurate prediction of TCs' therapeutic and preventive functions. Further, identification of the specific bacterial genes that aid the efficacy of TCs could be useful for many applications, such as bioengineering of commensal probiotics to resolve the long-standing issue of the low bioavailability of food bioactive compounds.

3. Expected Results

The overall objective of this dissertation is to evaluate how TCs interact with the gut microbiota and α-synuclein protein by examining behavioral changes, biochemical and physiological implications, and functional and structural modification of the gut microbiota of a mouse model. The expected results are:

1. Aggregation of A53T α-synuclein will be observed in transgenic A53T PD control mouse alongside behavioral deficits;

2. In comparison to control transgenic A53T mice, TC treatment groups will demonstrate (i) improved neurobehavioral outcomes; (ii) reduced aggregation of A53T α-synuclein; and (iii) increased circulation of TC and TC metabolites;
3. More neurodegenerative-induced dysbiosis of the gut microbiome in control transgenic A53T mice compared to TC treatment groups;
4. TC induced alterations of gut microbiota and genes which reduce α-synuclein aggregation in the transgenic PD mouse model will be identified;
5. Key microbial TC derived metabolites will be identified and characterized;
6. Information about how TCs alter the gut microbiome and gut-microbiota-dependent metabolome that will benefit neuroprotection will be obtained.

We hypothesize that TC intake will modify the composition of gut microbiota and gut-microbiota-dependent metabolic pathways, and consequently, the alternative microbes and their specific metabolites may lead to enhancement of neuroprotective effect of TCs via attenuating α-synuclein aggregation and pro-inflammatory response in PD pathogenesis and progression.

4. Conclusion

This dissertation investigates the impact of TCs on the gut microbiome and metabolome and the potential neuroprotective effects in a transgenic PD mouse model that over-expresses human α-synuclein. Our findings are expected to highlight the intricate interactions between TCs, the metabolome, and the gut microbiome, providing insights into how these natural compounds might mitigate or alleviate neurodegenerative pathways in PD. The anticipated outcomes include the identification of specific gut microbes and their metabolites that are modulated by TCs, leading to reduced α-synuclein protein aggregation and/or circulation and improved neurobehavioral outcomes. By elucidating the role of gut microbiota in enhancing the bioactivity of TCs, this study could add to the foundation for novel therapeutic strategies targeting the gut-brain axis in

PD. Ultimately, this research underscores the potential of dietary interventions to influence neurodegenerative disease progression, offering a promising avenue for future PD management and treatment.

References

1. Organization WH. Launch of WHO's Parkinson disease technical brief 2022 [cited 2024 January 22].
2. Bloem BR, Okun MS, Klein C. Parkinson's disease. *Lancet*. 2021 Jun 12;397(10291):2284-2303.
3. Abdelmoaty MM, Lu E, Kadry R, et al. Clinical biomarkers for Lewy body diseases. *Cell Bioscience*. 2023 Nov 14;13(1):209.
4. Burré, Jacqueline. 'The Synaptic Function of α-Synuclein'. *Journal of Parkinson's Disease*. 1 Jan. 2015 : 699 – 713.
5. Breydo L, Wu JW, Uversky VN. Alpha-synuclein misfolding and Parkinson's disease. *Biochim Biophys Acta*. 2012 Feb;1822(2):261-85.
6. Neupane S, De Cecco E, Aguzzi A. The Hidden Cell-to-Cell Trail of alpha-Synuclein Aggregates. *Journal of Molecular Biology*. 2023 Jun 15;435(12):167930.
7. Liu J, Xu F, Nie Z, Shao L. Gut Microbiota Approach-A New Strategy to Treat Parkinson's Disease. Front Cell Infect Microbiol. 2020 Oct 22;10:570658. doi: 10.3389/fcimb.2020.570658. PMID: 33194809; PMCID: PMC7643014.
8. Belvisi D, Pellicciari R, Fabbrini G, et al. Modifiable risk and protective factors in disease development, progression and clinical subtypes of Parkinson's disease: What do prospective studies suggest? *Neurobiol Dis*. 2020 Feb;134:104671.
9. Li C, Lin J, Yang T, et al. Green Tea Intake and Parkinson's Disease Progression: A Mendelian Randomization Study. *Frontiers in Nutrition*. 2022;9:848223.
10. Malar DS, Prasanth MI, Brimson JM, et al. Neuroprotective Properties of Green Tea (Camellia sinensis) in Parkinson's Disease: A Review. *Molecules*. 2020 Aug 27;25(17).

11. Payne A, Nahashon S, Taka E, et al. Epigallocatechin-3-Gallate (EGCG): New Therapeutic Perspectives for Neuroprotection, Aging, and Neuroinflammation for the Modern Age. *Biomolecules*. 2022 Feb 25;12(3).

12. Yang, C.S. (1999). Tea and health. *Nutrition* 15, 946-949.

13. Cabrera, C., Artacho, R., and Giménez, R. (2006). Beneficial effects of green tea--a review. *J Am Coll Nutr*. 25, 79-99.

14. Barranco Quintana, J.L., Allam, M.F., Del Castillo, A.S., and Navajas, R.F. (2009). Parkinson's disease and tea: a quantitative review. *J Am Coll Nutr*. 28, 1-6.

15. Caruana, M., and Vassallo, N. (2015). Tea Polyphenols in Parkinson's Disease. *Adv Exp Med Biol.* 863, 117-137.

16. Clemente, J.C., Ursell, L.K., Parfrey, L.W., and Knight, R. (2012). The impact of the gut microbiota on human health: an integrative view. Cell 148, 1258-1270.

17. Nicholson, J.K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., and Pettersson, S. (2012). Host-gut microbiota metabolic interactions. *Science.* 336, 1262-1267.

18. Sampson, T.R., Debelius, J.W., Thron, T., Janssen, S., Shastri, G.G., Ilhan, Z.E., Challis, C., Schretter, C.E., Rocha, S., Gradinaru, V., *et al.* (2016). Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. Cell *167*, 1469-1480.e1412.

19. Rani, L., and Mondal, A.C. (2021). Unravelling the role of gut microbiota in Parkinson's disease progression: Pathogenic and therapeutic implications. Neurosci Res.

20. Wang, Q., Luo, Y., Chaudhuri, K.R., Reynolds, R., Tan, E.K., and Pettersson, S. (2021). The role of gut dysbiosis in Parkinson's disease: mechanistic insights and therapeutic options. Brain.

21. Keshavarzian, A.; Green, S.J.; Engen, P.A.; Voigt, R.M.; Naqib, A.; Forsyth, C.B.; Mutlu, E.; Shannon, K.M. Colonic bacterial composition in Parkinson’s disease. Mov. Disord. 2015, 30, 1351–1360.

22. Aho, V.T.E., Houser, M.C., Pereira, P.A.B., Chang, J., Rudi, K., Paulin, L., Hertzberg, V., Auvinen, P., Tansey, M.G., and Scheperjans, F. (2021). Relationships of gut microbiota, short-chain fatty acids, inflammation, and the gut barrier in Parkinson's disease. Mol Neurodegener *16*, 6.

23. Wallen, Z.D.; Appah, M.; Dean, M.N.; Sesler, C.L.; Factor, S.A.; Molho, E.; Zabetian, C.P.; Standaert, D.G.; Payami, H. Characterizing dysbiosis of gut microbiome in PD: Evidence for overabundance of opportunistic pathogens. NPJ Park. Dis 2020, 6, 11.

24. Nishiwaki, H.; Ito, M.; Ishida, T.; Hamaguchi, T.; Maeda, T.; Kashihara, K.; Tsuboi, Y.; Ueyama, J.; Shimamura, T.; Mori, H.; et al. Meta-Analysis of Gut Dysbiosis in Parkinson’s Disease. Mov. Disord. 2020, 35, 1626–1635.

25. Li, W.; Wu, X.; Hu, X.; Wang, T.; Liang, S.; Duan, Y.; Jin, F.; Qin, B. Structural changes of gut microbiota in Parkinson’s disease and its correlation with clinical features. Sci. China Life Sci. 2017, 60, 1223–1233.

26. Cirstea, M.S.; Yu, A.C.; Golz, E.; Sundvick, K.; Kliger, D.; Radisavljevic, N.; Foulger, L.H.; Mackenzie, M.; Huan, T.; Finlay, B.B.; et al. Microbiota Composition and Metabolism Are Associated with Gut Function in Parkinson’s Disease. Mov. Disord. 2020, 35, 1208–1217.

27. Barichella, M.; Severgnini, M.; Cilia, R.; Cassani, E.; Bolliri, C.; Caronni, S.; Ferri, V.; Cancello, R.; Ceccarani, C.; Faierman, S.; et al. Unraveling gut microbiota in Parkinson’s disease and atypical parkinsonism. Mov. Disord. 2019, 34, 396–405.

28. Hill-Burns, E.M.; Debelius, J.W.; Morton, J.T.; Wissemann, W.T.; Lewis, M.R.; Wallen, Z.D.; Peddada, S.D.; Factor, S.A.; Molho, E.; Zabetian, C.P.; et al. Parkinson’s disease and Parkinson’s disease medications have distinct signatures of the gut microbiome. Mov. Disord. 2017, 32, 739–749.

29. Liu, Y.C., Li, X.Y., and Shen, L. (2020). Modulation effect of tea consumption on gut microbiota. Appl Microbiol Biotechnol *104*, 981-987.

30. Pérez-Burillo, S.; Navajas-Porras, B.; López-Maldonado, A.; Hinojosa-Nogueira, D.; Pastoriza, S.; Rufián-Henares, J.A. Green Tea and Its Relation to Human Gut Microbiome. Molecules 2021, 26, 3907.

31. Wang, J., Tang, L., Zhou, H., Zhou, J., Glenn, T.C., Shen, C.L., and Wang, J.S. (2018). Long-term treatment with green tea polyphenols modifies the gut microbiome of female sprague-dawley rats. J Nutr Biochem *56*, 55-64.

32. Guo, T., Song, D., Cheng, L., and Zhang, X. (2019). Interactions of tea catechins with intestinal microbiota and their implication for human health. Food Sci Biotechnol *28*, 1617-1625.

33. Carregosa, D., Carecho, R., Figueira, I., and C, N.S. (2020). Low-Molecular Weight Metabolites from Polyphenols as Effectors for Attenuating Neuroinflammation. J Agric Food Chem *68*, 1790-1807.

34. Pervin, M., Unno, K., Takagaki, A., Isemura, M., and Nakamura, Y. (2019). Function of Green Tea Catechins in the Brain: Epigallocatechin Gallate and its Metabolites. Int J Mol Sci *20*.

35. Zhou J, Tang L, Shen CL, Wang JS. Green tea polyphenols modify gut-microbiota dependent metabolisms of energy, bile constituents and micronutrients in female Sprague-Dawley rats. J Nutr Biochem. 2018;61:68-81. doi:10.1016/j.jnutbio.2018.07.018

36. Giasson, B.I., Duda, J.E., Quinn, S.M., Zhang, B., Trojanowski, J.Q., and Lee, V.M. (2002). Neuronal alpha-synucleinopathy with severe movement disorder in mice expressing A53T human alpha-synuclein. Neuron *34*, 521-533.

37. van der Putten, H., Wiederhold, K.H., Probst, A., Barbieri, S., Mistl, C., Danner, S., Kauffmann, S., Hofele, K., Spooren, W.P., Ruegg, M.A., et al. (2000). Neuropathology in mice expressing human alpha-synuclein. J Neurosci 20, 6021-6029.

CHAPTER 2

GUT–BRAIN AXIS IN FOCUS: POLYPHENOLS, MICROBIOTA, AND THEIR INFLUENCE ON A-SYNUCLEIN IN PARKINSON'S DISEASE [1]

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[1] Riegelman, E.; Xue, K.S.; Wang, J.-S.; Tang, L. Gut–Brain Axis in Focus: Polyphenols, Microbiota, and Their Influence on α-Synuclein in Parkinson's Disease. *Nutrients* 2024, 16, 2041. https://doi.org/10.3390/nu16132041

Abstract

With the recognition of the importance of the gut–brain axis in Parkinson's disease (PD) etiology, there is increased interest in developing therapeutic strategies that target α-synuclein, the hallmark abhorrent protein of PD pathogenesis, which may originate in the gut. Research has demonstrated that inhibiting the aggregation, oligomerization, and fibrillation of α-synuclein are key strategies for disease modification. Polyphenols, which are rich in fruits and vegetables, are drawing attention for their potential role in this context. In this paper, we reviewed how polyphenols influence the composition and functional capabilities of the gut microbiota and how the resulting microbial metabolites of polyphenols may potentially enhance the modulation of α-synuclein aggregation. Understanding the interaction between polyphenols and gut microbiota and identifying which specific microbes may enhance the efficacy of polyphenols is crucial for developing therapeutic strategies and precision nutrition based on the microbiome.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease, after Alzheimer's, affecting approximately 2–3% of the population more than 60 years of age and up to 5% of the population over 85 years of age [1,2]. It is estimated that there are 1.5 million people in the United States living with PD, and 60,000 Americans are diagnosed with this disease annually [3]. PD is a progressive neurological disorder that primarily affects motor function, leading to symptoms such as tremors, stiffness, slowness of movement, and impaired balance and coordination [4,5]. One of the hallmark pathologies of PD is the accumulation of alpha-synuclein (α-synuclein), a presynaptic neuronal protein that aggregates into insoluble fibrils, forming Lewy bodies (LBs) and Lewy neurites in the brain [6]. Although the exact function of α-synuclein remains partially understood, it has been linked to a range of neuronal functions, including neurotransmitter release, the modulation of a variety of enzymes and transporters, the dynamics of presynaptic vesicles, and the support of neuronal plasticity [7]. Its abnormal accumulation contributes to neuronal death and the subsequent manifestation of PD symptoms [8].

The pathogenesis of PD has not yet been fully elucidated. While the majority of earlier PD studies focused solely on brain pathologies, the gastrointestinal (GI) system is now recognized as a pivotal participant in the pathogenesis of PD [9–13]. Recent research has increasingly focused on the gut–brain axis, a complex bidirectional communication system that links the enteric nervous system (ENS) of the GI tract with the central nervous system (CNS). This axis not only plays a critical role in maintaining GI homeostasis but also appears to be involved in the pathogenesis of several neurological disorders, including PD. The emerging evidence suggests that α-synuclein pathology may originate in the gut and then spread to the brain via the vagus nerve, a concept supported by observations of GI abnormalities in PD patients years before the onset of motor symptoms. Moreover, alterations in the gut microbiota composition have been observed in PD patients, indicating a potential role of gut dysbiosis in the disease's progression.

Currently, there is no existing cure for PD, and drugs that are presently used in clinics only provide symptomatic treatment rather than preventing or slowing the pathogenic progression of neurodegeneration [14]. In addition, some of these drugs present many side effects in patients [15]. Therefore, there is an urgent need to develop novel therapeutic agents with lower side effects and a broader spectrum of targets to not only treat the symptoms but also potentially prevent or slow the pathogenic progression of PD. Given the interaction between the gut microbiota and the CNS, dietary interventions that modulate the gut microbiome have garnered interest as a potential therapeutic strategy for PD. Polyphenols, a diverse group of phytochemicals found in fruits, vegetables, tea, wine, and certain herbs, have garnered attention in this context. These compounds are known for their antioxidant and anti-inflammatory properties and have been shown to influence the composition and function of the gut microbiota. Importantly, polyphenols may also inhibit the aggregation of α-synuclein, offering a dual mechanism by which they could mitigate PD pathology. By modulating the gut microbial community, dietary polyphenols may reduce intestinal inflammation, enhance gut barrier function, and produce metabolites that could potentially inhibit the aggregation of α-synuclein. Therefore, understanding the mechanisms underlying the interactions between dietary polyphenols, the gut–brain axis, and α-synuclein pathology could open new avenues for preventing or slowing the progression of PD, highlighting the significance of diet and gut microbiota in the pathogenesis of neurodegenerative diseases. This review aims to integrate the current knowledge and recent discoveries to shed light on the complex interactions between dietary polyphenols, gut microbiota, and α-synuclein aggregation, offering insights into potential novel therapeutic strategies for PD that leverage the gut–brain axis.

2. α-Synuclein: A Key Player in Parkinson's Disease Pathology

α-synuclein is a protein comprising 140 amino acids encoded by the SNCA gene, which normally exists in naturally occurring monomers. Under pathological conditions, natively unfolded

monomers can undergo self-aggregation, forming pathological oligomers that further mature into fibrils [16]. The accumulation of α-synuclein aggregates (particularly into oligomers and fibrils) disrupts normal cellular processes, causes mitochondrial dysfunction, triggers inflammation, and leads to neuronal death. Therefore, treatments that target α-synuclein oligomers and/or fibrils may reduce neurodegeneration, which is a promising therapeutic strategy for PD [17].

2.1. α-Synuclein Structure and Physiological Function

α-synuclein is a relatively small protein within the synuclein family, which also includes β-synuclein and γ-synuclein. With a molecular weight of approximately 14 kDa, α-synuclein is predominantly located at the presynaptic terminals in the CNS and accounts for about 1% of all cytosolic proteins in the brain [7,18].

A schematic figure about the structure of α-synuclein is shown in Figure 1. The protein can be divided into three distinct domains: The N-terminal amphipathic domain, which contains the evolutionary conserved KTEGV motifs and the main mutations associated with familial PD, such as A53T and A30p [19]. This region is responsible for membrane binding as well. Additionally, there is a central hydrophobic segment known as the non-amyloid component (NAC), which confers the potential for a β-pleated sheet, and a highly negatively charged carboxyl tail at the C-terminal end, which contains a Ca 2 +-binding site and the main phosphorylation site at Ser129, modulating α-synuclein aggregation. The hydrophobic NAC region, in particular, is critical for the protein's propensity to adopt β-sheet configurations that can self-assemble into fibrillar aggregates, forming the core of LBs, a pathological hallmark found in PD brains [20]. Once α-synuclein fibrils are established in a neuron, they can act as a guide for the aggregation of endogenous α-synuclein protein [21], further initiating PD progression.

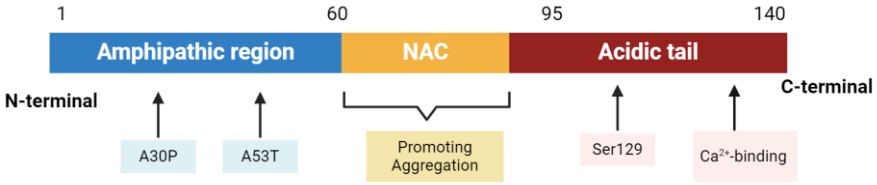

Figure 2.1 Scheme of α-synuclein regions with the number of corresponding residues.

Although the main function of α-synuclein in both the central and peripheral nervous systems remains unclear, it has been linked to a range of neuronal functions, including neurotransmitter release, the modulation of a variety of enzymes and transporters, the dynamics of presynaptic vesicles, and the support of neuronal plasticity [7]. Additionally, α-synuclein is involved in the formation of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex at presynaptic nerve terminals. This latter function is thought to be mediated by a chaperone activity that has not yet been identified, highlighting α-synuclein's potential role in ensuring efficient neurotransmission and synaptic function [20,22].

2.2. Role of α-Synuclein in PD

α-synuclein plays a significant role in the pathogenesis of both familial and sporadic forms of PD [23], as well as in other synucleinopathies [24]. Under normal conditions, native α-synuclein exists in a dynamic equilibrium between unfolded monomers and α-helically folded tetramers with a low propensity for aggregation [25]. The aggregation process of α-synuclein involves a conformational change whereby it adopts a β-sheet-rich structure that facilitates its aggregation into oligomers, protofibrils, and insoluble fibrils that finally accumulate in Lewy bodies within neurons [26]. Almost all pathologically aggregated α-synuclein is phosphorylated at the Ser129 site [27]. These aggregates disrupt various cellular processes, leading to neuronal dysfunction and, ultimately, cell death [28,29] (Figure 2).

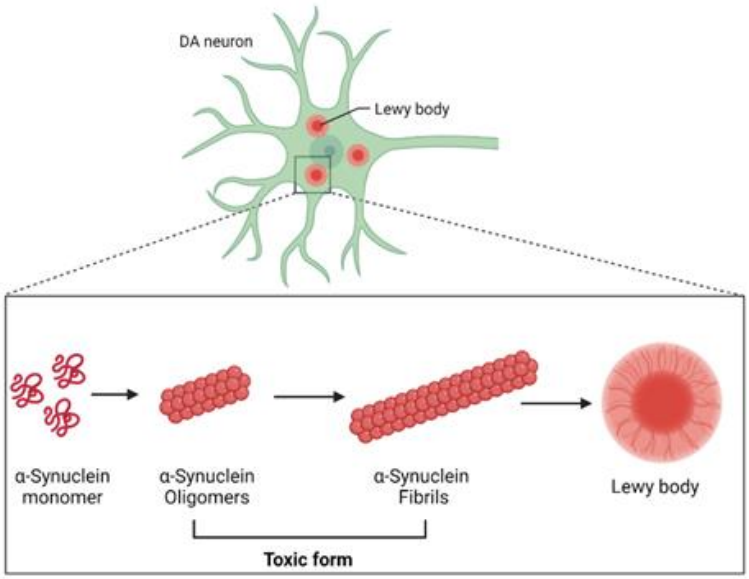

Figure 2.2. The role of α-synuclein in the pathogenesis of PD. DA: dopaminergic.

One of the critical ways that misfolded α-synuclein contributes to neuronal dysfunction is through the disruption of mitochondrial function [30–32]. Mitochondria are essential for energy production, and their dysfunction leads to reduced cellular energy levels, increased oxidative stress, and the activation of apoptotic pathways [33,34]. Misfolded α-synuclein aggregates can impair mitochondrial dynamics, including fission and fusion processes, and interfere with mitochondrial transport along axons, further exacerbating neuronal dysfunction [35].

Another significant impact of misfolded α-synuclein is on synaptic function [36]. Synapses are critical for neuronal communication; therefore, any disruption in their function can lead to significant impairments in neuronal signaling [37]. Misfolded α-synuclein can impair synaptic vesicle release and reuptake, leading to reduced neurotransmitter availability and synaptic transmission efficiency. This disruption contributes to the motor and cognitive symptoms observed in PD and related disorders [38,39].

2.3. α-Synuclein Propagation

Studies have suggested that α-synuclein can be transmitted between neurons [40] and can seed the formation of toxic aggregates in recipient neurons in a prion-like manner [41]. α-synuclein demonstrates several prion-like characteristics, notably the capacity of its aggregated forms to self-propagate by promoting the aggregation of normal α-synuclein, a process known as ‘seeding’. This mechanism can increase the aggregate burden within a cell. Similar to prions, misfolded α-synuclein can act as a template, inducing the misfolding of native α-synuclein in neighboring neurons. This templated misfolding can lead to the cell-to-cell transmission of pathological α-synuclein aggregates, contributing to the progressive nature of PD. For example, fetal dopamine cells transplanted into the striatum of patients with PD were found to develop Lewy pathology when examined neuropathologically 1–2 decades later [42]. Similar to the human transplants, α-synuclein has been observed in cell culture and rodent transplantation experiments, where it was transferred from one cell to another [40,43,44].

3. Gut–Brain Axis: A New Frontier in Parkinson's Disease Research

The gut–brain axis represents a complex communication network linking the GI tract and the CNS, fundamentally impacting health and disease [45]. Research has highlighted that gut microbiota might play a significant role in the onset and progression of PD [46]. Various studies indicate that alterations in the gut microbiome have been observed in PD patients, with changes in the abundance of certain bacterial species compared to healthy controls. Additionally, α-synuclein also accumulates in the ENS [47–49]. This accumulation can occur years before the typical motor symptoms of PD appear, suggesting that the gut might be an early site of disease pathology. Understanding the gut–brain axis in PD could open new avenues for early diagnosis and targeted treatments that modulate the gut microbiota or its metabolic outputs, potentially slowing disease progression or alleviating symptoms.

3.1. Gut–Brain Axis

The concept of the gut–brain axis describes bidirectional communication between the CNS and ENS of the GI tract, which is linked by neurons of the sympathetic and para-sympathetic nervous system [50,51]. Through this bidirectional communication network, signals from the brain can influence the motor, sensory, and secretory modalities of the GI tract and, conversely, visceral messages from the GI tract can influence brain function [52]. At present, it is suggested that gut microbes may serve as significant contributors to the bidirectional communication that takes place along the gut–brain axis. The microbiome community carries out important metabolic and physiological functions for the host and contributes to overall health and homeostasis [53–55]. Consequently, the gut microbiota has emerged as a potential diagnostic and therapeutic target in disorders as diverse as PD [56–59].

3.2. Dysbiosis of Microbiome in PD

Dysbiosis of the gut microbiome in PD patients is currently being investigated to determine which microbiota actively produce metabolites that are implemented in microglia activation, α-synuclein aggregation, and inflammation in the gut. Gut microbes play a crucial role in the gut–brain axis, which interacts with the ENS, enterocytes, and immune system to influence host health [60]. Evidence suggests that PD pathogenesis may be influenced by the interplay between the imbalance of gut microbes and altered bacterial metabolites [61–63]. Numerous findings in both observational PD patient studies and experimental animal studies have revealed that gut bacteria aid in the regulation of anti-inflammatory and pro-inflammatory profiles, suggesting that alterations within an individual's gut microbiome can influence the risk of developing PD [46,59,64–72]. Newly diagnosed PD patients often exhibit intestinal hyperpermeability, endotoxemia, and microbial dysbiosis, which bolsters the hypothesis that gut-derived inflammation promotes neuroinflammation and neurodegenerative changes in PD pathogenesis [67,70,72,73]. Clinical studies have shown an increase in intestinal permeability in PD, where bacterial endotoxins in the form of lipopolysaccharides are associated with increased α-synuclein accumulation within the GI tract [74,75]. Some PD patients have shown elevated lipopolysaccharide serum levels, which could correlate to increased intestinal permeability [76–78]. Further, the guts of PD patients are often colonized by lipopolysaccharide-producing bacteria such as *Helicobacter pylori*, which induces chronic inflammation and degradation of the gut mucosal lining [77,79–81].

Individuals with high-risk factors for PD or the diagnosis of PD have been documented to have significantly different compositions of gut microbes compared to those of healthy controls [13,82–84]. The relative abundances of anti-inflammatory bacteria such as *Blautia*, *Coprococcus*, *Roseburia*, *Fusicatenibacter*, *Faecalibacterium*, and *Lachnospira* are reduced in PD patients, while, in contrast, *Lactobacillus*, *Bifidobacterium*, and *Akkermansia* phyla are higher in PD

patients [67,78,85–89]. Numerous studies have documented the alterations in the composition of the gut microbiome in PD, as seen in Table 1 [46,70,85,86,90–109].

Table 2.1 Altered gut microbes in patients with PD compared to healthy controls.

Phyla	Class	Order	Family	Genus	Species	Increased	Decreased
Bacteroidetes (Bacteroidota)	Phylum Bacteroidetes						87, 100, and 103
	Bacteroidia	Bacteroidales	*Bacteroidaceae*	*Bacteroides*		87 and 104	
				Bacteroides	*fragilis*		88
				Bacteroides	*coprocola*		93 and 106
			Barnesiellaceae			98	
			Odoribacteraceae	*Butyricimonas*		97	
				Odoribacter		97	
			Porphyromonadaceae			100	
				Porphyromonas		89 and 97	
			Prevotellaceae				44, 87, 94, 100, and 103
				Prevotella		89 and 90	82 and 97
				Prevotella	*copri*		91
			Rikenellaceae	*Alistipes*		106	
				Alistipes	*shahii*	91	
			Tannerellaceae			83 and 97	
				Parabacteroides		83 and 97	
Firmicutes (Bacillota)	Phylum Firmicutes					91 and 103	68, 102, and 104
	Erysipelotrichia	Erysipelotrichales	*Coprobavillaceae*	*Coprobacillus*		83 and 97	
			Erysipelotrichaceae	*Eubacterium*	*biforme*		91
					hallii		91
					rectale		91
					eligens		91
	Clostridia	Eubacteriales	*Clostridiaceae*	*Butyricicoccus*			89
				Clostridium	*saccharolyticum*		91
					coccoides		88
				Hungatella		83	

			Christensenellaceae	*Christensenella*		92, 93, 95, and 96
				Christensenella	*minuta*	93
			Carabacteraceae	*Catabacter*		83 and 93
				Catabacter	*hongkongensis*	93
			Lachnospiraceae			68, 83, 89, 92, 95, 96, 101, and 102
				Agathobacter		89
				Blautia		68, 89, and 99
				Coprococcus		68 and 83
				Fusicatenibacter		89
				Lachnospira		89
				Roseburia		68, 83, 89, 92, 95, and 96
			Oscillopiraceae (Ruminococcaceae)			100
				Faecalibacterium		83, 89, 92, 95, and 96
				Faecalibacterium prausnitzii		87
				Hydrogenoanaerobacterium		83 and 100
				Oscillospira		68, 93, 95, 96, and 103
				Ruminiclostridium		89
				Ruminococcus		83
				Ruminococcus	*bromii*	95 and 100
				Papillibacter	*cinnamivorans*	93
	Bacilli	Lactobacillales	*Lactobacillaceae*			44, 83, 95, 98, and 101
						87

				Lactobacillus		88, 89, 93, 96, 97, and 104	
				Lactobacillus	*mucosae*	93	
			Enterococcaceae			98 and 101	87
				Enterococcus		97 and 99	
			Streptococcaceae	*Streptococcus*		99	
	Negativicutes	Veillonellales	*Veillonellaceae*	*Veillonella*		97	
Proteobacteria (Pseudomonadota)	Deltaproteobacteria	Desulfovibrionales	*Desulfovibrionaceae*	*Bilophilia*		83, 92, 97, and 103	
				Bilophila	*wadsworthia*	92 and 105	
	Gammaproteobacteria	Enterobacterales	*Enterobacteriaceae*			44, 87, 94, 95, and 101	
		Pasteurellales	*Pasteurellaceae*			100	83
				Haemophilus			83
Actinobacteria (Actinomycetota)	Actinomycetia	Bifidobacteriales	*Bifidobacteriaceae*	*Bifidobacterium*		82, 87, 89, 92, 93, 95, 96, and 102	104
		Corynebacteriales	*Corynebacteriaceae*			95	
				Corynebacterium		89	
	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Collinsella*		92, 95, and 103	
		Eggerthellales	*Eggerthellaceae*			83 and 103	
Verrucomicrobia	Phylum Verrucomicrobia					97, 100, 103, and 105	
	Verrucomicrobiae	Verrucomicrobiales	*Akkermansiaceae*	*Akkermansia*		68, 90, 92, 95, 96, 97, 100, 103, and 106	
				Akkermansia	*muciniphila*	87, 91, 105, and 106	
Synergistetes	Synergistia	Synergistales	*Synergistaceae*			83	
Deferribacteres	Deferribacteres	Deferribacterales	*Mucispirillaceae*	*Mucispirillum*		97	

Lower levels of fecal SCFAs have been observed in PD patients compared to healthy controls [85,90,110,111], and the evidence suggests that SCFA-producing bacteria may modify the microbiome's genetic potential to produce the enzymes need-ed for SCFA formation. A recent meta-analysis determined that PD patients had reduced levels of bacteria in the *Lachnospiraceae* and *Ruminococcaceae* families [67]. Similarly, another meta-analysis reported that PD patients had decreased levels of the genera *Roseburia* and *Faecalibacterium*, both belonging to the Firmicutes Phyla [86]. These bacteria are known SCFA producers, and reductions in their levels have been documented in other diseases with an inflammatory component. It is important to note, however, that not all studies have obtained the same results. Although it is not clear whether alterations in the microbiome are the origin of PD pathogenesis, it is evident that disease pathology influences the microbiota over time and vice versa.

3.3. Gut–Brain Axis and α-Synuclein

α-synuclein is a protein primarily found in the brain; however, recent evidence suggests that it also occurs in other peripheral tissues such as the GI tract [112–114]. Biopsies of GI tissues from PD patients have shown α-synuclein accumulation in the lower parts of the esophagus, stomach, duodenum, colon, and rectum [115–117]. Finding α-synuclein outside the CNS supports the hypothesis that the presence of α-synuclein in both the brain and the gut may result from a common pathological aggregation pathway involving the vagus nerve. This evidence indicates that the initial process of α-synuclein misfolding and pathology might start in the GI tract before spreading to the brain [118,119]. This observation supports Braak's hypothesis, which proposes that α-synuclein aggregates originate in the gut and then propagate through the gut–brain axis, triggering the onset of PD [120,121]. Further, the retrograde spread of α-synuclein towards the brain suggests that GI-derived α-synuclein could ascend the ENS and propagate through interconnected neurons toward the midbrain of the CNS via the vagus nerve, ultimately reaching

the *substantia nigra pars compacta* (SNpc) and forming fibril structures that cause motor dysfunction [49,119,120,122,123].

Research suggests that certain microbes may influence the misfolding and abnormal formation of α-synuclein through extracellular mechanisms [124–128]; an example of this may be *Helicobacter pylori*, a gram-negative spiral bacterium known as the causative agent of gastric ulcers, present in 50% of the world population [129]. *H. pylori* contains cholesterol-α-glucosyltransferase, which catalyzes the conversion of membrane cholesterol to cholesteryl glucosides. The glycosylated derivatives can exert a neurotoxic effect on dopamine neurons and promote the aggregation of α-synuclein in the vagus nerve from the level of the stomach [130,131]. Another gram-negative bacteria species, i.e., *Escherichia coli*, has been documented to be implicated in α-synuclein misfolding [132]. *E. coli* produces an amyloid fiber protein called curli, whose purpose is to promote cell community behavior through the formation of biofilms in the extracellular matrix. The administration of *E. coli* in animal models has demonstrated an increase in α-synuclein fibril reactivity and accumulation of insoluble α-synuclein in the *substantia nigra* portion of the midbrain compared to controls, indicating that exposure to microbial amyloids in the GI may accelerate α-synuclein aggregation in the gut and brain [133,134].

3.3.1. Braak's Hypothesis

Braak's hypothesis is a significant theory in the field of neurology, particularly concerning the pathology of PD. Proposed by Braak and his colleagues in the early 2000s, this hypothesis suggests that PD may start outside the brain, specifically in the gut or the nasal cavity, and then spread to the brain via the nervous system. According to Braak's hypothesis, an unknown pathogen (either a virus or bacterium) in the gut could trigger the onset of sporadic PD [135]. Along with this hypothesis, they presented a staging system for PD that is based on a specific pattern of the spread of α-synuclein [136]. Subsequent to these publications, the broader dual-hit

hypothesis was proposed, which posits that sporadic PD begins simultaneously in two locations: the neurons of the nasal cavity and the neurons in the gut [137,138]. From these places, the pathology is hypothesized to spread according to a specific pattern, i.e., via the olfactory tract and the vagus nerve, respectively, toward and within the CNS. There is experimental and clinical evidence supporting Braak's hypothesis [139–147]. Additionally, α-synuclein aggregations have been found in the GI tract of animal models of early and advanced PD [148–151].

Braak's hypothesis has had a substantial impact on research directions, focusing attention on the potential role of the gut–brain axis and the olfactory system in the early detection and understanding of PD [152–154]. It also supports the observation that non-motor symptoms, such as the loss of smell or GI issues, can precede the motor symptoms by several years.

3.3.2. α-Synuclein from the Gut to the Brain

The transmission of α-synuclein from the ENS to the CNS is a critical research area for understanding the pathogenesis of neurodegenerative diseases, particularly PD. This area has gained significant attention as it may explain the initiation and progression of PD [12,155,156]. Direct evidence of gut–brain α-synuclein transmission in rodents was provided by Holmqvist et al. [157] who demonstrated that α-synuclein fibrils derived from PD patients could migrate from the GI tract to the brain via the vagus nerve in rats. Challis et al. [158] complemented these findings by inoculating the duodenal walls of aged mice with α-synuclein pre-formed fibrils (PFFs) and observed the progression of α-synuclein pathology to the brain. Similarly, Kim et al. provided additional evidence by injecting mouse α-synuclein PFFs into the pylorus and duodenum, noting the subsequent detection of phosphorylated α-synuclein in the CNS, beginning in the dorsal motor nucleus of the vagus nerve and progressing to regions like the *locus coeruleus*, amygdala, *substantia nigra*, and prefrontal cortex [159]. This progression closely reflects the Braak staging scheme for PD. Crucially, the study showed that these effects were negated if α-synuclein PFFs were injected into the intestinal wall following truncal vagotomy,

which involves the severing of the vagus nerve. In this experimental setup, phosphorylated α-synuclein was still detectable in the upper duodenum 7 months after injection, but there was no spread to the *substantia nigra* [159]. These findings robustly support the notion that gut-derived α-synuclein is capable of propagating through the vagus nerve in a prion-like manner to induce CNS disease.

4. Polyphenols: Beyond Antioxidants

4.1. Types and Dietary Sources

Polyphenols are highly specific secondary metabolites of plants that have a variety of biological roles, from resistance to infection by pathogenic microorganisms to defense against ultraviolet radiation [160]. Generally speaking, “polyphenol” refers to compounds derived entirely from the shikimate/phenylpropanoid pathway and/or the polyketide pathway, characterized by the presence of more than one phenolic unit and devoid of nitrogen-based functions [161]. To date, thousands of polyphenols have been identified from natural dietary plants. In general, polyphenols are mainly classified as phenolic acids, flavonoids, lignans, coumarins, and stilbenes, as outlined in Figure 3 [162]. The presence of polyphenol compounds in food greatly depends on environmental factors and food processing, production, and storage [160].

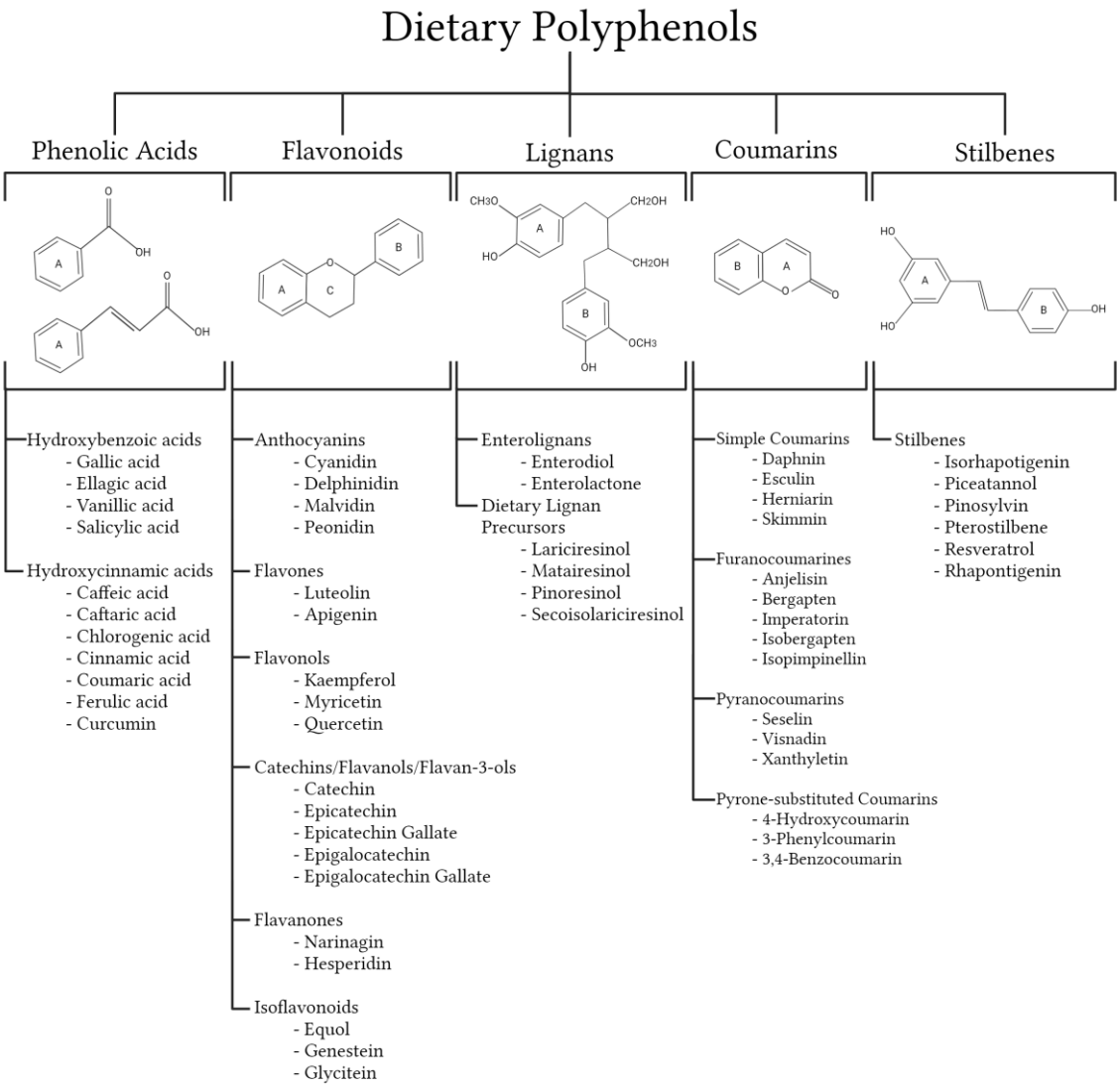

Figure 2.3 Classification of major dietary polyphenols.

4.1.1. Phenolic Acids

Phenolic acids are phytochemicals found in the majority of plant tissues and comprise a phenolic ring with an attached organic carboxylic acid. The secondary plant metabolites are aromatic acids that can be further classified into two broad groups, including hydroxycinnamic acids (HCAs) and hydroxybenzoic acids (HBAs), the latter of which are less common. HCAs are most concentrated in the outer ripened parts of fruits and are present in a variety of fruits, vegetables, and seeds [163,164]. The most abundant HCAs include the free forms of para-coumaric acid, caffeine, ferulic acid, and sinapic acid, whereas the bound forms include glycosylated derivatives of quinic acid, shikimic acid, and tartaric acid [165]. The most common HBAs include gallic acid, ellagic acid, syringic acid, and salicylic acid [166]. A wide variety of fruits and vegetables contain phenolic acids, including apples that contain chlorogenic acid and blueberries that contain para-coumaric, caffeic, and ferulic acids [167]. Phenolic acids are potent antioxidants due to their high potential to serve as hydrogen donors and oxygen quenchers and reduce metal-chelating agents [164,168,169]. Furthermore, HCAs and HBAs have been found to exhibit protective effects on neural, cardiac, and hepatic tissues, making this group of polyphenols an interesting target for research in the field of therapeutic medicine.

4.1.2. Coumarins

Coumarins belong to the benzopyrone family and comprise a six-membered benzene ring attached to an α-pyrone ring [170]. Coumarins are present in several plants but are highest in concentration in the tonka bean, vanilla grass, sweet clover, and cinnamon [171]. These small molecule candidates have been considered for drug development due to their high solubility, low molecular weight, low toxicity, and high bioavailability [171,172]. Coumarins act as secondary plant metabolites to protect against herbivores and microorganisms through interactions with plant growth hormones and respiration; how-ever, regarding biochemistry and physiology, coumarins are known to exhibit antioxidant effects and act as enzyme inhibitors [173]. Coumarins

can be further classified into four groups: simple coumarins, furanocoumarins, pyranocoumarins, and pyrone-substituted coumarins, whereas both the free and glycosidic forms of coumarin can be found in plants [174]. While coumarins pose as promising therapeutic agents for human health, there are certain derivatives of coumarins, such as coumarin chromen-2-one, which are potentially poisonous for human consumption [175]. Still, the majority of coumarins have been recognized as harmless compounds and are considered to be candidates for medicinal drugs with diverse pharmacological activities, high bioavailability, and generally low toxicity [173].

4.1.3. Stilbenes

Stilbenes accumulate in the vine tissues of plants under stressful biotic and abiotic conditions [176]. Over 400 natural stilbenes have been described [177], and their potential as antioxidants and chemopreventive agents is of interest to the scientific community. A major stilbene of interest, namely, resveratrol, has been researched for its potential cardio-protective effects. Resveratrol has been identified in at least 185 different plant species and is associated with antioxidant activity and its positive effect on lifespan and age-related diseases [178,179]. The regular consumption of stilbenes has been demonstrated to alleviate intracellular oxidative stress, reduce chronic inflammation, and suppress adipogenesis and lipogenesis [180].

4.1.4. Lignans

Lignans consist of two phenylpropanoid units coupled by a β-β'-bond and are found in various seeds, grains, vegetables, and fruits [181]. Lignan bioavailability depends heavily on the diet due to its relatively low concentrations [182]. As a polyphenol class, lignans have received growing attention given their potentially beneficial bioactive properties due to their steroid-analogous chemical structure. Technically considered phytoestrogens, lignans have been documented to exhibit anti-estrogenic, antioxidant, and anti-carcinogenic effects. The lignans most common in the human diet include lariciresinol, matairesinol, pinoresinol, and secoisolariciresional which are

most concentrated in sesame and flax seeds [181,183,184]. While the database on the content of lignans in foods is growing, more evidence needs to be added on the number of lignan dietary sources and the physiological role that the diverse compounds play *in vivo*.

4.1.5. Flavonoids

Flavonoids are the largest class of dietary polyphenols and, hence, contribute greatly to our understanding of the broad spectrum of their health-promoting effects. There are over 6,000 flavonoids that contribute to the pigments and protection of different fruits, vegetables, and medicinal plants [160]. All flavonoids contain at least 15 carbons with two benzene A and B rings and are further classified based on variations within the heterocyclic C ring [166]. The main groups of flavonoids are flavones, flavonols, catechins, isoflavones, flavanones, and anthocyanins. A broad spectrum of health-promoting effects has been attributed to the flavonoid group largely due to their ability to modulate key cellular enzyme functions and act as antioxidant, anti-inflammatory, anti-mutagenic, and anti-carcinogenic compounds [185]. Research has shown that flavones and catechins have the highest biological significance as antioxidants for protection against reactive oxygen species (ROS). Further, flavonoids are capable of inhibiting enzymes such as phosphodiesterase, lipoxygenase, Ca2+ ATPase, and COX, which are implicated in neurodegenerative diseases [185]. Researchers have also recognized flavonoids for their antimicrobial [186,187], antifungal [188], and antiviral [189,190] activities *in vivo*.

4.2. Gut Microbiota and Polyphenols

Dietary polyphenols can interfere directly with the enhancement or impairment of nutrient absorption. Given the antioxidant capabilities of many polyphenols, macro- and micronutrient oxidation may be prevented during metabolism, which could protect the quality of the nutrients. Carbohydrate absorption and glycemia may be decreased by polyphenols through their amylase-antagonizing activities [191,192], providing carbohydrates for bacteria such as *Bacteroides*,

Bifidobacterium, *Clostridium*, *Eubacterium*, *Lactobacillus*, and *Ruminococcus* [193]. In this sense, polyphenols can influence bacterial diversity, gut peptide synthesis, energy and nutrient absorption, and insulin sensitivity [166]. However, polyphenol's antioxidant capabilities may be a double-edged sword as polyphenols can interfere with mineral absorption. Gallic acid, chlorogenic acid, and poly-phenolic polymerization products have been demonstrated to inhibit iron absorption [194–197]. Tannins and gallic acid have been documented to have an affinity for binding to zinc, further impairing zinc absorption [198,199]. Regardless of the caveats that accompany dietary polyphenols, research has elucidated the beneficial potentials and relationships between the gut microbiome and commonly consumed polyphenol substances.

4.2.1. Curcumin

Curcumin, commonly known as turmeric, is a popular HCA that is known for its variety of pharmacological and restorative effects, including therapeutic approaches for chronic diseases, cardiovascular disease, depression, skin diseases, obesity, diabetes, and multiple types of cancer [200–203]. Studies that have observed the pharmacokinetics and bioavailability of curcumin have documented how poorly absorbed and rapidly metabolized the compound is, resulting in speculation about its clinical use [204]. Nevertheless, peripheral influences of curcumin may play a more important role in the CNS [205]. Curcumin may prevent the formation of ROS and glial cell activation, further hindering α-synuclein aggregation and neuronal cell apoptosis [205–208]. It has been proposed that curcumin can modulate gut signaling pathways and potentially exert effects on the microbiome gut–brain axis. Oral administration of curcumin has been demonstrated to promote beneficial gut bacteria growth, including *Bifidobacteria* and *Lactobacilli* strains, while reducing the abundance of pathogenic bacteria strains, including *Prevotellaceae*, *Coriobacterales*, *Enterobacteria*, and *Rikenellaceae* [209–211]. The primary metabolite of curcumin, i.e., tetrahydrocurcumin, has been documented to restore gut microbiome dysbiosis by lowering the relative abundance of *Actinobacteria* and *Proteobacteria* and by modifying the ratio of

Firmicutes to *Bacteroidetes* [212]. An *in vivo* study showed that curcumin intervention improved motor deficits, glial cell activation, and the aggregation of α-synuclein in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) PD mouse model. The microbiome data showed elevated levels of *Lactobacillaceae*, *Lachnospiraceae*, *Muribaculaceae*, and *Eggerthellaceae* in the treatment groups compared to controls [205]. These studies suggest that oral administration of curcumin may promote the growth and proliferation of beneficial gut microbes.

4.2.2. Anthocyanins

Anthocyanins are water-soluble pigments in the polyphenol family, found in flowers and fruits that have red, blue, or purple hues as well as in nuts and certain vegetables. Anthocyanins are a group of flavonoids with a wide range of uses, such as medicinal herbs, including antidiabetic, anticancer, anti-inflammatory, antimicrobial, and anti-obesity effects [213]. The most common types of anthocyanins include cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin, which are all potent nutraceutical or pharmaceutical components. These compounds have low bioavailability due to their rapid absorption in the stomach and small intestine, causing low absorption into the blood and circulatory system and high excretion rates and reducing the efficiency of anthocyanins as free-radical scavengers. An *in vitro* incubation study in 2012 assessed anthocyanins' effects on the growth of *Bifidobacterium* spp., *Lactobacillus* spp., and *Bateroides* spp. and found that the mixture of malvidin-3-glucoside, delphinidin-3-glucoside, peonidin-3-glucoside, petunidin-3-glucoside, and cyanidin-3-glucoside had a synergistic effect on the growth of beneficial bacteria [214]. One of the microbiota metabolites of anthocyanin, namely, gallic acid, was observed to have an inhibitory effect on a group of potentially harmful bacteria, including *Clostridium histolyticum*, without affecting the beneficial bacteria. Furthermore, gallic acid produced from the administration of the anthocyanin mixture significantly reduced the *Bacteroides* spp. group [214]. A 2012 intervention study aimed to evaluate the effect of the moderate intake of anthocyanins in red wine on certain gut microbial

groups on host health benefits and found that red wine polyphenols could have a significant effect on the growth of select gut microbes, including *Proteobacteria*, *Fusobacteria*, *Firmicutes*, and *Bacteroidetes* [215].

4.2.3. Tea Polyphenols

The evergreen shrub *Camellia sinesis* can be consumed as unfermented green tea, semi fermented oolong tea, and fermented (oxidized) black tea [216,217]. Tea leaves contain a variety of components, including polyphenols, terpenoids, purine alkaloids, the amino acid L-theanine, carbohydrates, caffeine, theaflavins, and a mixture of natural flavonoids called tea catechins (TCs). The health benefits of tea have been attributed in part to their antioxidant and anti-inflammatory properties; however, some benefits have been linked to the relationship between tea bioactive compounds and the gut microbiota [218]. Tea polyphenols have a low bioavailability; additionally, it has been estimated that 90–95% of the dietary polyphenol compounds travel to the large intestine where they are in direct contact with the gut microbiota [219]. Both green and black teas are rich in flavonoids, comprising up to 30% of their dried volume [220].

Theaflavins

Theaflavin (3,4,5-trihydroxybenzocyclohepten-6-one) is a polyphenol and biflavonoid responsible for the red pigment in black and oolong tea formed by oxidation during fermentation [221,222]. Theaflavins (TFs) are further divided into theaflavin (TF1), theaflavin-3-gallated (TF2A), theaflavin-3’-gallate (TF2B), and theaflavin-3,3’-digallate (TF3) [223]. TFs consist of a benzotropolone skeleton that is formed by the oxidation of epicatechin (EC) and epigallocatechin-3-gallate (EGCG) in the presence of polyphenol oxidase and peroxidase enzymes [224]. TFs contribute to the health-promoting effects of the gut microbiota due to the bacteria-mediated metabolism that occurs in the lower GI tract. TFs that reach the large intestine are modified by ring-cleavage, reduction, hydrolysis, de-carboxylation, and dihydroxylation reactions [225,226].

An *in vitro* fecal fermentation study demonstrated that TF administration promoted the growth of *Bacteroides*, *Lachnoclostridium*, *Faecalibacterium*, *Parabacteroides*, and *Bifidobacterium*, whereas *Prevotella* and *Fusobacterium* growth was inhibited [227]. TF3 was shown to inhibit the growth of gram-negative bacteria, including *Klebsiella aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*, and gram-positive bacteria, including *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Mycobacterium smegmatis* [228].

The exerted health benefits may be due to the regulation of various cellular signaling pathways, including signaling to mitigate cellular inflammatory responses and blocking of the mitogen-activated protein kinase [229]. TFs have several therapeutic properties, including a protective effect on neuronal cell damage, promotion of immune response, and protection against certain disease initiation via the induction of apoptosis, cell cycle arrest, and suppression of inflammation [230,231]. TFs have been documented to act as strong free-radical scavengers, inhibit oxygen radical-mediated lipid peroxidation, and induce activation in different antioxidant enzymes [223,232]. TFs have been demonstrated in vivo to have the ability to penetrate the blood–brain barrier (BBB) and offer neuroprotection via radical scavenging, antioxidative, antiapoptotic, and cell-regulating pathways [230,233,234]. When TFs are hydrolyzed by Bifidobacteria and Lactobacilli species, the corresponding metabolites TF1, TF2A, TF2B, gallic acid, and pyrogallol become available metabolites in circulation [226,235]. Additionally, two microbial metabolites of TFs, i.e., 3-4’-hydroxyphenylpropionic acid and gallic acid, can increase tyrosine hydroxylase (TH) and dopamine transporter (DAT) immune responses. TFs should be considered promising prebiotic components due to their capacity to increase the abundance of beneficial bacteria and decrease the abundance of potentially harmful bacteria.

Green Tea Catechins

There are four major TC compounds found in green tea leaves, identified as (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate

(EGCG) [236], whereas EGCG is the most abundant and the most active component among catechins. TCs offer a spectrum of health benefits to animals and humans, including protection from cardiovascular diseases [237], cancers [238], and neurodegenerative diseases [239,240]. Several cell-based and animal experiments have provided evidence demonstrating that tea polyphenols and TCs have beneficial effects against PD [68,217,241–243]. The mode of action of TCs is mainly through the prevention of aggregate formation and dopamine loss, alleviating mitochondrial dysfunction, antioxidative stress, and anti-neuroinflammation, as well as the activation of neurotrophic factor and signaling pathways [244]. Metabolites of TCs are readily absorbed into circulation and may lead to the enhanced bioavailability and bioactivity of TCs [245–247]. TCs have been documented to modify gut-microbiota-dependent metabolisms of bile constituents and micronutrients [248], including the TCA cycle, bile acid metabolism, and metabolism of purine, pyrimidine, and amino acids [249]. EGCG has been reported to cross the BBB after ingestion, leading to increased activity of antioxidants, iron chelation, and antimutagenic effects [240]. Additionally, aromatic rings and hydroxyl groups produced by TC metabolism may provide neuroprotection by reducing lipid peroxidation and antioxidant activity [250].

Overall, the evidence has demonstrated that TCs can significantly impact the biodiversity of host gut microbiota and overall health by stimulating or hindering the growth of certain microbial species [218,251]. Previous studies [242] have shown that long-term supplementation with TCs significantly affected the compositions of the gut microbiome in time-dependent and dose-dependent patterns in Sprague–Dawley (SD) rats. *Bacteroidetes* and *Oscillospira*, both beneficial microbial families, were significantly enriched, while *Peptostreptococcaceae*, a gram-positive bacterium that is overrepresented in the gut of colon cancer patients, was significantly diminished. TCs have shown inhibitory effects on certain bacteria, such as *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfringens*, *E. coli*, *H. pylori*, *Legionella pneumophila*, and

Mycobacterium species [252]. Gram-negative bacteria are surrounded by a negatively charged lipopolysaccharide membrane that repels catechins [253]; however, some evidence shows that TC supplementation can inhibit the growth of harmful gram-negative bacteria. A 2013 study [254] found that in vitro EGCG, gallocatechin gallate (GCG), and EGCG 3"-methyl could significantly increase the abundance of beneficial bacteria, including *Bifidobacterium* spp., *Lactobacillus*, and *Enterococcus*, successively increasing the production of SCFAs. It was also observed that catechins were able to reduce the growth of *Bacteroides*, *Prevotella*, *Clostridium hystoliticum*, *Eubacterium*, and *Clostridium* species [254].

4.3. Polyphenols and α-Synuclein

Research has demonstrated that α-synuclein is a crucial therapeutic target for PD, and inhibiting its aggregation, oligomerization, and fibrillation are key strategies for dis-ease modification [255,256]. Numerous studies have identified a variety of polyphenolic compounds capable of inhibiting fibrils and oligomer formation, as well as stabilizing or disaggregating the α-synuclein oligomers [257–260]. This makes them promising therapeutic candidates for PD and related synucleinopathies. EGCG, quercetin, polyphenolic acids, curcumin, and their derivatives are some of the most well-known and effective polyphenolic inhibitors of α-synuclein.

4.3.1. EGCG

EGCG is a well-studied inhibitor of α-synuclein fibrillization, with a notable inhibition concentration (IC50) of 9.8 μM [261–264]. First reported by Ehrnhoefer in 2008 [261], EGCG inhibits α-synuclein by redirecting its aggregation into stable, spherical, and off-pathway oligomers and transforming pre-formed fibrils into unstructured aggregates. Apart from redirecting the aggregated α-synuclein to off-pathway oligomers, EGCG was reported to be able to transform already-formed fibrils into unstructured aggregates without releasing monomers [265]. The binding of EGCG with α-synuclein is non-specific, and it exhibits anti-amyloidogenic

properties against multiple proteins, including Aβ and huntingtin [266–268]. Further studies suggest that EGCG crosslinks α-synuclein into compact structures that cannot bind to the normal fibrils [269]. Importantly, EGCG was reported to bind to the same binding sites on α-synuclein fibrils as Thioflavin T(ThT) and substitute it, leading to misinterpretation of the aggregation readout [270]. Its efficacy, including in cells overexpressing α-synuclein or its A53T mutant [264], suggests that EGCG-induced aggregates are less likely to damage cell membranes compared to aggregates formed in its absence [271]. However, the full scope of EGCG's activity remains under investigation and is not yet fully understood [272].

4.3.2. Curcumin

Curcumin has been recognized for its potential to slow the progression of neuro-degenerative diseases such as PD [273]. Over the past few decades, the effects of curcumin on α-synuclein aggregation have been widely studied [274–281]. It has been reported that curcumin can inhibit α-synuclein fibrillization in vitro and disassemble already-formed fibrils [274,275,278]. Additionally, several modified analogs of curcumin with improved stability have also been proven effective in inhibiting α-synuclein amyloid aggregation and depolymerizing α-synuclein fibrils [277,282]. Interestingly, some studies suggested that curcumin may bind to oligomers and fibrils, thereby accelerating α-synuclein fibrillation, while producing less toxic α-synuclein aggregates [274,283]. Curcumin has also been shown to prevent hexokinase I (HKI) release and reactive oxygen species (ROS) enhancement triggered by α-synuclein fibrils in mitochondria [284,285]. Animal testing in an α-synuclein transgenic PD mouse model has demonstrated that the curcumin-rich diet showed improvements in mice motor behavior, although no changes in aggregate levels were detected [286].

Although *in vitro* data have shown that many polyphenols successfully modify α-synuclein, they still require systematic pharmacokinetic evaluations through in vivo studies. Additionally, many polyphenolic compounds face challenges in crossing the BBB due to their non-lipophilic nature,

potentially preventing them from reaching the concentrations necessary to exert effects in the brain [287,288]. Several factors, such as stability, solubility in an acidic environment at a gastric pH, absorption pattern, gut microbiota, enterohepatic circulation, first-pass metabolism, and metabolic fate concerning phase I or phase II metabolism, play a key role in achieving the ideal bioavailability of the phytochemicals in the brain [289,290].

5. Interplay between Polyphenols, Gut Microbiota, and α-Synuclein

With increasing recognition of the importance of the gut–brain axis in PD etiology, there is increasing interest in developing interventional strategies that target and attenuate α-synuclein aggregation and dispersion. As discussed in the above section, polyphenols are capable of regulating the gut microbiome composition and the metabolic pathways, mostly through the inhibition of pathogenic bacteria and the stimulation of beneficial bacteria [291–293]; in turn, polyphenols are extensively metabolized by the gut bacteria, resulting in the generation of bioactive secondary metabolites, which enhance bioavailability [294]. These gut bacterial metabolites inhibit fibrils and oligomer formation and stabilize or disaggregate the α-synuclein oligomers. More recent research suggested that the microbial metabolites of polyphenols can inhibit the spread of α-synuclein in a cell-based system [257,295] (Figure 4). However, the mechanisms of such an interaction between polyphenols, gut microbiota, and α-synuclein are largely unknown.

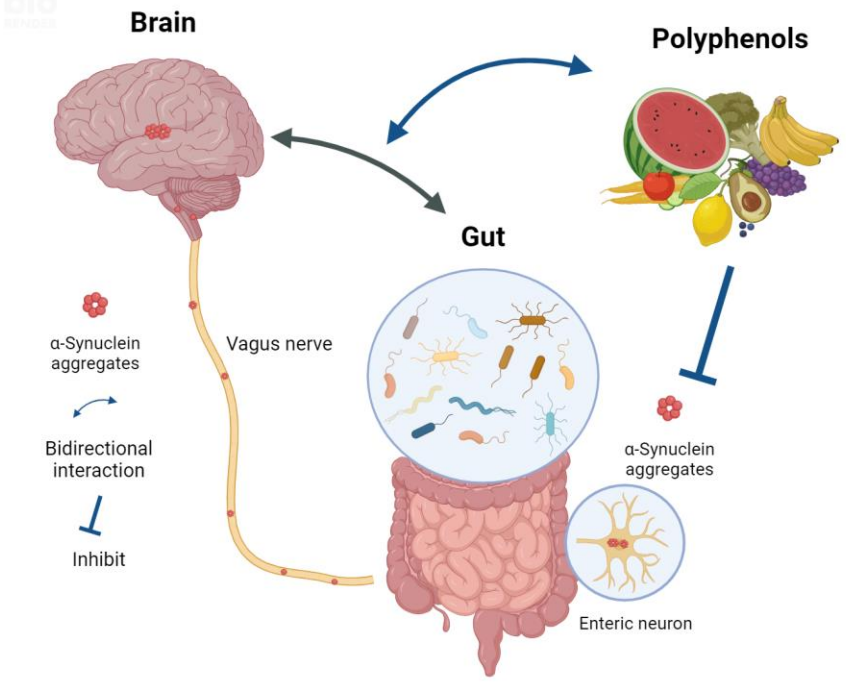

Figure 2.4. The interactions between polyphenols, the gut–brain axis, and α-synuclein.

5.1. Bioactivity and Efficacy of Polyphenols Are Affected by Gut Microbiota

Polyphenols that are most commonly found in dietary components are glycosides [296], which are not completely absorbed in the upper GI tract; only aglycones and some glucosides can be absorbed in the intestinal mucosa [297]. Recent evidence shows that the bioactivity and efficacy of polyphenols are significantly influenced by the gut microbiota [294,298–300]. The microbes in the gut can transform polyphenols into various metabolites that may have different bioactive properties from the original compounds [301,302]. Additionally, the composition of an individual's gut microbiota, which varies greatly among individuals, can determine the degree to which polyphenols are metabolized and utilized [303]. For example, interpersonal heterogeneity in gut microbiota may lead to interpersonal variabilities for their efficacy in metabolizing dietary flavanols into certain biologically available phenolic acid metabolites that interfere with α-synuclein misfolding [304]. Preclinical investigations have demonstrated that dietary supplementation with select bioactive polyphenol-rich dietary preparations, such as a grape seed polyphenol ex-tract (GSPE) and a standardized Bioactive Dietary Polyphenol Preparation (BDPP, comprised of a select Concord grape juice, GSPE, and resveratrol) is mechanistically effective in modulating diverse neuropathologic phenotypes [305]. Furthermore, treatment with a flavanol-rich preparation (FRP) in gnotobiotic mice yielded brain-bioavailable polyphenol metabolites [306]. A recent review highlighted that polyphenols' neuroprotective effects can be direct, after the metabolites of polyphenols cross the BBB, or indirect, with influences on gut microbial communities [307]. Clinical trials have demonstrated that polyphenol metabolites are positively associated with cerebral blood flow and oxygenation, resulting in direct neuroprotective effects [308,309]. Meanwhile, the gut microbiome can produce active metabolites of polyphenols, which indirectly enhance their neuroprotective capacity, an example of which is curcumin being transformed into demethylcurcumin and bisdemethoxycurcumin [307,310]. Therefore, understanding the interaction between polyphenols and gut microbiota, and identifying the

specific microbes that enhance the efficacy of polyphenols is crucial for developing therapeutic strategies and precision nutrition based on the microbiome.

5.2. Anti-α-Synuclein Microbial Polyphenol Metabolites

As α-synuclein is a crucial therapeutic target for PD, inhibiting its aggregation, oligomerization, and fibrillation are key strategies for disease modification [311]. Polyphenols are among the emerging therapeutic options for combatting abhorrent α-synuclein as they exist in a wide variety of chemical compounds and have the ability to produce secondary metabolites after undergoing microbial transformation by gut microbiota. These metabolites are known for their varied pharmacological properties, which have been extensively documented in scientific reviews [302,303,312,313].

Ono and colleagues [304] established two experimental groups of humanized gnotobiotic mice with diverse gut bacteria compositions and administered a polyphenol-rich preparation orally. They detected 15 polyphenol-derived microbial metabolites, which are generated by the gut microbiota fermentation of grape seed polyphenol extract, in the cecal compartment, including caffeic acid, ferulic acid (FA), gallic acid (GA), and vanillic acid at μM to sub-μM concentrations. Notably, three metabolites—3,4-dihydroxybenzoic acid (3,4-diHBA), 3-hydroxybenzoic acid (3-HBA), and 3-(3′-hydroxyphenyl) propionic acid (3-HPPA)—were found to accumulate in the brain. The in vitro study confirmed that 3-HBA, 3,4-diHBA, and 3-HPPA inhibit α-synuclein aggregation, including the formation of low-order oligomers such as dimers and trimers. The findings align with reports from another study that showed that 3-HBA and 3-HPPA prevent the misfolding and assembly of Aβ peptides into neurotoxic aggregates, such as Aβ oligomers [314]. Furthermore, using the A53T mutant Drosophila model of PD, these metabolites effectively improved motor dysfunction, indicating a mitigating effect on mutant α-synuclein-mediated neurotoxicity [315]. Very recently, 3-HPPA, 3,4-diHBA, 3-HBA, and 4-HBA were found to significantly attenuate intracellular α-synuclein seeding aggregation in a cell-based system, and

the findings were confirmed using insoluble α-synuclein aggregates extracted from post-mortem Multiple System Atrophy (MSA) and PD brain specimens [257].

6. Conclusion

Dietary polyphenols are promising protective agents for PD prevention because of their abundance and relatively low toxicity [316]. Previous studies have shown that polyphenols can combat PD through multiple mechanisms, including reducing neuronal apoptosis, attenuating oxidative stress, and downregulating neuroinflammation. Recent evidence has also demonstrated that polyphenols inhibit the formation and spread of α-synuclein aggregates, a hallmark of PD pathology, which may originate from the gut. Furthermore, polyphenols are capable of regulating the gut microbiota composition and its metabolic pathways, mostly through the inhibition of pathogenic bacteria and the stimulation of beneficial bacteria [291]; in turn, polyphenols are extensively metabolized by gut bacteria, resulting in the generation of bioactive secondary metabolites that enhance their bioavailability.

While polyphenols showed benefits in animal models of PD, evidence from epidemiological studies primarily demonstrated the association between the dietary intake of polyphenols and a reduced risk of developing PD. However, data from prospective randomized controlled trials in patients with pre-existing PD are limited. Therefore, future clinical studies are necessary to evaluate the effectiveness of dietary polyphenols in slowing the progression of PD. Although the existing evidence points to the potential of polyphenols to favorably modulate the gut–brain axis, it is evident that more focused research is needed to fully understand the mechanisms between polyphenols, the gut microbiota, and α-synuclein in order to establish the therapeutic viability of polyphenols in clinical settings.

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References

1. Tysnes, O.B.; Storstein, A. Epidemiology of Parkinson's disease. J. Neural Transm. 2017, 124, 901–905. [Google Scholar] [CrossRef] [PubMed]
2. Cerri, S.; Mus, L.; Blandini, F. Parkinson's Disease in Women and Men: What's the Difference? J. Parkinson's Dis. 2019, 9, 501–515. [Google Scholar] [CrossRef] [PubMed]
3. Marras, C.; Beck, J.C.; Bower, J.H.; Roberts, E.; Ritz, B.; Ross, G.W.; Abbott, R.D.; Savica, R.; Van Den Eeden, S.K.; Willis, A.W.; et al. Prevalence of Parkinson's disease across North America. NPJ Parkinson's Dis. 2018, 4, 21. [Google Scholar] [CrossRef] [PubMed]
4. Armstrong, M.J.; Okun, M.S. Diagnosis and Treatment of Parkinson Disease: A Review. JAMA 2020, 323, 548–560. [Google Scholar] [CrossRef] [PubMed]
5. Fahn, S. Description of Parkinson's disease as a clinical syndrome. Ann. N. Y. Acad. Sci. 2003, 991, 1–14. [Google Scholar] [CrossRef] [PubMed]
6. Forno, L.S. The Lewy body in Parkinson's disease. Adv. Neurol. 1987, 45, 35–43. [Google Scholar] [PubMed]
7. Goedert, M. Alpha-synuclein and neurodegenerative diseases. Nat. Rev. Neurosci. 2001, 2, 492–501. [Google Scholar] [CrossRef] [PubMed]
8. Bras, I.C.; Xylaki, M.; Outeiro, T.F. Mechanisms of alpha-synuclein toxicity: An update and outlook. Prog. Brain Res. 2020, 252, 91–129. [Google Scholar]
9. Omotosho, A.O.; Tajudeen, Y.A.; Oladipo, H.J.; Yusuff, S.I.; AbdulKadir, M.; Muili, A.O.; Egbewande, O.M.; Yusuf, R.O.; Faniran, Z.O.; Afolabi, A.O.; et al. Parkinson's disease: Are gut microbes involved? Brain Behav. 2023, 13, e3130. [Google Scholar] [CrossRef]
10. Li, Q.; Meng, L.-B.; Chen, L.-J.; Shi, X.; Tu, L.; Zhou, Q.; Yu, J.-L.; Liao, X.; Zeng, Y.; Yuan, Q.-Y. The role of the microbiota-gut-brain axis and intestinal microbiome

dysregulation in Parkinson's disease. Front. Neurol. 2023, 14, 1185375. [Google Scholar] [CrossRef]

11. Pant, A.; Bisht, K.S.; Aggarwal, S.; Maiti, T.K. Human gut microbiota and Parkinson's disease. Prog. Mol. Biol. Transl. Sci. 2022, 192, 281–307. [Google Scholar] [PubMed]
12. Dogra, N.; Mani, R.J.; Katare, D.P. The Gut-Brain Axis: Two Ways Signaling in Parkinson's Disease. Cell. Mol. Neurobiol. 2022, 42, 315–332. [Google Scholar] [CrossRef] [PubMed]
13. Menozzi, E.; Macnaughtan, J.; Schapira, A.H.V. The gut-brain axis and Parkinson disease: Clinical and pathogenetic relevance. Ann. Med. 2021, 53, 611–625. [Google Scholar] [CrossRef]
14. Elsworth, J.D. Parkinson's disease treatment: Past, present, and future. J. Neural Transm. 2020, 127, 785–791. [Google Scholar] [CrossRef] [PubMed]
15. Solla, P.; Cannas, A.; Congia, S.; Floris, G.; Aste, R.; Tacconi, P.; Marrosu, M.G. Levodopa/carbidopa/entacapone-induced acute Pisa syndrome in a Parkinson's disease patient. J. Neurol. Sci. 2008, 275, 154–156. [Google Scholar] [CrossRef] [PubMed]
16. Spillantini, M.G.; Schmidt, M.L.; Lee, V.M.; Trojanowski, J.Q.; Jakes, R.; Goedert, M. Alpha-synuclein in Lewy bodies. Nature 1997, 388, 839–840. [Google Scholar] [CrossRef] [PubMed]
17. Dehay, B.; Bourdenx, M.; Gorry, P.; Przedborski, S.; Vila, M.; Hunot, S.; Singleton, A.; Olanow, C.W.; Merchant, K.M.; Bezard, E.; et al. Targeting α-synuclein for treatment of Parkinson's disease: Mechanistic and therapeutic considerations. Lancet Neurol. 2015, 14, 855–866. [Google Scholar] [CrossRef] [PubMed]
18. Norris, E.H.; Giasson, B.I.; Lee, V.M. Alpha-synuclein: Normal function and role in neurodegenerative diseases. Curr. Top. Dev. Biol. 2004, 60, 17–54. [Google Scholar] [PubMed]

19. Singleton, A.B.; Farrer, M.; Johnson, J.; Singleton, A.; Hague, S.; Kachergus, J.; Hulihan, M.; Peuralinna, T.; Dutra, A.; Nussbaum, R.; et al. α-Synuclein locus triplication causes Parkinson's disease. Science 2003, 302, 841. [Google Scholar] [CrossRef]

20. Karpowicz, R.J.; Trojanowski, J.Q.; Lee, V.M.-Y. Transmission of alpha-synuclein seeds in neurodegenerative disease: Recent developments. Lab. Investig. 2019, 99, 971–981. [Google Scholar] [CrossRef]

21. Burré, J.; Sharma, M.; Tsetsenis, T.; Buchman, V.; Etherton, M.R.; Südhof, T.C. Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. Science 2010, 329, 1663–1667. [Google Scholar] [CrossRef] [PubMed]

22. Walker, L.C.; Jucker, M. Neurodegenerative diseases: Expanding the prion concept. Annu. Rev. Neurosci. 2015, 38, 87–103. [Google Scholar]

23. Lashuel, H.A.; Overk, C.R.; Oueslati, A.; Masliah, E. The many faces of α-synuclein: From structure and toxicity to therapeutic target. Nat. Rev. Neurosci. 2013, 14, 38–48. [Google Scholar] [CrossRef] [PubMed]

24. Mehra, S.; Sahay, S.; Maji, S.K. α-Synuclein misfolding and aggregation: Implications in Parkinson's disease pathogenesis. Biochim. Biophys. Acta (BBA)-Proteins Proteom. 2019, 1867, 890–908. [Google Scholar] [CrossRef] [PubMed]

25. Anderson, J.P.; Walker, D.E.; Goldstein, J.M.; de Laat, R.; Banducci, K.; Caccavello, R.J.; Barbour, R.; Huang, J.; Kling, K.; Lee, M.; et al. Phosphorylation of Ser-129 is the dominant pathological modification of α-synuclein in familial and sporadic Lewy body disease. J. Biol. Chem. 2006, 281, 29739–29752. [Google Scholar] [CrossRef] [PubMed]

26. Yasuda, T.; Nakata, Y.; Mochizuki, H. Alpha-Synuclein and neuronal cell death. Mol. Neurobiol. 2013, 47, 466–483. [Google Scholar] [CrossRef]

27. Benskey, M.J.; Perez, R.G.; Manfredsson, F.P. The contribution of alpha synuclein to neuronal survival and function—Implications for Parkinson's disease. J. Neurochem. 2016, 137, 331–359. [Google Scholar] [CrossRef]

28. Pozo Devoto, V.M.; Falzone, T.L. Mitochondrial dynamics in Parkinson's disease: A role for alpha-synuclein? Dis. Model. Mech. 2017, 10, 1075–1087. [Google Scholar] [CrossRef]

29. Lee, S.J. Alpha-synuclein aggregation: A link between mitochondrial defects and Parkinson's disease? Antioxid. Redox Signal. 2003, 5, 337–348. [Google Scholar] [CrossRef]

30. Mullin, S.; Schapira, A. Alpha-Synuclein and mitochondrial dysfunction in Parkinson's disease. Mol. Neurobiol. 2013, 47, 587–597. [Google Scholar] [CrossRef]

31. Ozawa, T.; Tanaka, M.; Suzuki, H.; Nishikimi, M. Structure and function of mitochondria: Their organization and disorders. Brain Dev. 1987, 9, 76–81. [Google Scholar] [CrossRef] [PubMed]

32. Sherratt, H.S. Mitochondria: Structure and function. Rev. Neurol. 1991, 147, 417–430. [Google Scholar] [PubMed]

33. Sohrabi, T.; Mirzaei-Behbahani, B.; Zadali, R.; Pirhaghi, M.; Morozova-Roche, L.A.; Meratan, A.A. Common Mechanisms Underlying alpha-Synuclein-Induced Mitochondrial Dysfunction in Parkinson's Disease. J. Mol. Biol. 2023, 435, 167992. [Google Scholar] [CrossRef] [PubMed]

34. Bellani, S.; Sousa, V.L.; Ronzitti, G.; Valtorta, F.; Meldolesi, J.; Chieregatti, E. The regulation of synaptic function by alpha-synuclein. Commun. Integr. Biol. 2010, 3, 106–109. [Google Scholar] [CrossRef] [PubMed]

35. Burre, J. The Synaptic Function of alpha-Synuclein. J. Parkinson's Dis. 2015, 5, 699–713. [Google Scholar] [CrossRef] [PubMed]

36. Parra-Rivas, L.A.; Madhivanan, K.; Aulston, B.D.; Wang, L.; Prakashchand, D.D.; Boyer, N.P.; Saia-Cereda, V.M.; Branes-Guerrero, K.; Pizzo, D.P.; Bagchi, P.; et al. Serine-129 phosphorylation of alpha-synuclein is an activity-dependent trigger for physiologic

protein-protein interactions and synaptic function. Neuron 2023, 111, 4006–4023.e10. [Google Scholar] [CrossRef] [PubMed]

37. Sharma, M.; Burre, J. Alpha-Synuclein in synaptic function and dysfunction. Trends Neurosci. 2023, 46, 153–166. [Google Scholar] [CrossRef] [PubMed]

38. Desplats, P.; Lee, H.-J.; Bae, E.-J.; Patrick, C.; Rockenstein, E.; Crews, L.; Spencer, B.; Masliah, E.; Lee, S.-J. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of α-synuclein. Proc. Natl. Acad. Sci. USA 2009, 106, 13010–13015. [Google Scholar] [CrossRef] [PubMed]

39. Mougenot, A.-L.; Nicot, S.; Bencsik, A.; Morignat, E.; Verchère, J.; Lakhdar, L.; Legastelois, S.; Baron, T. Prion-like acceleration of a synucleinopathy in a transgenic mouse model. Neurobiol. Aging 2012, 33, 2225–2228. [Google Scholar] [CrossRef]

40. Kordower, J.H.; Chu, Y.; Hauser, R.A.; Olanow, C.; Freeman, T.B. Transplanted dopaminergic neurons develop PD pathologic changes: A second case report. Mov. Disord. 2008, 23, 2303–2306. [Google Scholar]

41. Conway, K.A.; Harper, J.D.; Lansbury, P.T. Accelerated in vitro fibril formation by a mutant α-synuclein linked to early-onset Parkinson disease. Nat. Med. 1998, 4, 1318–1320. [Google Scholar] [CrossRef] [PubMed]

42. Hansen, C.; Angot, E.; Bergström, A.-L.; Steiner, J.A.; Pieri, L.; Paul, G.; Outeiro, T.F.; Melki, R.; Kallunki, P.; Fog, K.; et al. α-Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. J. Clin. Investig. 2011, 121, 715–725. [Google Scholar] [CrossRef] [PubMed]

43. Ullah, H.; Arbab, S.; Tian, Y.; Liu, C.-Q.; Chen, Y.; Qijie, L.; Khan, M.I.U.; Hassan, I.U.; Li, K. The gut microbiota-brain axis in neurological disorder. Front. Neurosci. 2023, 17, 1225875. [Google Scholar] [CrossRef] [PubMed]

44. Scheperjans, F.; Aho, V.; Pereira, P.A.B.; Koskinen, K.; Paulin, L.; Pekkonen, E.; Haapaniemi, E.; Kaakkola, S.; Eerola-Rautio, J.; Pohja, M.; et al. Gut microbiota are

related to Parkinson's disease and clinical phenotype. Mov. Disord. 2015, 30, 350–358. [Google Scholar] [CrossRef] [PubMed]

45. Yang, Z.-X.; Zhang, Y.; Wang, Q.; Zhang, L.; Liu, Y.-F.; Zhang, Y.; Ren, Y.; Zhou, C.; Gao, H.-W.; Zhang, N.-X.; et al. Addition of α-synuclein aggregates to the intestinal environment recapitulates Parkinsonian symptoms in model systems. Acta Pharmacol. Sin. 2024, 45, 36–51. [Google Scholar] [CrossRef] [PubMed]

46. Wang, K.; Zhang, C.; Zhang, B.; Li, G.; Shi, G.; Cai, Q.; Huang, M. Gut dysfunction may be the source of pathological aggregation of alpha-synuclein in the central nervous system through Paraquat exposure in mice. Ecotoxicol. Environ. Saf. 2022, 246, 114152. [Google Scholar] [CrossRef] [PubMed]

47. Holmqvist, S.; Chutna, O.; Bousset, L.; Aldrin-Kirk, P.; Li, W.; Björklund, T.; Wang, Z.-Y.; Roybon, L.; Melki, R.; Li, J.-Y. Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. Acta Neuropathol. 2014, 128, 805–820. [Google Scholar] [CrossRef] [PubMed]

48. Quigley, E.M.M. Microbiota-Brain-Gut Axis and Neurodegenerative Diseases. Curr. Neurol. Neurosci. Rep. 2017, 17, 94. [Google Scholar] [CrossRef] [PubMed]

49. Cryan, J.F.; O'Riordan, K.J.; Cowan, C.S.; Sandhu, K.V.; Bastiaanssen, T.F.; Boehme, M.; Codagnone, M.G.; Cussotto, S.; Fulling, C.; Golubeva, A.V.; et al. The Microbiota-Gut-Brain Axis. Physiol. Rev. 2019, 99, 1877–2013. [Google Scholar] [CrossRef]

50. O'Mahony, S.M.; Hyland, N.P.; Dinan, T.G.; Cryan, J.F. Maternal separation as a model of brain–gut axis dysfunction. Psychopharmacology 2011, 214, 71–88. [Google Scholar] [CrossRef]

51. Rhee, S.H.; Pothoulakis, C.; Mayer, E.A. Principles and clinical implications of the brain–gut–enteric microbiota axis. Nat. Rev. Gastroenterol. Hepatol. 2009, 6, 306–314. [Google Scholar] [CrossRef] [PubMed]

52. Carabotti, M.; Scirocco, A.; Maselli, M.A.; Severi, C. The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems. Ann. Gastroenterol. 2015, 28, 203–209. [Google Scholar] [PubMed]

53. Ma, Q.; Xing, C.; Long, W.; Wang, H.Y.; Liu, Q.; Wang, R.-F. Impact of microbiota on central nervous system and neurological diseases: The gut-brain axis. J. Neuroinflammation 2019, 16, 53. [Google Scholar] [CrossRef] [PubMed]

54. Yarandi, S.S.; Peterson, D.A.; Treisman, G.J.; Moran, T.H.; Pasricha, P.J. Modulatory effects of gut microbiota on the central nervous system: How gut could play a role in neuropsychiatric health and diseases. J. Neurogastroenterol. Motil. 2016, 22, 201. [Google Scholar] [CrossRef] [PubMed]

55. Raj, K.; Singh, S.; Chib, S.; Mallan, S. Microbiota-Brain-Gut-Axis Relevance to Parkinson's Disease: Potential Therapeutic Effects of Probiotics. Curr. Pharm. Des. 2022, 28, 3049–3067. [Google Scholar] [CrossRef] [PubMed]

56. Loh, J.S.; Mak, W.Q.; Tan, L.K.S.; Ng, C.X.; Chan, H.H.; Yeow, S.H.; Foo, J.B.; Ong, Y.S.; How, C.W.; Khaw, K.Y. Microbiota-gut-brain axis and its therapeutic applications in neurodegenerative diseases. Signal Transduct. Target Ther. 2024, 9, 37. [Google Scholar] [PubMed]

57. Chen, S.-J.; Lin, C.-H. Gut microenvironmental changes as a potential trigger in Parkinson's disease through the gut-brain axis. J. Biomed. Sci. 2022, 29, 54. [Google Scholar] [CrossRef] [PubMed]

58. Dowling, L.R.; Strazzari, M.R.; Keely, S.; Kaiko, G.E. Enteric nervous system and intestinal epithelial regulation of the gut-brain axis. J. Allergy Clin. Immunol. 2022, 150, 513–522. [Google Scholar] [CrossRef] [PubMed]

59. dos Santos, J.C.C.; Oliveira, L.F.; Noleto, F.M.; Gusmão, C.T.P.; Brito, G.A.d.C.; Viana, G.S.d.B. Gut-microbiome-brain axis: The crosstalk between the vagus nerve, alpha-

synuclein and the brain in Parkinson's disease. Neural Regen. Res. 2023, 18, 2611–2614. [Google Scholar] [CrossRef]

60. Tan, A.H.; Chong, C.W.; Lim, S.; Yap, I.K.S.; Teh, C.S.J.; Loke, M.F.; Song, S.; Tan, J.Y.; Ang, B.H.; Tan, Y.Q.; et al. Gut Microbial Ecosystem in Parkinson Disease: New Clinicobiological Insights from Multi-Omics. Ann. Neurol. 2021, 89, 546–559. [Google Scholar] [CrossRef]

61. Elfil, M.; Kamel, S.; Kandil, M.; Koo, B.B.; Schaefer, S.M. Implications of the Gut Microbiome in Parkinson's Disease. Mov. Disord. 2020, 35, 921–933. [Google Scholar] [CrossRef]

62. Nie, S.; Ge, Y. The link between the gut microbiome, inflammation, and Parkinson's disease. Appl. Microbiol. Biotechnol. 2023, 107, 6737–6749. [Google Scholar] [CrossRef]

63. Nie, S.; Wang, J.; Deng, Y.; Ye, Z.; Ge, Y. Inflammatory microbes and genes as potential biomarkers of Parkinson's disease. NPJ Biofilms Microbiomes 2022, 8, 101. [Google Scholar] [CrossRef] [PubMed]

64. Zhu, M.; Liu, X.; Ye, Y.; Yan, X.; Cheng, Y.; Zhao, L.; Chen, F.; Ling, Z. Gut Microbiota: A Novel Therapeutic Target for Parkinson's Disease. Front. Immunol. 2022, 13, 937555. [Google Scholar] [CrossRef] [PubMed]

65. Romano, S.; Savva, G.M.; Bedarf, J.R.; Charles, I.G.; Hildebrand, F.; Narbad, A. Meta-analysis of the Parkinson's disease gut microbiome suggests alterations linked to intestinal inflammation. NPJ Parkinson's Dis. 2021, 7, 27. [Google Scholar] [CrossRef]

66. Sampson, T.R.; Debelius, J.W.; Thron, T.; Janssen, S.; Shastri, G.G.; Ilhan, Z.E.; Challis, C.; Schretter, C.E.; Rocha, S.; Gradinaru, V.; et al. Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. Cell 2016, 167, 1469–1480.e12. [Google Scholar] [CrossRef]

67. Clairembault, T.; Leclair-Visonneau, L.; Coron, E.; Bourreille, A.; Le Dily, S.; Vavasseur, F.; Heymann, M.-F.; Neunlist, M.; Derkinderen, P. Structural alterations of the intestinal epithelial barrier in Parkinson's disease. Acta Neuropathol. Commun. 2015, 3, 12. [Google Scholar] [CrossRef]

68. Keshavarzian, A.; Green, S.J.; Engen, P.A.; Voigt, R.M.; Naqib, A.; Forsyth, C.B.; Mutlu, E.; Shannon, K.M. Colonic bacterial composition in Parkinson's disease. Mov. Disord. 2015, 30, 1351–1360. [Google Scholar] [CrossRef] [PubMed]

69. Devos, D.; Lebouvier, T.; Lardeux, B.; Biraud, M.; Rouaud, T.; Pouclet, H.; Coron, E.; Bruley des Varannes, S.; Naveilhan, P.; Nguyen, J.-M.; et al. Colonic inflammation in Parkinson's disease. Neurobiol. Dis. 2013, 50, 42–48. [Google Scholar] [CrossRef]

70. Forsyth, C.B.; Shannon, K.M.; Kordower, J.H.; Voigt, R.M.; Shaikh, M.; Jaglin, J.A.; Estes, J.D.; Dodiya, H.B.; Keshavarzian, A. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. PLoS ONE 2011, 6, e28032. [Google Scholar] [CrossRef]

71. Li, Y.; Chen, Y.; Jiang, L.; Zhang, J.; Tong, X.; Chen, D.; Le, W. Intestinal Inflammation and Parkinson's Disease. Aging Dis. 2021, 12, 2052–2068. [Google Scholar] [CrossRef]

72. Lubomski, M.; Tan, A.H.; Lim, S.-Y.; Holmes, A.J.; Davis, R.L.; Sue, C.M. Parkinson's disease and the gastrointestinal microbiome. J. Neurol. 2020, 267, 2507–2523. [Google Scholar] [CrossRef] [PubMed]

73. Tan, A.H.; Hor, J.W.; Chong, C.W.; Lim, S. Probiotics for Parkinson's disease: Current evidence and future directions. JGH Open 2021, 5, 414–419. [Google Scholar] [CrossRef] [PubMed]

74. Brown, G.C. The endotoxin hypothesis of neurodegeneration. J. Neuroinflammation 2019, 16, 180. [Google Scholar] [CrossRef] [PubMed]

75. Silva, D.F.; Empadinhas, N.; Cardoso, S.M.; Esteves, A.R. Neurodegenerative Microbially-Shaped Diseases: Oxidative Stress Meets Neuroinflammation. Antioxidants 2022, 11, 2141. [Google Scholar] [CrossRef] [PubMed]

76. Hirayama, M.; Ohno, K. Parkinson's Disease and Gut Microbiota. Ann. Nutr. Metab. 2021, 77 (Suppl. S2), 28–35. [Google Scholar] [CrossRef] [PubMed]

77. Çamcı, G.; Oğuz, S. Association between Parkinson's Disease and Helicobacter Pylori. J. Clin. Neurol. 2016, 12, 147–150. [Google Scholar] [CrossRef] [PubMed]

78. Deng, I.; Corrigan, F.; Zhai, G.; Zhou, X.-F.; Bobrovskaya, L. Lipopolysaccharide animal models of Parkinson's disease: Recent progress and relevance to clinical disease. Brain Behav. Immun. Health 2020, 4, 100060. [Google Scholar] [CrossRef] [PubMed]

79. Cryan, J.F.; O'Riordan, K.J.; Sandhu, K.; Peterson, V.; Dinan, T.G. The gut microbiome in neurological disorders. Lancet Neurol. 2020, 19, 179–194. [Google Scholar] [CrossRef] [PubMed]

80. Heinzel, S.; Aho, V.T.E.; Suenkel, U.; Von Thaler, A.K.; Schulte, C.; Deuschle, C.; Paulin, L.; Hantunen, S.; Brockmann, K.; Eschweiler, G.W.; et al. Gut Microbiome Signatures of Risk and Prodromal Markers of Parkinson Disease. Ann. Neurol. 2021, 90, E1–E12. [Google Scholar] [CrossRef]

81. Rani, L.; Mondal, A.C. Unravelling the role of gut microbiota in Parkinson's disease progression: Pathogenic and therapeutic implications. Neurosci. Res. 2021, 168, 100–112. [Google Scholar] [CrossRef] [PubMed]

82. Aho, V.T.E.; Houser, M.C.; Pereira, P.A.B.; Chang, J.; Rudi, K.; Paulin, L.; Hertzberg, V.; Auvinen, P.; Tansey, M.G.; Scheperjans, F. Relationships of gut microbiota, short-chain fatty acids, inflammation, and the gut barrier in Parkinson's disease. Mol. Neurodegener. 2021, 16, 6. [Google Scholar] [CrossRef] [PubMed]

83. Nishiwaki, H.; Ito, M.; Ishida, T.; Hamaguchi, T.; Maeda, T.; Kashihara, K.; Tsuboi, Y.; Ueyama, J.; Shimamura, T.; Mori, H.; et al. Meta-Analysis of Gut Dysbiosis in Parkinson's Disease. Mov. Disord. 2020, 35, 1626–1635. [Google Scholar] [CrossRef]

84. Toh, T.S.; Chong, C.W.; Lim, S.-Y.; Bowman, J.; Cirstea, M.; Lin, C.-H.; Chen, C.-C.; Appel-Cresswell, S.; Finlay, B.B.; Tan, A.H. Gut microbiome in Parkinson's disease: New insights from meta-analysis. Park. Relat. Disord. 2022, 94, 1–9. [Google Scholar] [CrossRef]

85. Shen, T.; Yue, Y.; He, T.; Huang, C.; Qu, B.; Lv, W.; Lai, H.Y. The Association between the Gut Microbiota and Parkinson's Disease, a Meta-Analysis. Front. Aging Neurosci. 2021, 13, 636545. [Google Scholar] [CrossRef]

86. Zhou, S.; Li, B.; Deng, Y.; Yi, J.; Mao, G.; Wang, R.; Zeng, W.; Liu, B.; Wu, D.; Liu, F. Meta-analysis of the relations between gut microbiota and pathogens and Parkinson's disease. Adv. Clin. Exp. Med. 2023, 32, 613–621. [Google Scholar] [CrossRef] [PubMed]

87. Unger, M.M.; Spiegel, J.; Dillmann, K.-U.; Grundmann, D.; Philippeit, H.; Bürmann, J.; Faßbender, K.; Schwiertz, A.; Schäfer, K.H. Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. Park. Relat. Disord. 2016, 32, 66–72. [Google Scholar] [CrossRef]

88. Hasegawa, S.; Goto, S.; Tsuji, H.; Okuno, T.; Asahara, T.; Nomoto, K.; Shibata, A.; Fujisawa, Y.; Minato, T.; Okamoto, A.; et al. Intestinal Dysbiosis and Lowered Serum Lipopolysaccharide-Binding Protein in Parkinson's Disease. PLoS ONE 2015, 10, e0142164. [Google Scholar] [CrossRef]

89. Wallen, Z.D.; Appah, M.; Dean, M.N.; Sesler, C.L.; Factor, S.A.; Molho, E.; Zabetian, C.P.; Standaert, D.G.; Payami, H. Characterizing dysbiosis of gut microbiome in PD: Evidence for overabundance of opportunistic pathogens. NPJ Park. Dis 2020, 6, 11. [Google Scholar] [CrossRef]

90. Heintz-Buschart, A.; Pandey, U.; Wicke, T.; Sixel-Döring, F.; Janzen, A.; Sittig-Wiegand, E.; Trenkwalder, C.; Oertel, W.H.; Mollenhauer, B.; Wilmes, P. The nasal and gut microbiome in Parkinson's disease and idiopathic rapid eye movement sleep behavior disorder. Mov. Disord. 2018, 33, 88–98. [Google Scholar] [CrossRef]

91. Bedarf, J.R.; Hildebrand, F.; Coelho, L.P.; Sunagawa, S.; Bahram, M.; Goeser, F.; Bork, P.; Wüllner, U. Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naive Parkinson's disease patients. Genome Med. 2017, 9, 39. [Google Scholar] [CrossRef]

92. Cirstea, M.S.; Yu, A.C.; Golz, E.; Sundvick, K.; Kliger, D.; Radisavljevic, N.; Foulger, L.H.; Mackenzie, M.; Huan, T.; Finlay, B.B.; et al. Microbiota Composition and Metabolism Are Associated with Gut Function in Parkinson's Disease. Mov. Disord. 2020, 35, 1208–1217. [Google Scholar] [CrossRef]

93. Petrov, V.A.; Saltykova, I.V.; Zhukova, I.A.; Alifirova, V.M.; Zhukova, N.G.; Dorofeeva, Y.B.; Tyakht, A.V.; Kovarsky, B.A.; Alekseev, D.G.; Kostryukova, E.S.; et al. Analysis of Gut Microbiota in Patients with Parkinson's Disease. Bull. Exp. Biol. Med. 2017, 162, 734–737. [Google Scholar] [CrossRef] [PubMed]

94. Caputi, V.; Giron, M.C. Microbiome-Gut-Brain Axis and Toll-Like Receptors in Parkinson's Disease. Int. J. Mol. Sci. 2018, 19, 1689. [Google Scholar] [CrossRef]

95. Barichella, M.; Severgnini, M.; Cilia, R.; Cassani, E.; Bolliri, C.; Caronni, S.; Ferri, V.; Cancello, R.; Ceccarani, C.; Faierman, S.; et al. Unraveling gut microbiota in Parkinson's disease and atypical parkinsonism. Mov. Disord. 2019, 34, 396–405. [Google Scholar] [CrossRef]

96. Hill-Burns, E.M.; Debelius, J.W.; Morton, J.T.; Wissemann, W.T.; Lewis, M.R.; Wallen, Z.D.; Peddada, S.D.; Factor, S.A.; Molho, E.; Zabetian, C.P.; et al. Parkinson's disease and Parkinson's disease medications have distinct signatures of the gut microbiome. Mov. Disord. 2017, 32, 739–749. [Google Scholar] [CrossRef] [PubMed]

97. Lin, C.-H.; Chen, C.-C.; Chiang, H.-L.; Liou, J.-M.; Chang, C.-M.; Lu, T.-P.; Chuang, E.Y.; Tai, Y.-C.; Cheng, C.; Lin, H.-Y.; et al. Altered gut microbiota and inflammatory cytokine responses in patients with Parkinson's disease. J. Neuroinflammation 2019, 16, 129. [Google Scholar] [CrossRef] [PubMed]

98. Hopfner, F.; Künstner, A.; Müller, S.H.; Künzel, S.; Zeuner, K.E.; Margraf, N.G.; Deuschl, G.; Baines, J.F.; Kuhlenbäumer, G. Gut microbiota in Parkinson disease in a northern German cohort. Brain Res. 2017, 1667, 41–45. [Google Scholar] [CrossRef]

99. Li, W.; Wu, X.; Hu, X.; Wang, T.; Liang, S.; Duan, Y.; Jin, F.; Qin, B. Structural changes of gut microbiota in Parkinson's disease and its correlation with clinical features. Sci. China Life Sci. 2017, 60, 1223–1233. [Google Scholar] [CrossRef]

100. Li, F.; Wang, P.; Chen, Z.; Sui, X.; Xie, X.; Zhang, J. Alteration of the fecal microbiota in North-Eastern Han Chinese population with sporadic Parkinson's disease. Neurosci. Lett. 2019, 707, 134297. [Google Scholar] [CrossRef]

101. Pietrucci, D.; Cerroni, R.; Unida, V.; Farcomeni, A.; Pierantozzi, M.; Mercuri, N.B.; Biocca, S.; Stefani, A.; Desideri, A. Dysbiosis of gut microbiota in a selected population of Parkinson's patients. Park. Relat. Disord. 2019, 65, 124–130. [Google Scholar] [CrossRef] [PubMed]

102. Lin, A.; Zheng, W.; He, Y.; Tang, W.; Wei, X.; He, R.; Huang, W.; Su, Y.; Huang, Y.; Zhou, H.; et al. Gut microbiota in patients with Parkinson's disease in southern China. Park. Relat. Disord. 2018, 53, 82–88. [Google Scholar] [CrossRef] [PubMed]

103. Zhang, F.; Yue, L.; Fang, X.; Wang, G.; Li, C.; Sun, X.; Jia, X.; Yang, J.; Song, J.; Zhang, Y.; et al. Altered gut microbiota in Parkinson's disease patients/healthy spouses and its association with clinical features. Park. Relat. Disord. 2020, 81, 84–88. [Google Scholar] [CrossRef] [PubMed]

104. Mehanna, M.; AbuRaya, S.; Ahmed, S.M.; Ashmawy, G.; Ibrahim, A.; AbdelKhaliq, E. Study of the gut microbiome in Egyptian patients with Parkinson's Disease. BMC Microbiol. 2023, 23, 196. [Google Scholar] [CrossRef] [PubMed]

105. Hertel, J.; Harms, A.C.; Heinken, A.; Baldini, F.; Thinnes, C.C.; Glaab, E.; Vasco, D.A.; Pietzner, M.; Stewart, I.; Wareham, N.; et al. Integrated Analyses of Microbiome and Longitudinal Metabolome Data Reveal Microbial-Host Interactions on Sulfur Metabolism in Parkinson's Disease. Cell Rep. 2019, 29, 1767–1777.e8. [Google Scholar] [CrossRef] [PubMed]

106. Qian, Y.; Yang, X.; Xu, S.; Huang, P.; Li, B.; Du, J.; He, Y.; Su, B.; Xu, L.-M.; Wang, L.; et al. Gut metagenomics-derived genes as potential biomarkers of Parkinson's disease. Brain 2020, 143, 2474–2489. [Google Scholar] [CrossRef] [PubMed]

107. Chen, S.-J.; Chen, C.-C.; Liao, H.-Y.; Lin, Y.-T.; Wu, Y.-W.; Liou, J.-M.; Wu, M.-S.; Kuo, C.-H.; Lin, C.-H. Association of Fecal and Plasma Levels of Short-Chain Fatty Acids with Gut Microbiota and Clinical Severity in Patients with Parkinson Disease. Neurology 2022, 98, e848–e858. [Google Scholar] [CrossRef]

108. Kalyanaraman, B.; Cheng, G.; Hardy, M. Gut microbiome, short-chain fatty acids, alpha-synuclein, neuroinflammation, and ROS/RNS: Relevance to Parkinson's disease and therapeutic implications. Redox Biol. 2024, 71, 103092. [Google Scholar] [CrossRef]

109. Schaeffer, E.; Kluge, A.; Bottner, M.; Zunke, F.; Cossais, F.; Berg, D.; Arnold, P. Alpha Synuclein Connects the Gut-Brain Axis in Parkinson's Disease Patients—A View on Clinical Aspects, Cellular Pathology and Analytical Methodology. Front. Cell. Dev. Biol. 2020, 8, 573696. [Google Scholar] [CrossRef]

110. Böttner, M.; Zorenkov, D.; Hellwig, I.; Barrenschee, M.; Harde, J.; Fricke, T.; Deuschl, G.; Egberts, J.-H.; Becker, T.; Fritscher-Ravens, A.; et al. Expression pattern and localization of alpha-synuclein in the human enteric nervous system. Neurobiol. Dis. 2012, 48, 474–480. [Google Scholar] [CrossRef]

111. Barrenschee, M.; Zorenkov, D.; Böttner, M.; Lange, C.; Cossais, F.; Scharf, A.B.; Deuschl, G.; Schneider, S.A.; Ellrichmann, M.; Fritscher-Ravens, A.; et al. Distinct pattern of enteric phospho-alpha-synuclein aggregates and gene expression profiles in patients with Parkinson's disease. Acta Neuropathol. Commun. 2017, 5, 1. [Google Scholar] [CrossRef]

112. Sánchez-Ferro, Á.; Rábano, A.; Catalán, M.J.; Rodríguez-Valcárcel, F.C.; Díez, S.F.; Herreros-Rodríguez, J.; García-Cobos, E.; Álvarez-Santullano, M.M.; López-Manzanares, L.; Mosqueira, A.J.; et al. In vivo gastric detection of alpha-synuclein inclusions in Parkinson's disease. Mov. Disord. 2015, 30, 517–524. [Google Scholar] [CrossRef] [PubMed]

113. Shannon, K.M.; Keshavarzian, A.; Mutlu, E.; Dodiya, H.B.; Daian, D.; Jaglin, J.A.; Kordower, J.H. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. Mov. Disord. 2012, 27, 709–715. [Google Scholar] [CrossRef]

114. Fayyad, M.; Salim, S.; Majbour, N.; Erskine, D.; Stoops, E.; Mollenhauer, B.; El-Agnaf, O.M.A. Parkinson's disease biomarkers based on alpha-synuclein. J. Neurochem. 2019, 150, 626–636. [Google Scholar] [CrossRef] [PubMed]

115. Hawkes, C.H.; Del Tredici, K.; Braak, H. A timeline for Parkinson's disease. Park. Relat. Disord. 2010, 16, 79–84. [Google Scholar] [CrossRef]

116. Schmitt, V.; Masanetz, R.K.; Weidenfeller, M.; Ebbinghaus, L.S.; Süß, P.; Rosshart, S.P.; von Hörsten, S.; Zunke, F.; Winkler, J.; Xiang, W. Gut-to-brain spreading of pathology in synucleinopathies: A focus on molecular signalling mediators. Behav. Brain Res. 2023, 452, 114574. [Google Scholar] [CrossRef]

117. Braak, H.; Rüb, U.; Gai, W.P.; Del Tredici, K. Idiopathic Parkinson's disease: Possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. J. Neural Transm. 2003, 110, 517–536. [Google Scholar] [CrossRef] [PubMed]

118. Visanji, N.P.; Brooks, P.L.; Hazrati, L.-N.; Lang, A.E. The prion hypothesis in Parkinson's disease: Braak to the future. Acta Neuropathol. Commun. 2013, 1, 2. [Google Scholar] [CrossRef]

119. Kim, S.; Kwon, S.-H.; Kam, T.-I.; Panicker, N.; Karuppagounder, S.S.; Lee, S.; Lee, J.H.; Kim, W.R.; Kook, M.; Foss, C.A.; et al. Transneuronal Propagation of Pathologic alpha-Synuclein from the Gut to the Brain Models Parkinson's Disease. Neuron 2019, 103, 627–641.e7. [Google Scholar] [CrossRef]

120. Dautan, D.; Paslawski, W.; Montejo, S.G.; Doyon, D.C.; Marangiu, R.; Kaplitt, M.G.; Chen, R.; Dawson, V.L.; Zhang, X.; Dawson, T.M.; et al. Gut-Initiated Alpha Synuclein Fibrils Drive Parkinson's Disease Phenotypes: Temporal Mapping of non-Motor Symptoms and REM Sleep Behavior Disorder. bioRxiv 2024. [Google Scholar] [CrossRef]

121. Anis, E.; Xie, A.; Brundin, L.; Brundin, P. Digesting recent findings: Gut alpha-synuclein, microbiome changes in Parkinson's disease. Trends Endocrinol. Metab. 2023, 34, 426. [Google Scholar] [CrossRef] [PubMed]

122. Yan, Y.; Ren, S.; Duan, Y.; Lu, C.; Niu, Y.; Wang, Z.; Inglis, B.; Ji, W.; Zheng, Y.; Si, W. Gut microbiota and metabolites of alpha-synuclein transgenic monkey models with early stage of Parkinson's disease. NPJ Biofilms Microbiomes 2021, 7, 69. [Google Scholar] [CrossRef] [PubMed]

123. Nielsen, S.D.; Pearson, N.M.; Seidler, K. The link between the gut microbiota and Parkinson's Disease: A systematic mechanism review with focus on α-synuclein transport. Brain Res. 2021, 1769, 147609. [Google Scholar] [CrossRef] [PubMed]

124. de Ora, L.O.; Balsamo, J.M.; Uyeda, K.S.; Bess, E.N. Discovery of a Gut Bacterial Metabolic Pathway that Drives alpha-Synuclein Aggregation. ACS Chem. Biol. 2024, 19, 1011–1021. [Google Scholar] [CrossRef] [PubMed]

125. Parikh, N.S.; Ahlawat, R. Helicobacter Pylori; StatPearls: Treasure Island, FL, USA, 2023. [Google Scholar]

126. Fernandez-Espejo, E. Microorganisms associated with increased risk of Parkinson's disease. Neurologia 2022, 38, 495–503. [Google Scholar] [CrossRef] [PubMed]

127. Hawkes, C.H.; Del Tredici, K.; Braak, H. Parkinson's disease: A dual-hit hypothesis. Neuropathol. Appl. Neurobiol. 2007, 33, 599–614. [Google Scholar] [CrossRef] [PubMed]

128. Barnhart, M.M.; Chapman, M.R. Curli biogenesis and function. Annu. Rev. Microbiol. 2006, 60, 131–147. [Google Scholar] [CrossRef] [PubMed]

129. Chen, S.G.; Stribinskis, V.; Rane, M.J.; Demuth, D.R.; Gozal, E.; Roberts, A.M.; Jagadapillai, R.; Liu, R.; Choe, K.; Shivakumar, B.; et al. Exposure to the Functional Bacterial Amyloid Protein Curli Enhances Alpha-Synuclein Aggregation in Aged Fischer 344 Rats and Caenorhabditis elegans. Sci. Rep. 2016, 6, 34477. [Google Scholar] [CrossRef]

130. Sampson, T.R.; Challis, C.; Jain, N.; Moiseyenko, A.; Ladinsky, M.S.; Shastri, G.G.; Thron, T.; Needham, B.D.; Horvath, I.; Debelius, J.W.; et al. A gut bacterial amyloid promotes alpha-synuclein aggregation and motor impairment in mice. eLife 2020, 9, e53111. [Google Scholar] [CrossRef]

131. Braak, H.; Del Tredici, K.; Rüb, U.; de Vos, R.A.; Steur, E.N.J.; Braak, E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol. Aging 2003, 24, 197–211. [Google Scholar] [CrossRef]

132. Hawkes, C.H.; Del Tredici, K.; Braak, H. Parkinson's disease: The dual hit theory revisited. Ann. N. Y. Acad. Sci. 2009, 1170, 615–622. [Google Scholar] [CrossRef] [PubMed]

133. Beach, T.G.; White, C.L.; Hladik, C.L.; Sabbagh, M.N.; Connor, D.J.; Shill, H.A.; Sue, L.I.; Sasse, J.; Bachalakuri, J.; Henry-Watson, J. Olfactory bulb α-synucleinopathy has

high specificity and sensitivity for Lewy body disorders. Acta Neuropathol. 2009, 117, 169–174. [Google Scholar] [CrossRef] [PubMed]

134. Cersosimo, M.G.; Benarroch, E.E. Pathological correlates of gastrointestinal dysfunction in Parkinson's disease. Neurobiol. Dis. 2012, 46, 559–564. [Google Scholar] [CrossRef] [PubMed]

135. Doty, R.L. Olfaction in Parkinson's disease and related disorders. Neurobiol. Dis. 2012, 46, 527–552. [Google Scholar] [CrossRef] [PubMed]

136. Kuo, Y.-M.; Li, Z.; Jiao, Y.; Gaborit, N.; Pani, A.K.; Orrison, B.M.; Bruneau, B.G.; Giasson, B.I.; Smeyne, R.J.; Gershon, M.D.; et al. Extensive enteric nervous system abnormalities in mice transgenic for artificial chromosomes containing Parkinson disease-associated α-synuclein gene mutations precede central nervous system changes. Hum. Mol. Genet. 2010, 19, 1633–1650. [Google Scholar] [CrossRef] [PubMed]

137. Wakabayashi, K.; Takahashi, H.; Takeda, S.; Ohama, E.; Ikuta, F. Parkinson's disease: The presence of Lewy bodies in Auerbach's and Meissner's plexuses. Acta Neuropathol. 1988, 76, 217–221. [Google Scholar] [CrossRef] [PubMed]

138. Bloch, A.; Probst, A.; Bissig, H.; Adams, H.; Tolnay, M. α-Synuclein pathology of the spinal and peripheral autonomic nervous system in neurologically unimpaired elderly subjects. Neuropathol. Appl. Neurobiol. 2006, 32, 284–295. [Google Scholar] [CrossRef]

139. Ross, G.W.; Petrovitch, H.; Abbott, R.D.; Tanner, C.M.; Popper, J.; Masaki, K.; Launer, L.; White, L.R. Association of olfactory dysfunction with risk for future Parkinson's disease. Ann. Neurol. 2008, 63, 167–173. [Google Scholar] [CrossRef]

140. Dickson, D.W.; Fujishiro, H.; DelleDonne, A.; Menke, J.; Ahmed, Z.; Klos, K.J.; Josephs, K.A.; Frigerio, R.; Burnett, M.; Parisi, J.E.; et al. Evidence that incidental Lewy body disease is pre-symptomatic Parkinson's disease. Acta Neuropathol. 2008, 115, 437–444. [Google Scholar] [CrossRef] [PubMed]

141. Noorian, A.R.; Rha, J.; Annerino, D.M.; Bernhard, D.; Taylor, G.M.; Greene, J.G. Alpha-synuclein transgenic mice display age-related slowing of gastrointestinal motility associated with transgene expression in the vagal system. Neurobiol. Dis. 2012, 48, 9–19. [Google Scholar] [CrossRef]

142. Wang, L.; Magen, I.; Yuan, P.; Subramaniam, S.R.; Richter, F.; Chesselet, M.; Taché, Y. Mice overexpressing wild-type human alpha-synuclein display alterations in colonic myenteric ganglia and defecation. Neurogastroenterol. Motil. 2012, 24, e425–e436. [Google Scholar] [CrossRef] [PubMed]

143. Kelly, L.P.; Carvey, P.M.; Keshavarzian, A.; Shannon, K.M.; Shaikh, M.; Bakay, R.A.E.; Kordower, J.H. Progression of intestinal permeability changes and alpha-synuclein expression in a mouse model of Parkinson's disease. Mov. Disord. 2014, 29, 999–1009. [Google Scholar] [CrossRef] [PubMed]

144. Natale, G.; Kastsiushenka, O.; Fulceri, F.; Ruggieri, S.; Paparelli, A.; Fornai, F. MPTP-induced parkinsonism extends to a subclass of TH-positive neurons in the gut. Brain Res. 2010, 1355, 195–206. [Google Scholar] [CrossRef] [PubMed]

145. Hallett, P.J.; McLean, J.R.; Kartunen, A.; Langston, J.W.; Isacson, O. Alpha-synuclein overexpressing transgenic mice show internal organ pathology and autonomic deficits. Neurobiol. Dis. 2012, 47, 258–267. [Google Scholar] [CrossRef] [PubMed]

146. Anselmi, L.; Bove, C.; Coleman, F.H.; Le, K.; Subramanian, M.P.; Venkiteswaran, K.; Subramanian, T.; Travagli, R.A. Ingestion of subthreshold doses of environmental toxins induces ascending Parkinsonism in the rat. NPJ Park. Dis 2018, 4, 30. [Google Scholar] [CrossRef] [PubMed]

147. Donlon, E.; Lynch, R.; Murphy, O.C.; Farrell, M.; Noel, J.; Keogan, M.; O'Connell, M.; Lynch, T. Braak's Unfinished Hypothesis: A Clinicopathological Case Report of α-Synuclein Peripheral Neuropathy Preceding Parkinsonism by 20 Years. Mov. Disord. Clin. Pract. 2021, 8, 1129–1133. [Google Scholar] [CrossRef] [PubMed]

148. Harsanyiova, J.; Buday, T.; Trancikova, A.K. Parkinson's Disease and the Gut: Future Perspectives for Early Diagnosis. Front. Neurosci. 2020, 14, 626. [Google Scholar] [CrossRef] [PubMed]

149. Chen, M.; Mor, D.E. Gut-to-Brain α-Synuclein Transmission in Parkinson's Disease: Evidence for Prion-like Mechanisms. Int. J. Mol. Sci. 2023, 24, 7205. [Google Scholar] [CrossRef] [PubMed]

150. Videlock, E.J.; Xing, T.; Yehya, A.H.S.; Travagli, R.A. Experimental models of gut-first Parkinson's disease: A systematic review. Neurogastroenterol. Motil. 2023, 35, e14604. [Google Scholar] [CrossRef] [PubMed]

151. Challis, C.; Hori, A.; Sampson, T.R.; Yoo, B.B.; Challis, R.C.; Hamilton, A.M.; Mazmanian, S.K.; Volpicelli-Daley, L.A.; Gradinaru, V. Gut-seeded α-synuclein fibrils promote gut dysfunction and brain pathology specifically in aged mice. Nat. Neurosci. 2020, 23, 327–336. [Google Scholar] [CrossRef]

152. Pandey, K.B.; Rizvi, S.I. Plant polyphenols as dietary antioxidants in human health and disease. Oxid. Med. Cell. Longev. 2009, 2, 270–278. [Google Scholar] [CrossRef] [PubMed]

153. Quideau, S.; Deffieux, D.; Douat-Casassus, C.; Pouységu, L. Plant polyphenols: Chemical properties, biological activities, and synthesis. Angew. Chem. Int. Ed. Engl. 2011, 50, 586–621. [Google Scholar] [CrossRef] [PubMed]

154. Hano, C.; Tungmunnithum, D. Plant Polyphenols, More than Just Simple Natural Antioxidants: Oxidative Stress, Aging and Age-Related Diseases. Medicines 2020, 7, 26. [Google Scholar] [CrossRef] [PubMed]

155. Kumar, N.; Goel, N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnol. Rep. 2019, 24, e00370. [Google Scholar] [CrossRef] [PubMed]

156. Sova, M.; Saso, L. Natural Sources, Pharmacokinetics, Biological Activities and Health Benefits of Hydroxycinnamic Acids and Their Metabolites. Nutrients 2020, 12, 2190. [Google Scholar] [CrossRef]

157. Aryal, S.; Skinner, T.; Bridges, B.; Weber, J.T. The Pathology of Parkinson's Disease and Potential Benefit of Dietary Polyphenols. Molecules 2020, 25, 4382. [Google Scholar] [CrossRef] [PubMed]

158. Martinez, K.B.; Mackert, J.D.; McIntosh, M.K. Polyphenols and Intestinal Health. In Nutritional and Functional Foods for Healthy Aging; Watson, R.R., Ed.; Academic Press: Cambridge, MA, USA, 2017; pp. 191–210. [Google Scholar]

159. Naczk, M.; Shahidi, F. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. J. Pharm. Biomed. Anal. 2006, 41, 1523–1542. [Google Scholar] [CrossRef] [PubMed]

160. Tsao, R.; Deng, Z. Separation procedures for naturally occurring antioxidant phytochemicals. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 2004, 812, 85–99. [Google Scholar] [CrossRef] [PubMed]

161. Liang, T.; Yue, W.; Li, Q. Comparison of the phenolic content and antioxidant activities of Apocynum venetum L. (Luo-Bu-Ma) and two of its alternative species. Int. J. Mol. Sci. 2010, 11, 4452–4464. [Google Scholar] [CrossRef] [PubMed]

162. PPatil, S.A.; Kandathil, V.; Sobha, A.; Somappa, S.B.; Feldman, M.R.; Bugarin, A.; Patil, S.A. Comprehensive Review on Medicinal Applications of Coumarin-Derived Imine-Metal Complexes. Molecules 2022, 27, 5220. [Google Scholar] [CrossRef]

163. Sharifi-Rad, J.; Cruz-Martins, N.; López-Jornet, P.; Lopez, E.P.-F.; Harun, N.; Yeskaliyeva, B.; Beyatli, A.; Sytar, O.; Shaheen, S.; Sharopov, F.; et al. Natural Coumarins: Exploring the Pharmacological Complexity and Underlying Molecular Mechanisms. Oxid. Med. Cell. Longev. 2021, 2021, 6492346. [Google Scholar] [CrossRef] [PubMed]

164. Annunziata, F.; Pinna, C.; Dallavalle, S.; Tamborini, L.; Pinto, A. An Overview of Coumarin as a Versatile and Readily Accessible Scaffold with Broad-Ranging Biological Activities. Int. J. Mol. Sci. 2020, 21, 4618. [Google Scholar] [CrossRef] [PubMed]

165. Onder, A. Anticancer activity of natural coumarins for biological targets. In Studies in Natural Products Chemistry; Rahman, A.-U., Ed.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 85–105. [Google Scholar]

166. Akkol, E.K.; Genç, Y.; Karpuz, B.; Sobarzo-Sánchez, E.; Capasso, R. Coumarins and Coumarin-Related Compounds in Pharmacotherapy of Cancer. Cancers 2020, 12, 1959. [Google Scholar] [CrossRef] [PubMed]

167. Abraham, K.; Wöhrlin, F.; Lindtner, O.; Heinemeyer, G.; Lampen, A. Toxicology and risk assessment of coumarin: Focus on human data. Mol. Nutr. Food Res. 2010, 54, 228–239. [Google Scholar] [CrossRef] [PubMed]

168. De Rosso, M.; Mayr, C.M.; Girardi, G.; Vedova, A.D.; Flamini, R. High-resolution mass spectrometry metabolomics of grape chemical markers to reveal use of not-allowed varieties in the production of Amarone and Recioto wines. Metabolomics 2018, 14, 124. [Google Scholar] [CrossRef] [PubMed]

169. Shen, T.; Wang, X.-N.; Lou, H.-X. Natural stilbenes: An overview. Nat. Prod. Rep. 2009, 26, 916–935. [Google Scholar] [CrossRef] [PubMed]

170. Sirerol, J.A.; Rodríguez, M.L.; Mena, S.; Asensi, M.A.; Estrela, J.M.; Ortega, A.L. Role of Natural Stilbenes in the Prevention of Cancer. Oxid. Med. Cell. Longev. 2016, 2016, 3128951. [Google Scholar] [CrossRef]

171. Li, Y.; Li, S.; Lin, C. Effect of resveratrol and pterostilbene on aging and longevity. Biofactors 2018, 44, 69–82. [Google Scholar] [CrossRef] [PubMed]

172. Mompeo, O.; Spector, T.D.; Hernandez, M.M.; Le Roy, C.; Istas, G.; Le Sayec, M.; Mangino, M.; Jennings, A.; Rodriguez-Mateos, A.; Valdes, A.M.; et al. Consumption of

Stilbenes and Flavonoids is Linked to Reduced Risk of Obesity Independently of Fiber Intake. Nutrients 2020, 12, 1871. [Google Scholar] [CrossRef] [PubMed]

173. Durazzo, A.; Zaccaria, M.; Polito, A.; Maiani, G.; Carcea, M. Lignan Content in Cereals, Buckwheat and Derived Foods. Foods 2013, 2, 53–63. [Google Scholar] [CrossRef]

174. Rodriguez-Garcia, C.; Sanchez-Quesada, C.; Toledo, E.; Delgado-Rodriguez, M.; Gaforio, J.J. Naturally Lignan-Rich Foods: A Dietary Tool for Health Promotion? Molecules 2019, 24, 917. [Google Scholar] [CrossRef] [PubMed]

175. Milder, I.E.J.; Arts, I.C.W.; van de Putte, B.; Venema, D.P.; Hollman, P.C.H. Lignan contents of Dutch plant foods: A database including lariciresinol, pinoresinol, secoisolariciresinol and matairesinol. Br. J. Nutr. 2005, 93, 393–402. [Google Scholar] [CrossRef] [PubMed]

176. Thompson, L.U.; Boucher, B.A.; Liu, Z.; Cotterchio, M.; Kreiger, N. Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan. Nutr. Cancer 2006, 54, 184–201. [Google Scholar] [CrossRef]

177. Panche, A.N.; Diwan, A.D.; Chandra, S.R. Flavonoids: An overview. J. Nutr. Sci. 2016, 5, e47. [Google Scholar] [CrossRef] [PubMed]

178. Li, M.; Xu, Z. Quercetin in a lotus leaves extract may be responsible for antibacterial activity. Arch. Pharm. Res. 2008, 31, 640–644. [Google Scholar] [CrossRef] [PubMed]

179. Cushnie, T.P.T.; Lamb, A.J. Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents 2005, 26, 343–356. [Google Scholar] [CrossRef]

180. Nguyen, W.; Grigori, L.; Just, E.; Santos, C.; Seleem, D. The in vivo anti-Candida albicans activity of flavonoids. J. Oral Biosci. 2021, 63, 120–128. [Google Scholar] [CrossRef] [PubMed]

181. Kaul, T.N.; Middleton, E., Jr.; Ogra, P.L. Antiviral effect of flavonoids on human viruses. J. Med. Virol. 1985, 15, 71–79. [Google Scholar] [CrossRef]

182. Badshah, S.L.; Faisal, S.; Muhammad, A.; Poulson, B.G.; Emwas, A.H.; Jaremko, M. Antiviral activities of flavonoids. Biomed. Pharmacother. 2021, 140, 111596. [Google Scholar] [CrossRef]

183. Thompson, L.U.; Yoon, J.H.; Jenkins, D.J.A.; Wolever, T.M.S.; Jenkins, A.L. Relationship between polyphenol intake and blood glucose response of normal and diabetic individuals. Am. J. Clin. Nutr. 1984, 39, 745–751. [Google Scholar] [CrossRef]

184. Madan, J.; Desai, S.; Moitra, P.; Salis, S.; Agashe, S.; Battalwar, R.; Mehta, A.; Kamble, R.; Kalita, S.; Phatak, A.G.; et al. Effect of Almond Consumption on Metabolic Risk Factors-Glucose Metabolism, Hyperinsulinemia, Selected Markers of Inflammation: A Randomized Controlled Trial in Adolescents and Young Adults. Front. Nutr. 2021, 8, 668622. [Google Scholar] [CrossRef] [PubMed]

185. Maukonen, J.; Saarela, M. Human gut microbiota: Does diet matter? Proc. Nutr. Soc. 2015, 74, 23–36. [Google Scholar] [CrossRef] [PubMed]

186. Hurrell, R.F.; Reddy, M.; Cook, J.D. Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. Br. J. Nutr. 1999, 81, 289–295. [Google Scholar] [CrossRef] [PubMed]

187. Smith, A.H.; Zoetendal, E.; Mackie, R.I. Bacterial mechanisms to overcome inhibitory effects of dietary tannins. Microb. Ecol. 2005, 50, 197–205. [Google Scholar] [CrossRef] [PubMed]

188. Ma, Q.; Kim, E.; Lindsay, E.A.; Han, O. Bioactive dietary polyphenols inhibit heme iron absorption in a dose-dependent manner in human intestinal Caco-2 cells. J. Food Sci. 2011, 76, H143–H150. [Google Scholar] [CrossRef]

189. Duda-Chodak, A.; Tarko, T. Possible Side Effects of Polyphenols and Their Interactions with Medicines. Molecules 2023, 28, 2536. [Google Scholar] [CrossRef] [PubMed]

190. Kim, E.-Y.; Pai, T.-K.; Han, O. Effect of bioactive dietary polyphenols on zinc transport across the intestinal Caco-2 cell monolayers. J. Agric. Food Chem. 2011, 59, 3606–3612. [Google Scholar] [CrossRef] [PubMed]

191. Islam, T.; Albracht-Schulte, K.; Ramalingam, L.; Schlabritz-Lutsevich, N.; Park, O.-H.; Zabet-Moghaddam, M.; Kalupahana, N.S.; Moustaid-Moussa, N. Anti-inflammatory mechanisms of polyphenols in adipose tissue: Role of gut microbiota, intestinal barrier integrity and zinc homeostasis. J. Nutr. Biochem. 2023, 115, 109242. [Google Scholar] [CrossRef] [PubMed]

192. Nouri-Vaskeh, M.; Mahdavi, A.M.; Afshan, H.; Alizadeh, L.; Zarei, M. Effect of curcumin supplementation on disease severity in patients with liver cirrhosis: A randomized controlled trial. Phytother. Res. 2020, 34, 1446–1454. [Google Scholar] [CrossRef]

193. Mantzorou, M.; Pavlidou, E.; Vasios, G.; Tsagalioti, E.; Giaginis, C. Effects of curcumin consumption on human chronic diseases: A narrative review of the most recent clinical data. Phytother. Res. 2018, 32, 957–975. [Google Scholar] [CrossRef]

194. Shabbir, U.; Rubab, M.; Daliri, E.B.-M.; Chelliah, R.; Javed, A.; Oh, D.-H. Curcumin, Quercetin, Catechins and Metabolic Diseases: The Role of Gut Microbiota. Nutrients 2021, 13, 206. [Google Scholar] [CrossRef] [PubMed]

195. Perrone, D.; Ardito, F.; Giannatempo, G.; Dioguardi, M.; Troiano, G.; Russo, L.L.; DE Lillo, A.; Laino, L.; Muzio, L.L. Biological and therapeutic activities, and anticancer properties of curcumin. Exp. Ther. Med. 2015, 10, 1615–1623. [Google Scholar] [CrossRef] [PubMed]

196. Siviero, A.; Gallo, E.; Maggini, V.; Gori, L.; Mugelli, A.; Firenzuoli, F.; Vannacci, A. Curcumin, a golden spice with a low bioavailability. J. Herb. Med. 2015, 5, 57–70. [Google Scholar] [CrossRef]

197. Cui, C.; Han, Y.; Li, H.; Yu, H.; Zhang, B.; Li, G. Curcumin-driven reprogramming of the gut microbiota and metabolome ameliorates motor deficits and neuroinflammation in a mouse model of Parkinson's disease. Front. Cell. Infect. Microbiol. 2022, 12, 887407. [Google Scholar] [CrossRef]

198. Chen, J.; Tang, X.Q.; Zhi, J.L.; Cui, Y.; Yu, H.M.; Tang, E.H.; Sun, S.N.; Feng, J.Q.; Chen, P.X. Curcumin protects PC12 cells against 1-methyl-4-phenylpyridinium ion-induced apoptosis by bcl-2-mitochondria-ROS-iNOS pathway. Apoptosis 2006, 11, 943–953. [Google Scholar] [CrossRef] [PubMed]

199. Lee, H.S.; Jung, K.K.; Cho, J.Y.; Rhee, M.H.; Hong, S.; Kwon, M.; Kim, S.H.; Kang, S.Y. Neuroprotective effect of curcumin is mainly mediated by blockade of microglial cell activation. Pharmazie 2007, 62, 937–942. [Google Scholar] [PubMed]

200. Jiang, T.-F.; Zhang, Y.-J.; Zhou, H.-Y.; Wang, H.-M.; Tian, L.-P.; Liu, J.; Ding, J.-Q.; Chen, S.-D. Curcumin ameliorates the neurodegenerative pathology in A53T alpha-synuclein cell model of Parkinson's disease through the downregulation of mTOR/p70S6K signaling and the recovery of macroautophagy. J. Neuroimmune Pharmacol. 2013, 8, 356–369. [Google Scholar] [CrossRef] [PubMed]

201. Shen, L.; Liu, L.; Ji, H.-F. Regulative effects of curcumin spice administration on gut microbiota and its pharmacological implications. Food Nutr. Res. 2017, 61, 1361780. [Google Scholar] [CrossRef] [PubMed]

202. Mcfadden, R.M.T.; Larmonier, C.B.; Shehab, K.W.; Midura-Kiela, M.; Ramalingam, R.; Harrison, C.A.; Besselsen, D.G.; Chase, J.H.; Caporaso, J.G.; Jobin, C.; et al. The Role of Curcumin in Modulating Colonic Microbiota During Colitis and Colon Cancer Prevention. Inflamm. Bowel Dis. 2015, 21, 2483–2494. [Google Scholar] [CrossRef]

203. Zam, W. Gut Microbiota as a Prospective Therapeutic Target for Curcumin: A Review of Mutual Influence. J. Nutr. Metab. 2018, 2018, 1367984. [Google Scholar] [CrossRef]

204. Yuan, T.; Yin, Z.; Yan, Z.; Hao, Q.; Zeng, J.; Li, L.; Zhao, J. Tetrahydrocurcumin ameliorates diabetes profiles of db/db mice by altering the composition of gut microbiota and up-regulating the expression of GLP-1 in the pancreas. Fitoterapia 2020, 146, 104665. [Google Scholar] [CrossRef]

205. Khoo, H.E.; Azlan, A.; Tang, S.T.; Lim, S.M. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr. Res. 2017, 61, 1361779. [Google Scholar] [CrossRef] [PubMed]

206. Hidalgo, M.; Oruna-Concha, M.J.; Kolida, S.; Walton, G.E.; Kallithraka, S.; Spencer, J.P.E.; Gibson, G.R.; de Pascual-Teresa, S. Metabolism of anthocyanins by human gut microflora and their influence on gut bacterial growth. J. Agric. Food Chem. 2012, 60, 3882–3890. [Google Scholar] [CrossRef]

207. Queipo-Ortuño, M.I.; Boto-Ordóñez, M.; Murri, M.; Gomez-Zumaquero, J.M.; Clemente-Postigo, M.; Estruch, R.; Cardona Diaz, F.; Andrés-Lacueva, C.; Tinahones, F.J. Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. Am. J. Clin. Nutr. 2012, 95, 1323–1334. [Google Scholar] [CrossRef] [PubMed]

208. Xing, L.; Zhang, H.; Qi, R.; Tsao, R.; Mine, Y. Recent Advances in the Understanding of the Health Benefits and Molecular Mechanisms Associated with Green Tea Polyphenols. J. Agric. Food Chem. 2019, 67, 1029–1043. [Google Scholar] [CrossRef]

209. Caruana, M.; Vassallo, N. Tea Polyphenols in Parkinson's Disease. Adv. Exp. Med. Biol. 2015, 863, 117–137. [Google Scholar]

210. Pérez-Burillo, S.; Navajas-Porras, B.; López-Maldonado, A.; Hinojosa-Nogueira, D.; Pastoriza, S.; Rufián-Henares, J.A. Green Tea and Its Relation to Human Gut Microbiome. Molecules 2021, 26, 3907. [Google Scholar] [CrossRef]

211. Clifford, M.N. Diet-derived phenols in plasma and tissues and their implications for health. Planta Med. 2004, 70, 1103–1114. [Google Scholar] [CrossRef]

212. Stangl, V.; Lorenz, M.; Stangl, K. The role of tea and tea flavonoids in cardiovascular health. Mol. Nutr. Food Res. 2006, 50, 218–228. [Google Scholar] [CrossRef] [PubMed]

213. Shan, Z.; Nisar, M.F.; Li, M.; Zhang, C.; Wan, C.C. Theaflavin Chemistry and Its Health Benefits. Oxid. Med. Cell. Longev. 2021, 2021, 6256618. [Google Scholar] [CrossRef]

214. Fatima, M.; Kesharwani, R.K.; Misra, K.; Rizvi, S.I. Protective effect of theaflavin on erythrocytes subjected to in vitro oxidative stress. Biochem. Res. Int. 2013, 2013, 649759. [Google Scholar] [CrossRef] [PubMed]

215. Imran, A.; Butt, M.S.; Arshad, M.S.; Arshad, M.U.; Saeed, F.; Sohaib, M.; Munir, R. Exploring the potential of black tea based flavonoids against hyperlipidemia related disorders. Lipids Health Dis. 2018, 17, 57. [Google Scholar] [CrossRef] [PubMed]

216. Tanaka, T.; Mine, C.; Inoue, K.; Matsuda, M.; Kouno, I. Synthesis of theaflavin from epicatechin and epigallocatechin by plant homogenates and role of epicatechin quinone in the synthesis and degradation of theaflavin. J. Agric. Food Chem. 2002, 50, 2142–2148. [Google Scholar] [CrossRef] [PubMed]

217. Ma, H.; Hu, Y.; Zhang, B.; Shao, Z.; Roura, E.; Wang, S. Tea polyphenol-gut microbiota interactions: Hints on improving the metabolic syndrome in a multi-element and multi-target manner. Food Sci. Hum. Wellness 2022, 11, 11–21. [Google Scholar] [CrossRef]

218. Shi, M.; Lu, Y.; Wu, J.; Zheng, Z.; Lv, C.; Ye, J.; Qin, S.; Zeng, C. Beneficial Effects of Theaflavins on Metabolic Syndrome: From Molecular Evidence to Gut Microbiome. Int. J. Mol. Sci. 2022, 23, 7595. [Google Scholar] [CrossRef] [PubMed]

219. Liu, Z.; de Bruijn, W.J.C.; Bruins, M.E.; Vincken, J.P. Microbial Metabolism of Theaflavin-3,3′-digallate and Its Gut Microbiota Composition Modulatory Effects. J. Agric. Food Chem. 2021, 69, 232–245. [Google Scholar] [CrossRef] [PubMed]

220. Yussof, A.; Cammalleri, B.; Fayemiwo, O.; Lopez, S.; Chu, T. Antibacterial and Sporicidal Activity Evaluation of Theaflavin-3,3′-digallate. Int. J. Mol. Sci. 2022, 23, 2153. [Google Scholar] [CrossRef] [PubMed]

221. Liu, W.; Li, J. Theaflavin-3,3′-Digallate Attenuates Rheumatoid Inflammation in Mice through the Nuclear Factor-kappaB and MAPK Pathways. Arch. Immunol. Ther. Exp. 2019, 67, 153–160. [Google Scholar] [CrossRef] [PubMed]

222. Chaturvedi, R.; Shukla, S.; Seth, K.; Chauhan, S.; Sinha, C.; Shukla, Y.; Agrawal, A. Neuroprotective and neurorescue effect of black tea extract in 6-hydroxydopamine-lesioned rat model of Parkinson's disease. Neurobiol. Dis. 2006, 22, 421–434. [Google Scholar] [CrossRef]

223. Gosslau, A.; Jao, D.L.E.; Huang, M.; Ho, C.; Evans, D.; Rawson, N.E.; Chen, K.Y. Effects of the black tea polyphenol theaflavin-2 on apoptotic and inflammatory pathways in vitro and in vivo. Mol. Nutr. Food Res. 2011, 55, 198–208. [Google Scholar] [CrossRef]

224. Trautwein, E.A.; Du, Y.; Meynen, E.; Yan, X.; Wen, Y.; Wang, H.; Molhuizen, H.O.F. Purified black tea theaflavins and theaflavins/catechin supplements did not affect serum lipids in healthy individuals with mildly to moderately elevated cholesterol concentrations. Eur. J. Nutr. 2010, 49, 27–35. [Google Scholar] [CrossRef] [PubMed]

225. Choi, J.-S.; Choi, Y.-J.; Shin, S.-Y.; Li, J.; Kang, S.-W.; Bae, J.-Y.; Kim, D.S.; Ji, G.-E.; Kang, J.-S.; Kang, Y.-H. Dietary flavonoids differentially reduce oxidized LDL-induced apoptosis in human endothelial cells: Role of MAPK- and JAK/STAT-signaling. J. Nutr. 2008, 138, 983–990. [Google Scholar] [CrossRef] [PubMed]

226. Banerjee, S.; Maity, P.; Mukherjee, S.; Sil, A.K.; Panda, K.; Chattopadhyay, D.; Chatterjee, I.B. Black tea prevents cigarette smoke-induced apoptosis and lung damage. J. Inflamm. 2007, 4, 3. [Google Scholar] [CrossRef] [PubMed]

227. Chen, H.; Parks, T.A.; Chen, X.; Gillitt, N.D.; Jobin, C.; Sang, S. Structural identification of mouse fecal metabolites of theaflavin 3,3′-digallate using liquid chromatography tandem mass spectrometry. J. Chromatogr. A 2011, 1218, 7297–7306. [Google Scholar] [CrossRef] [PubMed]

228. Yang, C.S. Tea and health. Nutrition 1999, 15, 946–949. [Google Scholar]

229. Cabrera, C.; Artacho, R.; Gimenez, R. Beneficial effects of green tea—A review. J. Am. Coll. Nutr. 2006, 25, 79–99. [Google Scholar] [CrossRef] [PubMed]

230. Yang, C.S.; Wang, X.; Lu, G.; Picinich, S.C. Cancer prevention by tea: Animal studies, molecular mechanisms and human relevance. Nat. Rev. Cancer 2009, 9, 429–439. [Google Scholar] [CrossRef]

231. Chen, S.-Q.; Wang, Z.-S.; Ma, Y.-X.; Zhang, W.; Lu, J.-L.; Liang, Y.-R.; Zheng, X.-Q. Neuroprotective Effects and Mechanisms of Tea Bioactive Components in Neurodegenerative Diseases. Molecules 2018, 23, 512. [Google Scholar] [CrossRef] [PubMed]

232. Quintana, J.L.B.; Allam, M.F.; Del Castillo, A.S.; Navajas, R.F.-C. Parkinson's disease and tea: A quantitative review. J. Am. Coll. Nutr. 2009, 28, 1–6. [Google Scholar] [CrossRef]

233. Malar, D.S.; Prasanth, M.I.; Brimson, J.M.; Sharika, R.; Sivamaruthi, B.S.; Chaiyasut, C.; Tencomnao, T. Neuroprotective Properties of Green Tea (Camellia sinensis) in Parkinson's Disease: A Review. Molecules 2020, 25, 3926. [Google Scholar] [CrossRef]

234. Wang, J.; Tang, L.; Zhou, H.; Zhou, J.; Glenn, T.C.; Shen, C.-L.; Wang, J.-S. Long-term treatment with green tea polyphenols modifies the gut microbiome of female sprague-dawley rats. J. Nutr. Biochem. 2018, 56, 55–64. [Google Scholar] [CrossRef]

235. Levites, Y.; Weinreb, O.; Maor, G.; Youdim, M.B.; Mandel, S. Green tea polyphenol (−)-epigallocatechin-3-gallate prevents N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurodegeneration. J. Neurochem. 2001, 78, 1073–1082. [Google Scholar] [CrossRef]

236. Dauer, W.; Przedborski, S. Parkinson's disease: Mechanisms and models. Neuron 2003, 39, 889–909. [Google Scholar] [CrossRef]

237. Carregosa, D.; Carecho, R.M.; Figueira, I.; Santos, C.N. Low-Molecular Weight Metabolites from Polyphenols as Effectors for Attenuating Neuroinflammation. J. Agric. Food Chem. 2020, 68, 1790–1807. [Google Scholar] [CrossRef]

238. Pervin, M.; Unno, K.; Takagaki, A.; Isemura, M.; Nakamura, Y. Function of Green Tea Catechins in the Brain: Epigallocatechin Gallate and its Metabolites. Int. J. Mol. Sci. 2019, 20, 3630. [Google Scholar] [CrossRef]

239. Guo, T.; Song, D.; Cheng, L.; Zhang, X. Interactions of tea catechins with intestinal microbiota and their implication for human health. Food Sci. Biotechnol. 2019, 28, 1617–1625. [Google Scholar] [CrossRef]

240. Zhou, J.; Tang, L.; Shen, C.-L.; Wang, J.-S. Green tea polyphenols modify gut-microbiota dependent metabolisms of energy, bile constituents and micronutrients in female Sprague-Dawley rats. J. Nutr. Biochem. 2018, 61, 68–81. [Google Scholar] [CrossRef]

241. Zhou, J.; Tang, L.; Shen, C.-L.; Wang, J.-S. Green tea polyphenols boost gut-microbiota-dependent mitochondrial TCA and urea cycles in Sprague-Dawley rats. J. Nutr. Biochem. 2020, 81, 108395. [Google Scholar] [CrossRef]

242. Pan, T.; Fei, J.; Zhou, X.; Jankovic, J.; Le, W. Effects of green tea polyphenols on dopamine uptake and on MPP+-induced dopamine neuron injury. Life Sci. 2003, 72, 1073–1083. [Google Scholar] [CrossRef]

243. Liu, Y.-C.; Li, X.-Y.; Shen, L. Modulation effect of tea consumption on gut microbiota. Appl. Microbiol. Biotechnol. 2020, 104, 981–987. [Google Scholar] [CrossRef]

244. Liu, Z.; Bruins, M.E.; Ni, L.; Vincken, J.-P. Green and Black Tea Phenolics: Bioavailability, Transformation by Colonic Microbiota, and Modulation of Colonic Microbiota. J. Agric. Food Chem. 2018, 66, 8469–8477. [Google Scholar] [CrossRef]

245. Yoda, Y.; Hu, Z.-Q.; Shimamura, T.; Zhao, W.-H. Different susceptibilities of Staphylococcus and Gram-negative rods to epigallocatechin gallate. J. Infect. Chemother. 2004, 10, 55–58. [Google Scholar] [CrossRef]

246. Zhang, X.; Zhu, X.L.; Sun, Y.K.; Hu, B.; Sun, Y.; Jabbar, S.; Zeng, X.X. Fermentation in vitro of EGCG, GCG and EGCG3″ Me isolated from Oolong tea by human intestinal microbiota. Food Res. Int. 2013, 54, 1589–1595. [Google Scholar] [CrossRef]

247. Teil, M.; Arotcarena, M.-L.; Faggiani, E.; Laferriere, F.; Bezard, E.; Dehay, B. Targeting alpha-synuclein for PD Therapeutics: A Pursuit on All Fronts. Biomolecules 2020, 10, 391. [Google Scholar] [CrossRef]

248. Kalsoom, I.; Wang, Y.; Li, B.; Wen, H. Research Progress of alpha-Synuclein Aggregation Inhibitors for Potential Parkinson's Disease Treatment. Mini Rev. Med. Chem. 2023, 23, 1959–1974. [Google Scholar] [CrossRef]

249. Yamasaki, T.R.; Ono, K.; Ho, L.; Pasinetti, G.M. Gut Microbiome-Modified Polyphenolic Compounds Inhibit alpha-Synuclein Seeding and Spreading in alpha-Synucleinopathies. Front. Neurosci. 2020, 14, 398. [Google Scholar] [CrossRef]

250. Caruana, M.; Neuner, J.; Högen, T.; Schmidt, F.; Kamp, F.; Scerri, C.; Giese, A.; Vassallo, N. Polyphenolic compounds are novel protective agents against lipid membrane damage by alpha-synuclein aggregates in vitro. Biochim. Biophys. Acta 2012, 1818, 2502–2510. [Google Scholar] [CrossRef]

251. Caruana, M.; Högen, T.; Levin, J.; Hillmer, A.; Giese, A.; Vassallo, N. Inhibition and disaggregation of alpha-synuclein oligomers by natural polyphenolic compounds. FEBS Lett. 2011, 585, 1113–1120. [Google Scholar] [CrossRef]

252. Ho, L.; Zhao, D.; Ono, K.; Ruan, K.; Mogno, I.; Tsuji, M.; Carry, E.; Brathwaite, J.; Sims, S.; Frolinger, T.; et al. Heterogeneity in gut microbiota drive polyphenol metabolism that influences alpha-synuclein misfolding and toxicity. J. Nutr. Biochem. 2019, 64, 170–181. [Google Scholar] [CrossRef]

253. Ehrnhoefer, D.E.; Bieschke, J.; Boeddrich, A.; Herbst, M.; Masino, L.; Lurz, R.; Engemann, S.; Pastore, A.; Wanker, E.E. EGCG redirects amyloidogenic polypeptides

into unstructured, off-pathway oligomers. Nat. Struct. Mol. Biol. 2008, 15, 558–566. [Google Scholar] [CrossRef]

254. Gonçalves, P.B.; Sodero, A.C.R.; Cordeiro, Y. Green Tea Epigallocatechin-3-gallate (EGCG) Targeting Protein Misfolding in Drug Discovery for Neurodegenerative Diseases. Biomolecules 2021, 11, 767. [Google Scholar] [CrossRef] [PubMed]

255. Fernandes, L.; Cardim-Pires, T.R.; Foguel, D.; Palhano, F.L. Green Tea Polyphenol Epigallocatechin-Gallate in Amyloid Aggregation and Neurodegenerative Diseases. Front. Neurosci. 2021, 15, 718188. [Google Scholar] [CrossRef] [PubMed]

256. Wang, Y.; Wu, S.; Li, Q.; Lang, W.; Li, W.; Jiang, X.; Wan, Z.; Chen, J.; Wang, H. Epigallocatechin-3-gallate: A phytochemical as a promising drug candidate for the treatment of Parkinson's disease. Front. Pharmacol. 2022, 13, 977521. [Google Scholar] [CrossRef] [PubMed]

257. Bieschke, J.; Russ, J.; Friedrich, R.P.; Ehrnhoefer, D.E.; Wobst, H.; Neugebauer, K.; Wanker, E.E. EGCG remodels mature alpha-synuclein and amyloid-beta fibrils and reduces cellular toxicity. Proc. Natl. Acad. Sci. USA 2010, 107, 7710–7715. [Google Scholar] [CrossRef] [PubMed]

258. Liu, Y.; Ho, L.H.; Carver, J.A.; Pukala, T.L. Ion Mobility Mass Spectrometry Studies of the Inhibition of Alpha Synuclein Amyloid Fibril Formation by (–)-Epigallocatechin-3-Gallate. Aust. J. Chem. 2011, 64, 36–40. [Google Scholar] [CrossRef]

259. Oliveri, V. Toward the discovery and development of effective modulators of α-synuclein amyloid aggregation. Eur. J. Med. Chem. 2019, 167, 10–36. [Google Scholar] [CrossRef]

260. Ehrnhoefer, D.E.; Duennwald, M.; Markovic, P.; Wacker, J.L.; Engemann, S.; Roark, M.; Legleiter, J.; Marsh, J.L.; Thompson, L.M.; Lindquist, S.; et al. Green tea (−)-epigallocatechin-gallate modulates early events in huntingtin misfolding and reduces toxicity in Huntington's disease models. Hum. Mol. Genet. 2006, 15, 2743–2751. [Google Scholar] [CrossRef] [PubMed]

261. Konijnenberg, A.; Ranica, S.; Narkiewicz, J.; Legname, G.; Grandori, R.; Sobott, F.; Natalello, A. Opposite Structural Effects of Epigallocatechin-3-gallate and Dopamine Binding to α-Synuclein. Anal. Chem. 2016, 88, 8468–8475. [Google Scholar] [CrossRef]

262. Palhano, F.L.; Lee, J.; Grimster, N.P.; Kelly, J.W. Toward the molecular mechanism(s) by which EGCG treatment remodels mature amyloid fibrils. J. Am. Chem. Soc. 2013, 135, 7503–7510. [Google Scholar] [CrossRef]

263. Lorenzen, N.; Nielsen, S.B.; Yoshimura, Y.; Vad, B.S.; Andersen, C.B.; Betzer, C.; Kaspersen, J.D.; Christiansen, G.; Pedersen, J.S.; Jensen, P.H.; et al. How epigallocatechin gallate can inhibit α-synuclein oligomer toxicity in vitro. J. Biol. Chem. 2014, 289, 21299–21310. [Google Scholar] [CrossRef]

264. Kelley, M.; Sant'anna, R.; Fernandes, L.; Palhano, F.L. Pentameric Thiophene as a Probe to Monitor EGCG's Remodeling Activity of Mature Amyloid Fibrils: Overcoming Signal Artifacts of Thioflavin T. ACS Omega 2021, 6, 8700–8705. [Google Scholar] [CrossRef] [PubMed]

265. Nebrisi, E.E. Neuroprotective Activities of Curcumin in Parkinson's Disease: A Review of the Literature. Int. J. Mol. Sci. 2021, 22, 11248. [Google Scholar] [CrossRef] [PubMed]

266. Singh, P.K.; Kotia, V.; Ghosh, D.; Mohite, G.M.; Kumar, A.; Maji, S.K. Curcumin modulates alpha-synuclein aggregation and toxicity. ACS Chem. Neurosci. 2013, 4, 393–407. [Google Scholar] [CrossRef] [PubMed]

267. Pandey, N.; Strider, J.; Nolan, W.C.; Yan, S.X.; Galvin, J.E. Curcumin inhibits aggregation of alpha-synuclein. Acta Neuropathol. 2008, 115, 479–489. [Google Scholar] [CrossRef] [PubMed]

268. Gautam, S.; Karmakar, S.; Bose, A.; Chowdhury, P.K. Beta-cyclodextrin and curcumin, a potent cocktail for disaggregating and/or inhibiting amyloids: A case study with alpha-synuclein. Biochemistry 2014, 53, 4081–4083. [Google Scholar] [CrossRef] [PubMed]

269. Ahsan, N.; Mishra, S.; Jain, M.K.; Surolia, A.; Gupta, S. Curcumin Pyrazole and its derivative (N-(3-Nitrophenylpyrazole) Curcumin inhibit aggregation, disrupt fibrils and modulate toxicity of Wild type and Mutant alpha-Synuclein. Sci. Rep. 2015, 5, 9862. [Google Scholar] [CrossRef] [PubMed]

270. Ahmad, B.; Lapidus, L.J. Curcumin prevents aggregation in alpha-synuclein by increasing reconfiguration rate. J. Biol. Chem. 2012, 287, 9193–9199. [Google Scholar] [CrossRef] [PubMed]

271. Xu, B.; Chen, J.; Liu, Y. Curcumin Interacts with alpha-Synuclein Condensates to Inhibit Amyloid Aggregation under Phase Separation. ACS Omega 2022, 7, 30281–30290. [Google Scholar] [CrossRef] [PubMed]

272. Li, J.-F.; Jiang, Z.-Q.; Cao, S.; Zhang, M.-X.; Wang, L.-H.; Liu, J.; Lu, Y.-H.; Wang, H.-Y.; Hong, X.-J.; Wang, Z.-G.; et al. Curcumin Inhibits alpha-Synuclein Aggregation by Acting on Liquid-Liquid Phase Transition. Foods 2024, 13, 1287. [Google Scholar] [CrossRef]

273. Kamelabad, M.R.; Sardroodi, J.J.; Ebrahimzadeh, A.R.; Ajamgard, M. Influence of curcumin and rosmarinic acid on disrupting the general properties of Alpha-Synuclein oligomer: Molecular dynamics simulation. J. Mol. Graph. Model. 2021, 107, 107963. [Google Scholar] [CrossRef]

274. Liu, W.; Zhang, W.; Xing, L.-Z.; Zhao, Y.-D.; Xu, J.; Li, R.-J.; Zhang, Y.-X. 4-Arylidene curcumin derivatives in vitro inhibit alpha-Synuclein aggregation and disaggregate the preformed fibril. Bioorg. Med. Chem. 2023, 96, 117529. [Google Scholar] [CrossRef] [PubMed]

275. Jha, N.N.; Ghosh, D.; Das, S.; Anoop, A.; Jacob, R.S.; Singh, P.K.; Ayyagari, N.; Namboothiri, I.N.N.; Maji, S.K. Effect of curcumin analogs onα-synuclein aggregation and cytotoxicity. Sci. Rep. 2016, 6, 28511. [Google Scholar] [CrossRef] [PubMed]

276. Wang, M.S.; Boddapati, S.; Emadi, S.; Sierks, M.R. Curcumin reduces alpha-synuclein induced cytotoxicity in Parkinson's disease cell model. BMC Neurosci. 2010, 11, 57. [Google Scholar] [CrossRef] [PubMed]

277. Dehghani, Z.; Meratan, A.A.; Saboury, A.A.; Nemat-Gorgani, M. Alpha-Synuclein fibrillation products trigger the release of hexokinase I from mitochondria: Protection by curcumin, and possible role in pathogenesis of Parkinson's disease. Biochim. Biophys. Acta Biomembr. 2020, 1862, 183251. [Google Scholar] [CrossRef] [PubMed]

278. Spinelli, K.J.; Osterberg, V.R.; Meshul, C.K.; Soumyanath, A.; Unni, V.K. Curcumin Treatment Improves Motor Behavior in α-Synuclein Transgenic Mice. PLoS ONE 2015, 10, e0128510. [Google Scholar] [CrossRef] [PubMed]

279. Pandareesh, M.D.; Mythri, R.; Bharath, M.S. Bioavailability of dietary polyphenols: Factors contributing to their clinical application in CNS diseases. Neurochem. Int. 2015, 89, 198–208. [Google Scholar] [CrossRef] [PubMed]

280. Pogačnik, L.; Pirc, K.; Palmela, I.; Skrt, M.; Kim, K.S.; Brites, D.; Brito, M.A.; Ulrih, N.P.; Silva, R.F. Potential for brain accessibility and analysis of stability of selected flavonoids in relation to neuroprotection in vitro. Brain Res. 2016, 1651, 17–26. [Google Scholar] [CrossRef] [PubMed]

281. Scholz, S.; Williamson, G. Interactions affecting the bioavailability of dietary polyphenols in vivo. Int. J. Vitam. Nutr. Res. 2007, 77, 224–235. [Google Scholar] [CrossRef] [PubMed]

282. Scalbert, A.; Williamson, G. Dietary intake and bioavailability of polyphenols. J. Nutr. 2000, 130 (Suppl. S8), 2073s–2085s. [Google Scholar] [CrossRef]

283. Zhang, W.; Dong, X.; Huang, R. Antiparkinsonian effects of polyphenols: A narrative review with a focus on the modulation of the gut-brain axis. Pharmacol. Res. 2023, 193, 106787. [Google Scholar] [CrossRef]

284. Láng, L.; McArthur, S.; Lazar, A.S.; Pourtau, L.; Gaudout, D.; Pontifex, M.G.; Müller, M.; Vauzour, D. Dietary (Poly)phenols and the Gut-Brain Axis in Ageing. Nutrients 2024, 16, 1500. [Google Scholar] [CrossRef] [PubMed]

285. Li, Y.; Peng, Y.; Shen, Y.; Zhang, Y.; Liu, L.; Yang, X. Dietary polyphenols: Regulate the advanced glycation end products-RAGE axis and the microbiota-gut-brain axis to prevent neurodegenerative diseases. Crit. Rev. Food Sci. Nutr. 2023, 63, 9816–9842. [Google Scholar] [CrossRef] [PubMed]

286. Ross, F.C.; Mayer, D.E.; Horn, J.; Cryan, J.F.; Del Rio, D.; Randolph, E.; Gill, C.I.R.; Gupta, A.; Ross, R.P.; Stanton, C.; et al. Potential of dietary polyphenols for protection from age-related decline and neurodegeneration: A role for gut microbiota? Nutr. Neurosci. 2024, 1–19. [Google Scholar] [CrossRef] [PubMed]

287. Galkin, M.; Priss, A.; Kyriukha, Y.; Shvadchak, V. Navigating alpha-Synuclein Aggregation Inhibition: Methods, Mechanisms, and Molecular Targets. Chem. Rec. 2024, 24, e202300282. [Google Scholar] [CrossRef]

288. Kemperman, R.A.; Bolca, S.; Roger, L.C.; Vaughan, E.E. Novel approaches for analysing gut microbes and dietary polyphenols: Challenges and opportunities. Microbiology 2010, 156 Pt 11, 3224–3231. [Google Scholar] [CrossRef] [PubMed]

289. Williamson, G.; Manach, C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. Am. J. Clin. Nutr. 2005, 81 (Suppl. S1), 243s–255s. [Google Scholar] [CrossRef] [PubMed]

290. Hollman, P.C.; Katan, M.B. Absorption, metabolism and health effects of dietary flavonoids in man. Biomed. Pharmacother. 1997, 51, 305–310. [Google Scholar] [CrossRef] [PubMed]

291. Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: Food sources and bioavailability. Am. J. Clin. Nutr. 2004, 79, 727–747. [Google Scholar] [CrossRef] [PubMed]

292. Chen, J.; Deng, Y.; Long, S.-Y.; Xu, H.-Y.; Zeng, Y.-T.; Peng, T.; Yang, C.-M.; Du, J.; Zhang, X.-Y. Metabolic flux and catabolic kinetics of prebiotic-like dietary polyphenol phlorizin in association with gut microbiota in vitro. Food Chem. 2024, 440, 138240. [Google Scholar] [CrossRef]

293. Shetty, S.S.; Kumari, N.S. Therapeutic efficacy of gut microbiota-derived polyphenol metabolite Urolithin A. Beni-Suef Univ. J. Basic Appl. Sci. 2024, 13, 31. [Google Scholar] [CrossRef]

294. Gade, A.; Kumar, M.S. Gut microbial metabolites of dietary polyphenols and their potential role in human health and diseases. J. Physiol. Biochem. 2023, 79, 695–718. [Google Scholar] [CrossRef] [PubMed]

295. Luca, S.V.; Macovei, I.; Bujor, A.; Miron, A.; Skalicka-Woźniak, K.; Aprotosoaie, A.C.; Trifan, A. Bioactivity of dietary polyphenols: The role of metabolites. Crit. Rev. Food Sci. Nutr. 2020, 60, 626–659. [Google Scholar] [CrossRef] [PubMed]

296. Frolinger, T.; Sims, S.; Smith, C.; Wang, J.; Cheng, H.; Faith, J.; Ho, L.; Hao, K.; Pasinetti, G.M. The gut microbiota composition affects dietary polyphenols-mediated cognitive resilience in mice by modulating the bioavailability of phenolic acids. Sci. Rep. 2019, 9, 3546. [Google Scholar] [CrossRef] [PubMed]

297. Pasinetti, G.M.; Singh, R.; Westfall, S.; Herman, F.; Faith, J.; Ho, L. The Role of the Gut Microbiota in the Metabolism of Polyphenols as Characterized by Gnotobiotic Mice. J. Alzheimer's Dis. 2018, 63, 409–421. [Google Scholar] [CrossRef] [PubMed]

298. Yan, L.; Guo, M.-S.; Zhang, Y.; Yu, L.; Wu, J.-M.; Tang, Y.; Ai, W.; Zhu, F.-D.; Law, B.Y.-K.; Chen, Q.; et al. Dietary Plant Polyphenols as the Potential Drugs in Neurodegenerative Diseases: Current Evidence, Advances, and Opportunities. Oxid. Med. Cell. Longev. 2022, 2022, 5288698. [Google Scholar] [CrossRef] [PubMed]

299. Leclerc, M.; Dudonne, S.; Calon, F. Can Natural Products Exert Neuroprotection without Crossing the Blood-Brain Barrier? Int. J. Mol. Sci. 2021, 22, 3356. [Google Scholar] [CrossRef] [PubMed]

300. Roberts, S.B.; Franceschini, M.A.; Silver, R.E.; Taylor, S.F.; De Sa, A.B.; Có, R.; Sonco, A.; Krauss, A.; Taetzsch, A.; Webb, P.; et al. Effects of food supplementation on cognitive function, cerebral blood flow, and nutritional status in young children at risk of undernutrition: Randomized controlled trial. BMJ 2020, 370, m2397. [Google Scholar] [CrossRef] [PubMed]

301. Burapan, S.; Kim, M.; Han, J. Curcuminoid Demethylation as an Alternative Metabolism by Human Intestinal Microbiota. J. Agric. Food Chem. 2017, 65, 3305–3310. [Google Scholar] [CrossRef] [PubMed]

302. Grosso Jasutkar, H.; Oh, S.E.; Mouradian, M.M. Therapeutics in the Pipeline Targeting alpha-Synuclein for Parkinson's Disease. Pharmacol. Rev. 2022, 74, 207–237. [Google Scholar] [CrossRef]

303. Koudoufio, M.; Desjardins, Y.; Feldman, F.; Spahis, S.; Delvin, E.; Levy, E. Insight into Polyphenol and Gut Microbiota Crosstalk: Are Their Metabolites the Key to Understand Protective Effects against Metabolic Disorders? Antioxidants 2020, 9, 982. [Google Scholar] [CrossRef]

304. Reddy, V.P.; Aryal, P.; Robinson, S.; Rafiu, R.; Obrenovich, M.; Perry, G. Polyphenols in Alzheimer's Disease and in the Gut-Brain Axis. Microorganisms 2020, 8, 199. [Google Scholar] [CrossRef] [PubMed]

305. Wang, D.; Ho, L.; Faith, J.; Ono, K.; Janle, E.M.; Lachcik, P.J.; Cooper, B.R.; Jannasch, A.H.; D'Arcy, B.R.; Williams, B.A.; et al. Role of intestinal microbiota in the generation of polyphenol-derived phenolic acid mediated attenuation of Alzheimer's disease β-amyloid oligomerization. Mol. Nutr. Food Res. 2015, 59, 1025–1040. [Google Scholar] [CrossRef] [PubMed]

306. Ali, Y.O.; Escala, W.; Ruan, K.; Zhai, R.G. Assaying locomotor, learning, and memory deficits in Drosophila models of neurodegeneration. J. Vis. Exp. 2011, 49, e2504. [Google Scholar]

307. Ascherio, A.; Zhang, S.M.; Hernán, M.A.; Kawachi, I.; Colditz, G.A.; Speizer, F.E.; Willett, W.C. Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. Ann. Neurol. 2001, 50, 56–63. [Google Scholar] [CrossRef]

CHAPTER 3

THERAPEUTIC POTENTIAL OF GREEN TEA CATECHINS ON THE DEVELOPMENT OF PARKINSON'S DISEASE SYMPTOMS IN A TRANSGENIC A53T MOUSE MODEL[1]

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[1] Riegelman, E.; Xue, K.S.; Wang, J.-S.; Tang, L. Therapeutic potential of green tea catechins on the development of Parkinson's disease symptoms in a transgenic A53T mouse model.

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Abstract

Objectives: This study aimed to evaluate the effects of green tea catechins on the prevention of Parkinson's disease neurobehavioral symptoms and α-synuclein blood plasma concentration in a hemizygous transgenic A53T mouse model.

Methods: Thirty 6-month-old male mice were randomly assigned to three groups (n=10/group): control, low-dose, and high-dose, receiving green tea polyphenol (GTP) treatment in their drinking water at 0%, 0.5%, and 1.5%, respectively, over a 90-day period. The efficacy of *ad libitum* dosing was assessed by analyzing the bioaccumulation of tea catechins in urine samples collected from metabolic cages on days 0, 30, 60, and 90, using LC/Q-TOF analysis. PD-related behavioral impairments were measured with open field and rotarod performance tests on days 0, 45, and 90. On day 90, plasma αsynuclein levels were analyzed via enzyme-linked immunosorbent assay (ELISA) to assess treatment effects.

Results: Circulating tea catechin metabolites were detected in treated groups by day 30, with levels progressively increasing through day 90. By day 90, control mice exhibited significant deficits in rotarod performance, while both low- and high-dose groups maintained or improved their maximum time on the rotarod. Open field testing indicated reduced anxiety-related behavior in control mice compared to treated groups. ELISA analysis revealed significantly lower circulating α-synuclein levels in high-dose mice compared to controls.

Conclusion: Our findings indicate that sustained administration of tea catechins significantly reduces circulating α-synuclein levels in blood plasma, improves motor coordination in a dose dependent manner, and modulates anxiety-related behaviors in a PD mouse model.

1. Introduction

Green tea, the dried unfermented leaves of the plant *Camellia sinensis*, has been consumed for millennia and is believed to possess numerous advantageous biochemical properties for human health. *Camellia sinensis* leaves contain over 2000 chemical classes, while the flavonols, a subclass of flavonoids that belong to the plant polyphenol group, represent approximately 30 – 40% of the solid mass of dried tea leaves [1]. Green tea polyphenols (GTP) are the primary focus of research on the advantages of green tea consumption due to their potential health-enhancing properties. The mixture of natural flavonols in green tea, also called tea catechins (TCs), is comprised of four main compounds including epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG), the latter of which is the most potent and bioactive (Figure 3.1) [1]. TCs are characterized by their benzopyran structure bearing at least one aromatic ring with a varying number and position of hydroxyl groups, which determine the ability of each compound to interact via hydrogen bonding or electron and hydrogen transfer [2]. As such, green tea has garnered global attention for its potential to combat various health ailments with inflammatory components, including the prevention of cardiovascular diseases [3,4], improvement in bone density [5,6], prevention of multiple types of cancers [7-11], and the prevention of neurodegenerative diseases such as Parkinson's disease (PD) [12-14].

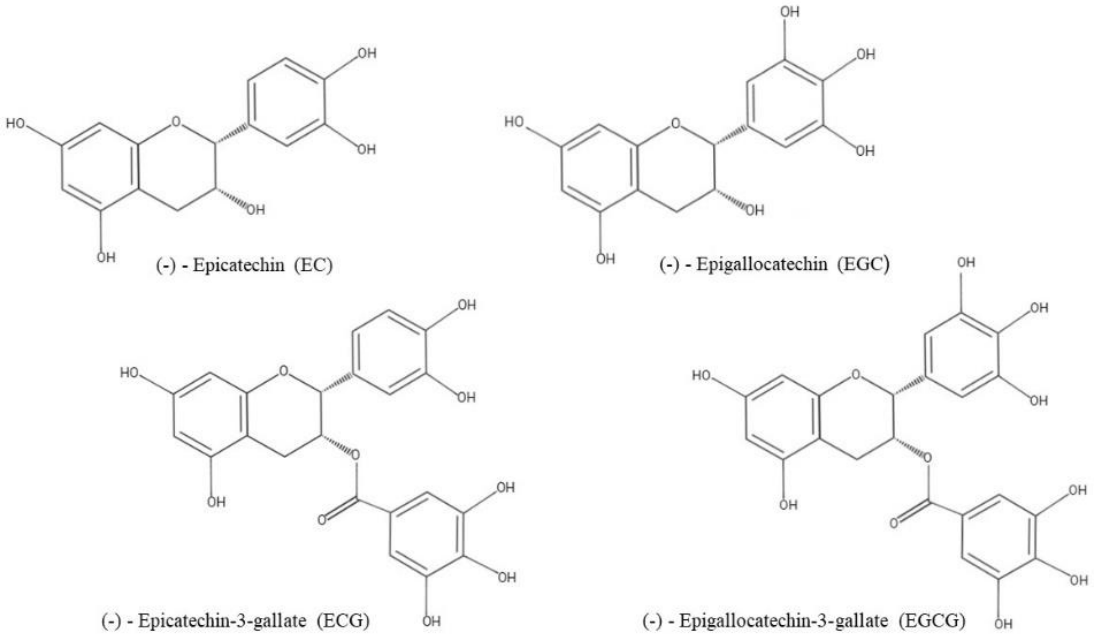

Figure 3.1. The four main tea catechins that are present in green tea.

PD is recognized as the second most common neurodegenerative disease behind Alzheimer's disease, and is the fastest-growing of all the neurological disorders included in the Global Burden of Disease Database [15]. PD is a complex neurological disorder characterized by the gradual deterioration of dopamine-producing neurons in the *substantia nigra* portion of the midbrain [16]. This neuronal loss, coupled with the accumulation of abnormal α-synuclein protein aggregates, disrupts normal neuron function which then manifests in a spectrum of motor and non-motor symptoms [17]. Although the precise physiological role of α-synuclein remains unclear, this protein is abundantly present in presynaptic regions, where it interacts with vesicles and membranes. At the synapse, pathological α-synuclein initiates its harmful effects by disrupting vesicle clustering and altering postsynaptic responses to neurotransmitters [18]. These disruptions contribute to impaired synaptic plasticity, which may underlie the early network dysfunctions responsible for motor impairment before significant neurodegeneration occurs [19].

In recent decades, over 20 genes, including *SNCA*, have been identified in association with PD, with implicated genes indicating multiple potential mechanisms by which genetic variation may increase disease risk [20,21]. However, the majority of PD cases are idiopathic in nature [16,22], suggesting a multifaceted etiology involving genetic susceptibility and environmental factors that influence mitochondrial dysfunction, inflammation, and aberrant α-synuclein aggregation [23]. The intricate interplay between genetic predispositions, as evidenced by genome-wide association studies [24], and environmental factors, such as exposure to pesticides [25,26], heavy metals [27,28], and diet [29,30] have been shown to play a pivotal role in both the manifestation and progression of PD. Inflammation is largely implicated in PD pathogenesis, with microglia becoming activated in response to diverse triggers, and then releasing pro-inflammatory molecules that contribute to neuroinflammation and neuronal damage [31]. However, the complete understanding of how these pathogenesis factors interact remains elusive.

An inverse correlation has emerged in research, suggesting that higher levels of tea consumption are linked to a reduced likelihood of developing PD [32-37], providing a compelling foundation for conducting experiments aimed at elucidating the mechanisms of action of TCs. Various potential mechanisms are proposed to understand the neuroprotective properties of TCs, one of which is TCs' rich antioxidant profile, which could enable it to counteract oxidative stress and mitigate mitochondrial dysfunction [32,38]. Evidence has shown where TCs may play a role in modulating the aggregation of α-synuclein protein and could reduce the formation of toxic aggregates through the disruption of α-synuclein protofibrils [39], oxidizing the N-terminal region and reducing α-synuclein affinity to permeabilize cell membranes [40], and inhibiting the conformational transition of α-synuclein to β-sheet and disaggregating amyloid fibrils of α-synuclein in a dose-dependent manner [41,42]. These findings collectively highlight the potential of TCs as a promising therapeutic strategy for mitigating the progression of PD by targeting multiple aspects of α-synuclein pathology.

Many *in vivo* studies have utilized the N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model to evaluate the impacts of TC on neurodegeneration and inflammation [43-46]. While the MPTP mouse model remains an effective tool for PD research, the difficulty of mimicking behavioral abnormalities expressed in human PD and the reduced likelihood of Lewy body formation [47] could limit its translational relevance and efficacy in fully capturing the complexity of the disease phenotype. For this study, the transgenic A53T model was chosen for its potential to investigate both the mechanisms of motor neuron degeneration and the role of α-synuclein in PD. Before cognitive and gross motor dysfunction sets in, hemizygous A53T mice exhibit notable abnormalities in fine motor skills, sensorimotor functions, and hippocampal synaptic activity. By employing the hemizygous A53T model, we aimed to assess the safety and efficacy of GTP administration in the prevention of PD pathology and symptoms, as A53T mice often display motor deficits and behavioral abnormalities reminiscent of PD in humans [48].

2. Materials and Methods

2.1. Chemicals and Reagents

Decaffeinated GTP (Lot # 2597074) was purchased from LKT Laboratories, Inc. (St. Paul, MN). Reference TC standards, including EC, ECG, EGC, and EGCG in dry powder form were purchased from Thomas Scientific (Chadds Ford Township, PA). High-performance liquid chromatography (HPLC) grade methanol and water were purchased from Thermo Fisher Scientific, while all other reagents were analytical grade. After analysis via LC/QTOF, the GTP extract consisted of 99.61% TCs, 70.12% of which were EGCG. The GTP extract was also analyzed by batch at LKT Laboratories for heavy metals and arsenic, both of which were under federal compliance levels (<10 ppm and <3 ppm, respectively) [49]. Sulfatase and β-glucuronidase were purchased from Sigma-Aldrich. Albumin standard (Pointe Scientific, Inc.), albumin reagent (Thermo Scientific), protein standard (Sigma), and protein assay dye reagent concentrate (Bio-RAD) were used for the analysis of total protein (TTP) and albumin in plasma. Mouse SNCA/Alpha-Synuclein ELISA Kit (Catalog no. LS-F23116) was purchased from Lifespan Biosciences (Lynnwood, WA). GTP powder was stored at 4°C during the study period while all other standards were stored at -20°C.

2.2 Animal Study Experimental Design

Six-month-old, male B6.Cg-2310039L15Rik$^{Tg(Prnp\text{-}SNCA*A53T)23Mkle}$/J mice (Strain #006823, Hualpha-Syn (A53T), Hemizygous transgenic line G2-3) (Jackson Laboratories), were utilized in this study. All mice were housed individually under a 12-12h light-dark cycle with free access to pelleted PicoLab Rodent Diet 5053 (LabDiet, Fort Worth, TX) and distilled drinking water during the study period. Two weeks before the start of the study, mice were acclimated to the single-house environment and daily handling procedures. All experiments were reviewed, approved, and carried out under the University of Georgia Animal Use and Care Committee. Mice (n=30) were

randomly assigned to one of three groups (n=10/group): control, low-dose (0.5% weight/volume GTP), or high-dose (1.5% weight/volume GTP). Distilled and GTP extract water was prepared fresh daily while the amount of water drank (mL) and the animal's body weight (g) were recorded. For the duration of the study, each animal was monitored once daily for any clinical indications of mortality and morbidity. At the conclusion of the study, animals were humanely euthanized and then subjected to a gross necropsy. The following organs, including the heart, lungs, spleen, liver, stomach, intestines, testes, preputial gland, brain, and thymus were dissected and trimmed carefully to remove fat or other contiguous tissues, then weighed immediately to minimize the effects of dry organ weight. The stomach and intestines were then promptly stored in appropriate containers at -80°C while the remaining organs were fixed in 10% formalin solution and stored at 4°C until future analyses.

2.2.1 Biological Sample Collection

The design of the sample collection is summarized in Figure 3.2. Mice were transferred to Nalgene metabolic cage systems (Rochester, NY, USA) on days 0, 30, 60, and 90. Urine was collected at two-time points over 24 hours into sterilized tubes, which were then promptly stored at -80°C until analysis. On day 90, mice were anesthetized via isoflurane inhalation and then euthanized via cardiac puncture followed by CO_2 inhalation. Blood from the cardiac puncture procedure was collected in EDTA-treated tubes and then centrifuged for 10 minutes at 2,000 g in a refrigerated centrifuge. The blood plasma supernatant was separated and then immediately stored at -20°C until analysis of TTP, albumin levels, and evaluation of α-synuclein concentration via ELISA.

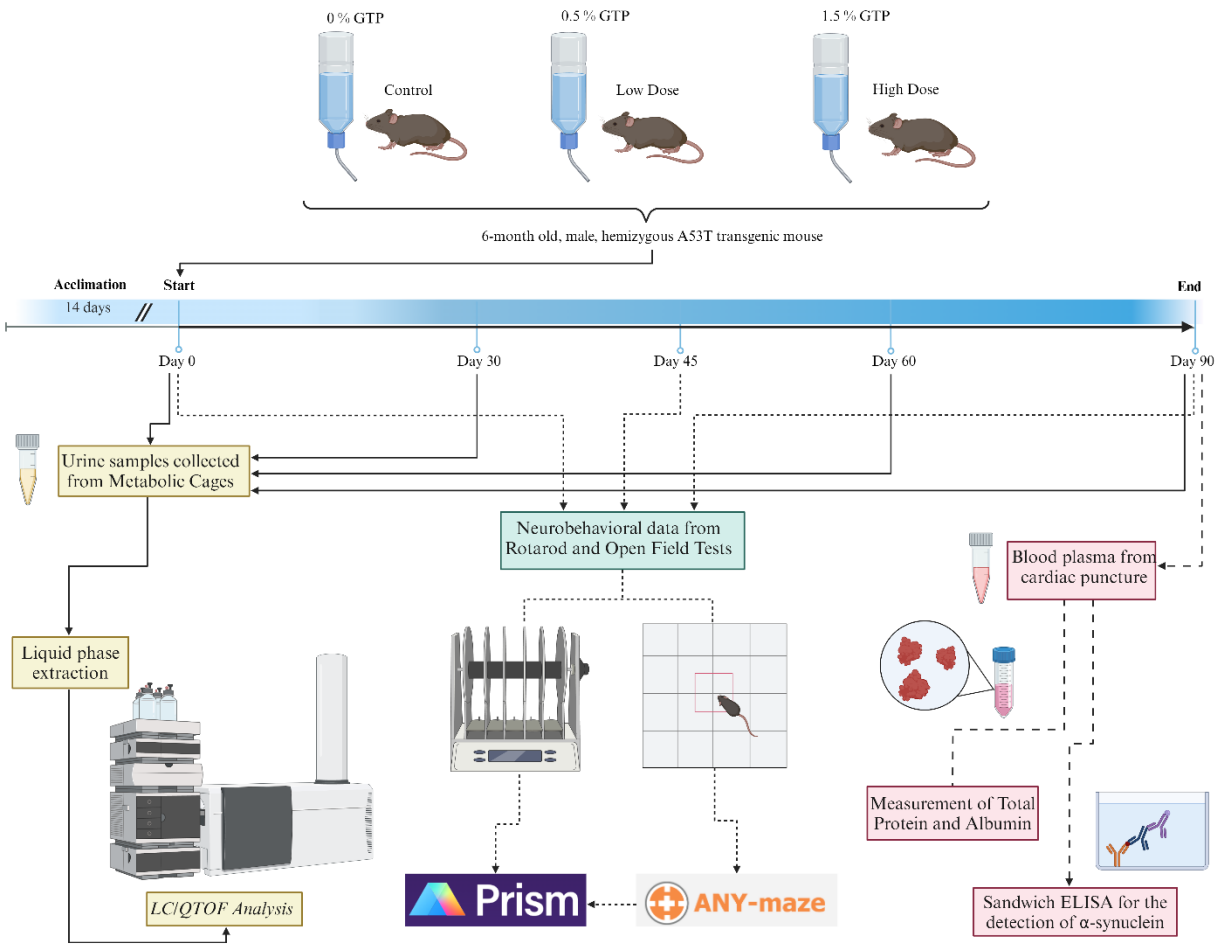

Figure 3.2. Graphical summary of experimental timeline.

6-month-old male A53T Transgenic mice were randomly assigned into the control, low-dose, or high-dose treatment groups and dosed over a 90-day period. Urine and fecal samples were collected via metabolic cages on days 0, 30, 60, and 90 in tandem with behavioral data collected from open field and rotarod performance tests on days 0, 45, and 90. At the conclusion of the study, mice were euthanized humanely through cardiac puncture followed by CO2 inhalation. Subsequently, necropsies were conducted to collect organs. (Created with BioRender.com).

2.3 Behavioral Experiments

Identical neurobehavioral assessments, including the open field and rotarod performance tests, were conducted on days 0, 45, and 90 of the 90-day treatment period. Testing was conducted in a quiet (50-60 dB ambient noise), temperature-controlled (70°F), and light-controlled (~300-400 lux) dedicated room. Before the start of each experiment, mice were acclimated to the testing room for 30 minutes.

2.3.1 Open field test

To examine spontaneous exploratory locomotor behavior, the open field test was employed. This test evaluates the exploration tendencies of mice and gauges their emotional responses when introduced to a substantially larger, brightly illuminated, and unfamiliar environment compared to their home cage [50]. Each mouse was placed in the center of a square (40 cm × 40 cm) arena and allowed to explore the arena for 5 minutes. ANY-maze tracking software (Version 7.35, Stoelting Co.) was employed to measure average speed, total distance traveled, time spent in the center region vs. outer region, grooming duration, and rearing activity. A 50% ethanol solution was used to cleanse the arena between each animal to mitigate odor.

2.3.2 Rotarod test

The rotarod test (Rotarod LE8500, Harvard Apparatus) is a commonly employed method for assessing the motor coordination of rodents, demonstrating particular effectiveness in detecting dysfunction within the cerebellum [51]. The acceleration protocol, adapted from Campos *et al*. [52], was comprised of a starting speed of 4 rpm increasing to 40 rpm over a span of 5 minutes, at which the length of time that each animal stayed on the rod was recorded as the test duration. Each animal underwent pre-training on the rotarod to achieve consistent performance levels and acclimate to the apparatus. Training consisted of three sessions over three consecutive days,

where the final test was performed in triplicate under the same accelerating protocol on the fourth day.

2.4 Biochemical Analyses

2.4.1 Measurement of total protein and albumin in blood plasma

For the measurement of total plasma proteins, the Bradford Assay was employed. Briefly, 5 µL of blood plasma was diluted with 95 µL of HPLC-grade water. Following vortexing, 4 µL of this solution, prepared at a 20-fold dilution, was introduced into a glass tube containing 796 µL of HPLC-grade water. 200 µL of a specialized protein assay dye reagent concentrate was added to the solution, followed by another round of vortexing to ensure homogeneity. The resultant mixture was subjected to spectrophotometric analysis at a λ of 595 nm. The determination of albumin concentration in plasma followed a parallel procedure. 10 µL of plasma was combined with 10 µL of HPLC-grade water within a glass tube. 980 µL of albumin reagent was added into the tube, followed by vortexing. The resulting solution was then subjected to spectrophotometric analysis at λ of 630 nm. Final concentrations of both TTP and albumin were calculated through the analysis of absorbance readings obtained from the spectrophotometric measurements.

2.4.2 Measurement of α-synuclein concentration in blood plasma

To evaluate the quantity of endogenous a-synuclein circulating in the blood, a sandwich ELISA protocol was employed. 100 µL of room temperature standard, blank, or sample was added to a pre-coated 96-well plate, covered, and incubated for 90 minutes at 37 °C. The liquid was then aspirated followed by the addition of 100 µL of biotinylated detection antibody, covered, gently mixed, then incubated for 60 minutes at 37 °C. The liquid was aspirated from the well, then washed three times with 350 µL of wash buffer. After three washes, the plate was tapped clean before adding 100 µL of horseradish peroxidase (HRP) conjugate working solution, covered, then incubated for 30 minutes at 37 °C. The liquid was then aspirated and washed five times with 350

µL of wash buffer. Then, 90 µL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution was added to each well, covered, then incubated for 15 minutes at 37 °C. Lastly, 50 µL of stop solution was added to each well, the plate gently mixed, then placed in a plate reader to determine the optical density of each well at 450 nm. All samples and standards were run in duplicate. Final concentrations were calculated through the analysis of averaged absorbance readings and subtracting the zero-standard reading.

2.5 Measurement of urinary TC metabolites

The procedure for analyzing the concentration of urinary TCs and the daughter metabolites, as shown in Figure 3.3, was determined following a method described in Wang et al. [53]. Thawed urine samples (50 µL) were centrifuged, then combined with 150 µL of 0.1 M NaH_2PO_4, 50 U of β-glucuronidase, 1 U of Sulfatase, and 20 µL of 20% Vit-C EDTA solution for a 1-hour incubation at 37°C to release conjugated polyphenols. Urine samples were quenched with ethyl acetate to stop the reaction, vortexed, and then centrifuged. The organic phase was extracted with ethyl acetate for a total of three washes. The pooled organic phases were dried *in vacuo* with a Labconco Centrivap concentrator (Marshall Scientific) and then reconstituted with 25% methanol for LC/Q-TOF injection.

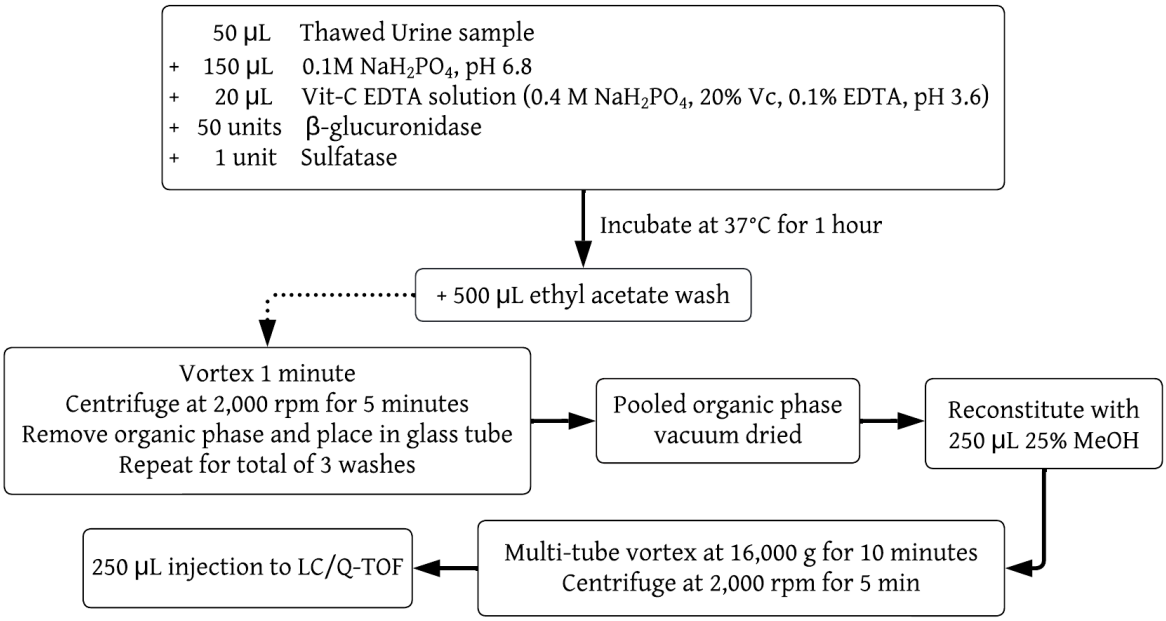

Figure 3.3. Workflow of liquid-phase extraction of urinary TCs.

2.5.1 LC/Q-TOF Targeted Analysis

Targeted metabolomics analysis of TC and daughter metabolites was performed on an Agilent 6546 LCMS in tandem with a quadrupole time of flight (Q-TOF) system (Agilent Technologies, Santa Clara, CA). Each sample was run in duplicate, employing a standard curve for the beginning and end of each run, and interspersing blanks after every 9 samples. Separation was performed on an Agilent Eclipse Plus C18 (4.6 mm × 150 mm, 5 μm) column. The binary solvent system was composed of 0.1% formic acid in HPLC-grade water (v/v, A) and 100% methanol (B). The gradient elution was 0.0 – 1.25 min: 10% B, 1.25 – 2.5 min: 15% B, 2.5 – 7.0 min: 27% B, 7.0 – 9.0 min: 40% B, 9.0 – 13.0 min: 95% B, 13.0 min: 25% B. Injection volume was set to 15μL, flow rate was 0.40 mL/min, and column temperature was controlled at 40°C. During the analysis, instrument parameters were set accordingly: capillary voltage 3500 V for the negative ion polarity mode, gas temperature 250°C, sheath gas temperature 300°C, sheath gas flow 12 l/min, gas flow 11 l/min, and nebulizer 35 psi. The mass scan range was 100 – 1500 m/z in negative ionization mode. LCMS/Q-TOF-MS/MS mode was employed to detect the daughter compounds, as described in Table 3.1. Collision energy (CE) was set to 25 mV. LC/Q-TOF data processing was performed with Agilent MassHunter “Quant-My-Way” Quantitative Analysis (version 10.2) and Qualitative analysis software (version 10.0). Targeted data processing was performed using the “Find Compounds by Formula” feature.

Table 3.1. Mass parameters for the analyzed GTP metabolites.

	Compound	$[M-H]^-$ (m/*z*)	Quant Ion	Qual Ion(s)	Rt (min)	Peak #
	Gallate	212	123		0.691	1
EGC	(-)-Epigallocatechin	305	261	221, 219	2.023	2
EGCG	(-)-Epigallocatechin-3-*O*-gallate	457	305	331, 169	2.788	3
EC	(-)-Epicatechin	289	245	205	3.06	4
ECG	(-)-Epicatechin-3-O-gallate	441	289	331, 169	3.714	5

2.6 Statistical Analysis

Statistical analyses were performed using GraphPad Prism® v.10.0.2. Statistical comparisons for changes in body weight, water consumption, food consumption, TC metabolites, and α-synuclein concentrations were analyzed using a one-way ANOVA test. A two-tailed T-test was used to determine significant differences between groups TTP and albumin levels. The effects of GTP treatment on rotarod performance and open-field maze performance data were analyzed using a two-way ANOVA, followed by *post-hoc* pairwise comparisons carried out using the Bonferroni method. All data are presented as mean ± SEM, and significance levels denoted as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

3. Results

On day 76, one animal in the control group was found deceased with no clinical history. Necropsy showed that the cause of mortality was likely a urinary obstruction. No other adverse findings were observed during daily clinical observations.

3.1 Body weight, water consumption, and food intake

The body weight of experimental mice from all three groups increased steadily during the 90-day study period. Figure 3.4 presents the body weight (BW) for the different treatments over the study period. At the baseline, there was no significant difference in BW amongst the treatment groups. By day 90, BW increased significantly in all groups compared to the baseline measurements (control $p = 0.0488$, low-dose $p = 0.0058$, high-dose $p = 0.0433$). Water intake increased over the study period compared to the baseline for all experimental groups. Figure 3.5 shows data on the weekly average water consumption for each treatment group.

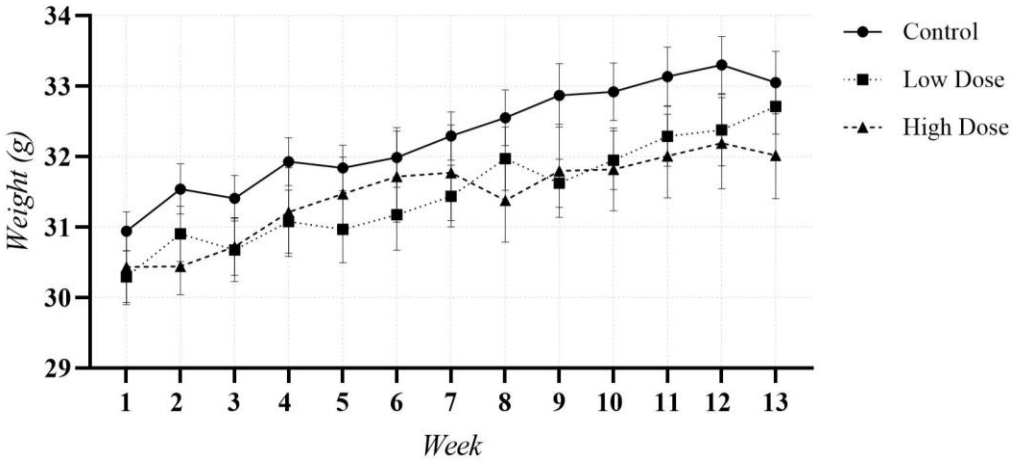

Figure 3.4. Average body weight of mice over 90-day study period. All groups had significantly greater body weight by the end of the study period compared to day 0. There was no significant difference in body weights between the treatment groups at the beginning or end of the study (ANOVA F: (38, 323) = 3.225). Data are presented as mean ± SEM.

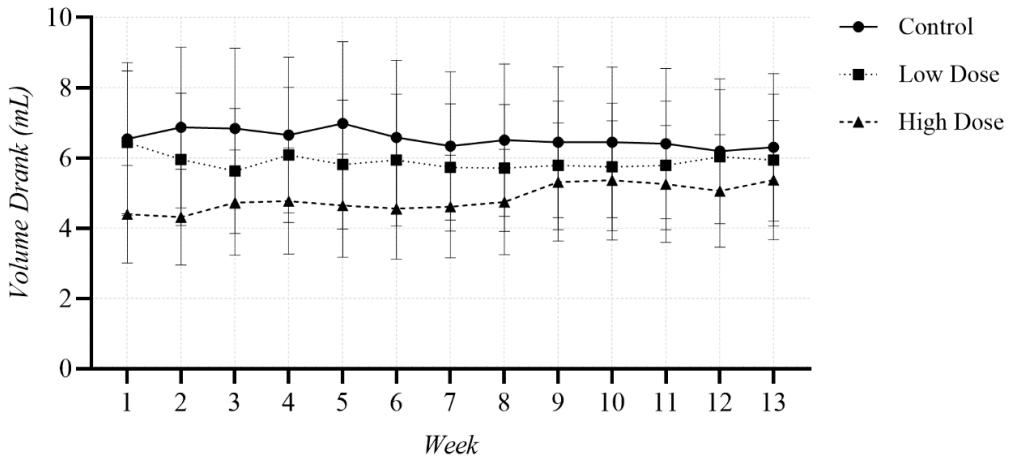

Figure 3.5. Average water consumption over 90-day study period. The amount of TC-treated water consumption was lower in the high-dose group compared to the control and low-dose groups. By week 7, there was no significant difference in average measured water consumption in the high-dose group (ANOVA F: (38, 231) = 12.07). Data are presented as mean ± SEM.

There was no significant difference in the amount of water consumed between the control and low-dose groups, while, in contrast, the high-dose group drank significantly less water over weeks 1-6 compared to the control and low-dose groups. By week 7, there was not a significant difference of water intake between treatment groups. Figure 3.6 presents the average food intake between treatment groups on days 0, 30, 60, and 90. There was no significant difference in the amount of food consumed between treatment groups. The control group had a relatively consistent average intake of food while the low-dose group showed a positive trend in food consumption and the high-dose group had a reduced trend in food consumption. The final organ weights, as shown in Table 3.2, did not exhibit any significant differences between treatment groups. The comparative analysis of TTP and albumin concentrations in blood plasma relative to body weight is summarized in Table 3.3. Our analysis demonstrated no statistically significant disparities among the control, low-dose, and high-dose groups.

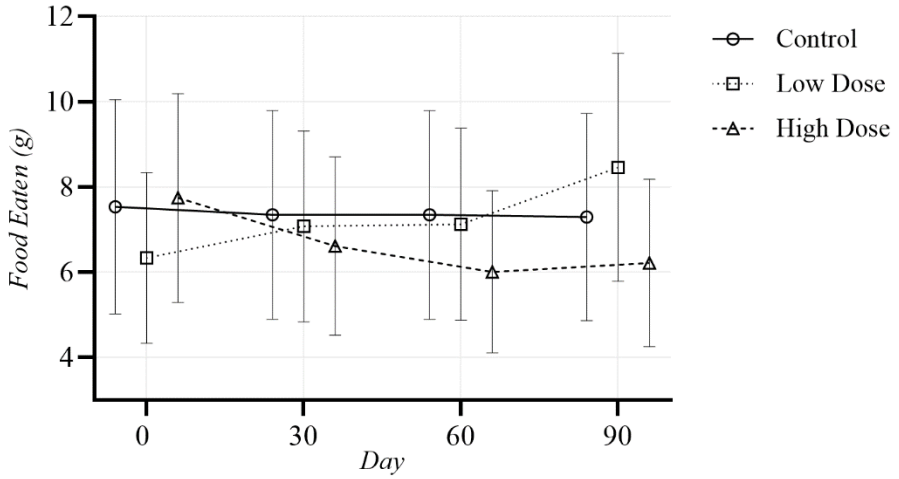

Figure 3.6. Average food consumption measured from metabolic cages on days 0, 30, 60, and 90. No significant differences were observed between treatment groups and control group over the study period (ANOVA F: (11, 104) = 2.632). Data are presented as mean ± SEM.

Table 3.2. Final organ weight. Data are represented as mean ± SEM.

	Control (n=9)	Low dose (n=10)	High dose (n=10)	*p*-value
Heart	0.244 ± 0.021	0.226 ± 0.010	0.209 ± 0.012	0.2541
Lungs	0.362 ± 0.028	0.331 ± 0.046	0.319 ± 0.024	0.6683
Spleen	0.132 ± 0.009	0.118 ± 0.012	0.12 ± 0.006	0.5275
Liver	1.896 ± 0.071	1.874 ± 0.044	1.809 ± 0.057	0.5497
Stomach	0.59 ± 0.031	0.589 ± 0.336	0.663 ± 0.055	0.3687
Intestines	3.69 ± 0.118	3.94 ± 0.128	3.88 ± 0.054	0.8219
Testes	0.411 ± 0.032	0.39 ± 0.022	0.349 ± 0.028	0.2822
Preputial gland	0.546 ± 0.035	0.548 ± 0.014	0.528 ± 0.015	0.7875
Brain	0.606 ± 0.025	0.579 ± 0.025	0.61 ± 0.016	0.5710
Thymus	0.093 ± 0.008	0.109 ± 0.006	0.092 ± 0.009	0.2311

Table 3.3. Levels of total protein and albumin in blood plasma on day 90. Data are represented as mean ± SEM.

	Body Weight (g)		Total Protein (g/L)	Albumin (g/L)
	Day 0	Day 90	Day 90	Day 90
Control (n=9)	30.94 ± 0.278	33.05 ± 0.445	44.72 ± 3.487	29.92 ± 1.134
Low Dose (n=10)	30.29 ± 0.366	32.71 ± 0.394	43.89 ± 5.658	30.51 ± 0.766
High Dose (n=10)	30.43 ± 0.535	32.02 ± 0.615	43.51 ± 4.233	30.37 ± 0.532

3.2 Biochemical Analyses

3.2.1 Urine TC concentration

The analysis of TC metabolites in urine involved assessing individual TC standard retention times (Figure 3.7), setting up calibration curves, and then measuring the concentration of TCs in urine in nanograms per mL (Figure 3.8). Metabolite concentrations were adjusted by nanograms per milligram of urinary creatinine (Figure 3.9). The major forms of TC ingredients in urine were Gallate, EC, ECG, EGCG. On day 30, the low-dose group had higher average concentrations of all 4 TCs compared to high-dose mice, likely due to the higher sustained consumption of TC water. At day 60 the high-dose group had higher average concentrations for all 4 TCs compared to control and low-dose groups (Figure 8) and by day 90, the high-dose group had sustained higher averages of EC, ECG, EGC, and EGCG (0.49 ug/mL, 0.89 ug/mL, 1.9 ug/mL, and 4.72 ug/mL, respectively). For EGCG specifically, we observed a 0.288% increase from day 30 to day 90 in the high-dose group, while the low-dose group demonstrated a 0.156% increase in EGCG metabolites over the same period. After adjusting for urinary creatinine, the high dose group showed significant increases of urinary concentration of EC from day 30 to day 90 ($p = 0.0058$), EGCG from day 30 to 60 ($p = 0.237$) and 30 to 90 ($p = 0.0137$), and EGC from day 30 to 90 ($p = 0.0129$) and 60 to 90 ($p = 0.0002$) (Figure 9). These results indicate a sustained presence and accumulation of TC metabolites in urine over the course of the study period, suggesting effective absorption and metabolism of TC following administration.

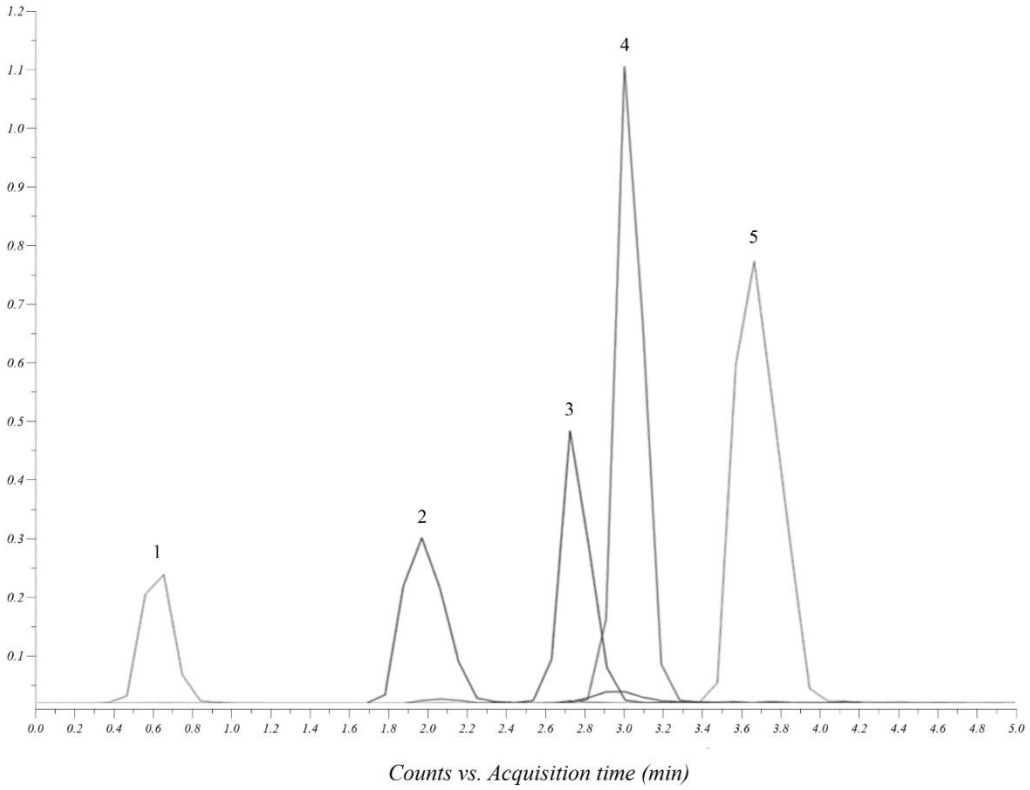

Figure 3.7. Overlapping of the extracted ion chromatograms of [M-H]⁻ ion signals of tea catechin standards identified in LC/Q-TOF targeted analysis.

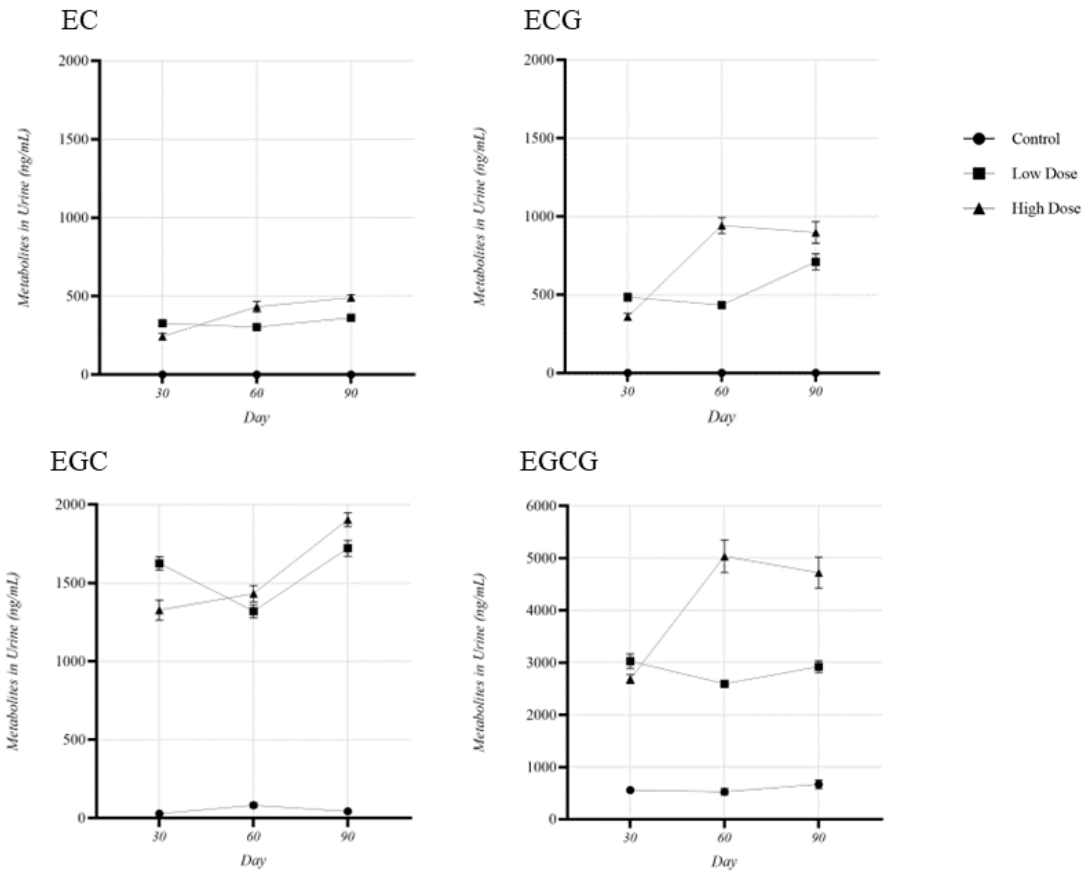

Figure 3.8. Urinary concentrations of four main TCs on day 30, 60, and 90 (ng/mL). Urine was collected via metabolic cages over a 24-hour period. Data are plotted as mean ± SEM.

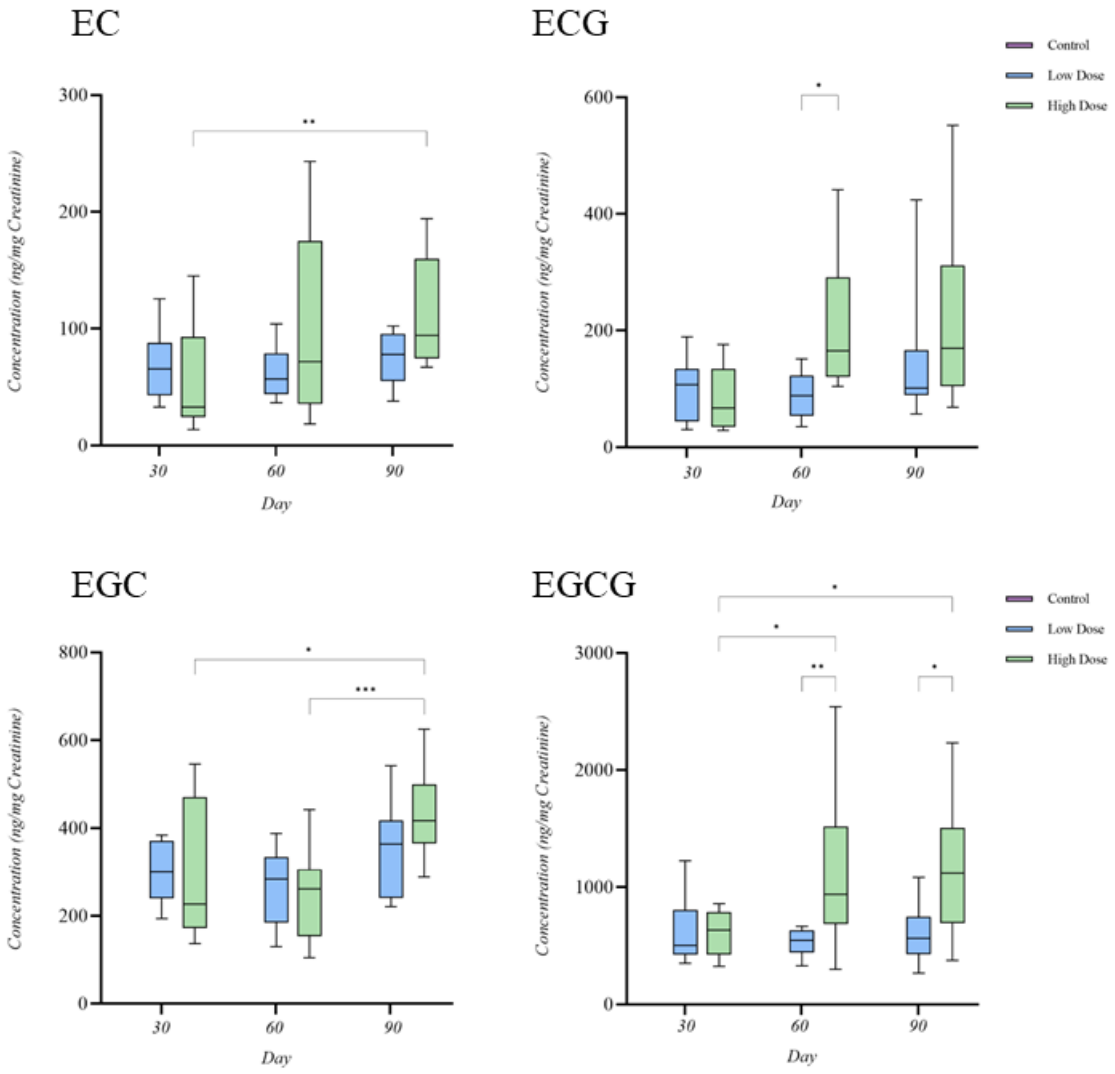

Figure 3.9. Adjusted urinary TC concentrations (ng/mg creatinine). Urine was collected via metabolic cages over a 24-hour period. (EC: ANOVA F: (8, 81) = 13.51), (ECG: ANOVA F: (4,45) = 8.622), (EGC: ANOVA F: (8, 81) = 37.06) , (EGCG: ANOVA F: (8, 81) = 17.21). Data are represented as mean and 5-95% confidence interval.

3.2.2 Concentration of α-synuclein in blood plasma

Elevated levels of α-synuclein in the plasma have been proposed as a potential biomarker for PD pathology [54,55]. As such, we employed a sandwich ELISA for the measurement of recombinant full-length mouse SNCα protein in blood plasma. The levels of α-synuclein in blood plasma of the control, low-dose, and high-dose groups were 189.9 ± 20.05 pg/mL (coefficient of variance (CV): 31.67%), 154.9 ± 9.376 pg/mL (CV: 19.14%), and 123.0 ± 7.090 pg/mL (CV: 18.22%), respectively. Brown-Forsythe ANOVA test (F: (2.000, 13.98) = 6.245) demonstrated where there was a significant difference among means ($p = 0.0115$). Dunnett's post hoc analysis demonstrated where the high-dose group had significantly less circulating α-synuclein in the blood plasma compared to controls and the low-dose group on day 90 ($p = 0.0293$, $p = 0.0421$, respectively) (Figure 3.10).

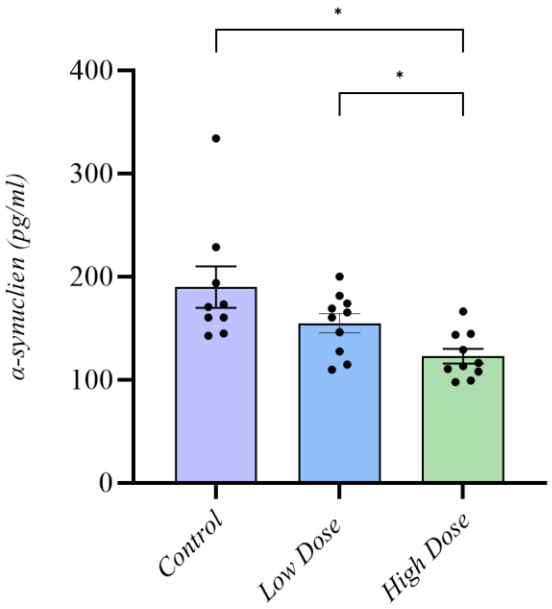

Figure 3.10. Scatter bar chart of plasma α-synuclein levels between the control, low-dose, and high-dose groups on day 90. Significant differences between the control and high dose (p = 0.0293) and low-dose and high-dose (p = 0.0421) were detected.

3.3 Behavioral Analyses

3.3.1 Open field

Given that animal models of PD usually exhibit diminished locomotor activity, especially as they age, we aimed to assess whether mice displayed altered movement in the context of GTP treatment. To determine changes in locomotor activity over the course of the study period, we utilized the open field test. By day 90, significant changes emerged regarding spatial positioning and behavior. High-dose mice spent significantly more time in the peripheral zone compared to control mice (control = 271.9 ±5.23/s; low-dose = 276.40 ± 4.49/s; high-dose = 287 ± 1.49/s) (p = 0.0393) (Figure 3.11A). Consequently, control mice spent significantly more time in the center region compared to the high-dose mice (control = 26.78 ± 5.1/s; low-dose = 22.53 ± 4.49/s; high-dose = 11.97 ± 1.42/s) (p = 0.0316) and demonstrated a significant increase in active time in the center region compared to the high-dose mice (p = 0.0009). These observations are consistent with previous reports of hyperactivity in A53T mutant α-synuclein mice [56,57], which suggest a potential connection between the expression of the A53T mutation and decreased anxiety-like behaviors. Additionally, high-dose mice exhibited significantly increased grooming behavior events compared to controls (control = 2.56 ± 0.69; low-dose = 3.80 ± 0.55; high-dose = 6.0 ± 0.72) (p = 0.0248), which may indicate alterations in stress response or anxiety-related behaviors as a result of GTP administration (Figure 3.11B). The grooming behavior of the low-dose mice showed a trend towards significance but fell short of the threshold when compared to the control groups (p = 0.0507). This trend suggests a potential dose-dependent effect of GTP treatment on anxiety related grooming behavior in an otherwise hyperactive and anti-anxiolytic mouse model.

3.3.2 Rotarod

As PD progresses, patients often experience difficulties with balance and coordination, which can significantly impact their daily activities. To evaluate these aspects, the rotarod test, a widely used

method for assessing motor coordination and balance in rodents, was employed. At the baseline, no significant differences were observed between the treatment groups (control = 130.9 ± 15.7/s; low-dose = 128.7 ± 14.61/s; high-dose = 109.5 ± 8.49/s), indicating similar motor performance prior to treatment initiation. All groups demonstrated a decrease in the maximum duration achieved on the rotarod at day 45 compared to the baseline (control = 77.53 ± 10.79/s; low-dose = 89.94 ± 16.92/s; high-dose = 81.21 ± 8.65/s). The control group exhibited significant reductions in maximum duration on the rotarod at day 45 ($p = 0.0199$) and day 90 ($p < 0.0001$), indicating deteriorating motor coordination and balance over time in control mice (Figure 3.11C). In contrast, both the low-dose and high-dose treatment groups showed longer maximum durations on the rotarod at day 90 compared to the control group (control = 54.05 ± 6.73/s; low-dose = 86.64 ± 6.97/s; high-dose = 96.55 ± 7.9/s). This suggests that the therapeutic intervention with GTP may have mitigated the decline in motor coordination and balance observed in control mice, with the improvement in rotarod performance in the treated groups implying a potential protective effect of the treatment on motor function.

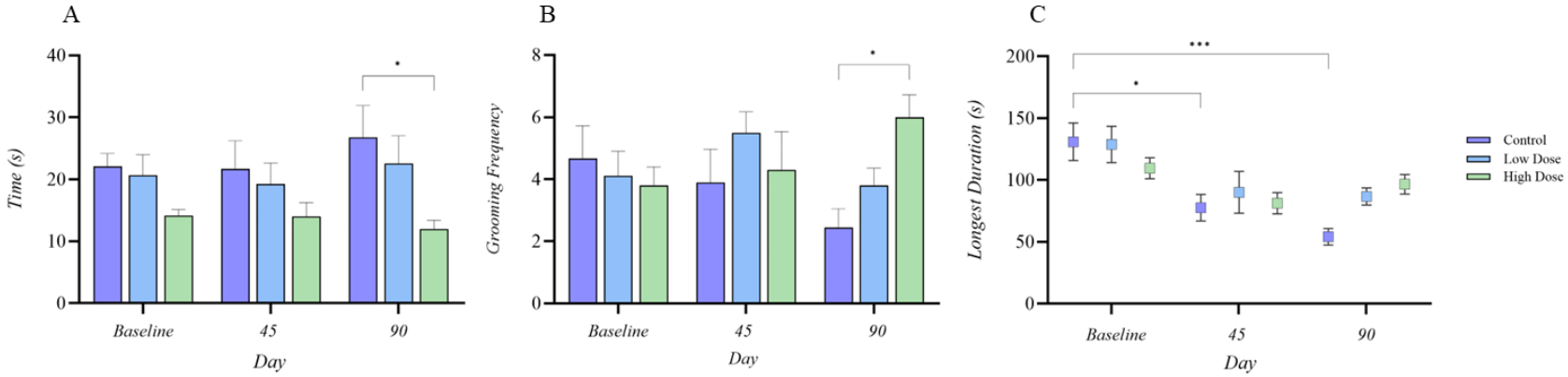

Figure 3.11. Behavioral activity recorded on the baseline, day 45, and day 90 of the experimental timeline. Data are plotted as mean ± SEM.

(A) Cumulative time (seconds) spent in the inner region of the maze over a 5-minute period; automated video tracking using ANY-maze software. On day 90, the high-dose group spent significantly less time in the center of the maze ($p = 0.0316$) and demonstrated significantly increased thigmotaxis ($p = 0.0393$) compared to the control group (ANOVA F: (8, 69) = 2.200).

(B) Grooming events measured over a 5-minute period, reported as grooming frequency. Mice in the high-dose group spent significantly more time grooming compared to controls ($p = 0.0248$). The grooming behavior of the low-dose mice exhibited a notable trend when compared with the control groups ($p = 0.0507$), narrowly missing the threshold for statistical significance (ANOVA F: (8, 69) = 1.851).

(C) Longest duration (seconds) recorded for the rotarod performance test. Motor coordination and balance was examined by rotarod performance testing the baseline, day 45, and day 90 of dosing. The control group had a significant decrease in maximum duration from day 0 to day 45 ($p = 0.0199$) and day 0 to day 90 ($p < 0.0001$) (ANOVA F: (8, 69) = 5.679)

4. Discussion

As global population aging escalates, there is a corresponding rise in the incidence of PD. PD not only profoundly impacts the health of older individuals, but also imposes a major economic and social burden on families and society [58]. Over time, the accumulation of α-synuclein in the *substantia nigra* exacerbates, leading to the progressive degeneration of dopaminergic neurons. Unfortunately, effective therapeutic options for PD patients remain limited. Conventional treatments, such as levodopa replacement therapy, merely offer symptomatic relief [59]. Consequently, there is a growing emphasis on researching early intervention and therapeutic strategies to prevent the onset and progression of PD. Nutritional factors seem to have a significant association with PD onset, prompting thorough investigation into the interplay between dietary habits and the risk of developing PD [60-63]. A Mendelian randomization study revealed that the consumption of green tea diminishes the likelihood of developing PD, indicating that a higher intake of green tea correlates with a slower progression of the disease [32]. The natural derivation of TCs and their capacity to traverse the digestive tract and penetrate the blood-brain barrier have garnered significant attention as a potential therapeutic for PD in recent years [64]. For this reason, assessment of the neuroprotective effects of TCs on PD progression with a hemizygous A53T mouse model that intrinsically overexpresses mutant human α-synuclein is significant.

Importantly, no abnormal treatment-related findings were observed, corroborating the overall safety and tolerability of the selected experimental dosages that have been reported in the literature [65,66]. Administration of 0.5% and 1.5% GTP did not have a significant impact on levels of TTP or albumin in blood plasma between controls and treatment groups. The TTP levels remained consistent across all groups and were parallel to the recorded levels observed in C57BL/6J mice [67]. There was a marginal increase noted in albumin concentrations of A53T

mice compared to C57BL/6J mice, suggesting a potential genotypic increase in endogenous albumin concentration.

Throughout the study duration, we noted a consistent increase in BW among mice in all three groups, with this increase becoming statistically significant by day 90 when compared to baseline measurements. The observed BW gain suggests that the experimental treatments did not adversely affect the overall health or nutritional status of the mice. Additionally, water intake increased over the study period in all experimental groups compared to baseline measurements. Interestingly, while there was no significant difference in water consumption between the control and low-dose groups, the high-dose group exhibited significantly lower water intake through weeks 1-6 of the study period. This could largely be due to the bitter taste of the TCs, as similar findings have been reported with TC administration via drinking water [68]. Daily intake of GTP water increased over the study period, as reflected in urine concentrations. Regarding food intake, no significant differences were observed between treatment groups in terms of the amount of food consumed. However, distinct trends were observed over time. The control group maintained relatively consistent food intake levels throughout the study period while the low-dose group showed a positive trend in food consumption by day 90, suggesting a potential increase in appetite or food preference with prolonged treatment. Conversely, the high-dose group exhibited a reduced trend in food consumption by day 90, which may indicate a decrease in appetite or alterations in feeding behavior associated with the high-dose treatment. EGCG has been documented to decrease weight gain and adipose tissue weight [69] through the induced secretion of anorexigenic gut hormones [70] or modulation of bile acid metabolism and gut microbiome composition [71,72]. These findings align with previous research indicating that EGCG may influence weight gain and metabolism through various mechanisms [73].

Despite the differences in amount of water consumption, a significant increase in TC concentrations in urine was observed in groups receiving GTP doses. Notably, levels of EC and

ECG were not detected in the control group's urine, but trace levels of EGCG (< 100 ng/ml) and EGC (< 25 ng/mL) were detected, largely due to exogenous substances in the diet that may have similar metabolic properties. The low-dose group had a slightly higher average concentration of TC metabolites on day 30, credible due to the difference in GTP drinking water volume consumed. By day 60, the high-dose group exhibited higher average concentrations of EC, ECG, and EGCG compared to the low-dose group, and by day 90 this trend extended to all of the targeted TC metabolites, with the high-dose group showing elevated levels for all measured TCs. Similar to Lambert et al. [74], urinary levels of conjugated EGCG were measured, primarily in the EGCG 3"Me ((−)-epigallocatechin-3-O-(3-O-methyl)-gallate) and EGCG 4″Me ((−)-epigallocatechin-3-O-(4-O-methyl)-gallate) forms. Liu et al reported similar urinary concentrations of EGCG on day 87 of their long-term oral administration study [75], however EGC was reported to be their highest metabolite in urine. Our results indicate where EGCG was the highest metabolite in urine, which could be due to the differences in prepared TC mixture or analytical parameters. The results suggest that frequent consumption of GTP enables the body to maintain a high level of circulating tea polyphenols, and the persistent increase in TC metabolite levels over the duration of the study suggests effective absorption and metabolism of TCs that likely contribute to *in vivo* biological effects.

Although there have been numerous biomarker-based studies conducted using peripheral tissues and body fluids to detect and quantify a-synuclein levels, there is still no consensus on which form serves as a reliable biomarker for early diagnosis or monitoring disease progression [76]. Because plasma α-synuclein levels have been documented to exhibit moderate sensitivity and high specificity in PD patients compared to healthy controls [77], an ELISA assay specific to mouse SNCA/ α-synuclein was employed for the quantification of circulating α-synuclein in blood plasma. We found that the high-dose group had significantly lower levels of circulating α-synuclein compared to the low-dose and control groups on day 90 of the study. This reduction in

α-synuclein suggests evidence of protective effects of high-dose GTP treatment, of which there are several potential mechanisms, including TC's antioxidant activity, anti-inflammatory properties, and inhibition of amyloid-beta aggregation, which may contribute to the observed reduction in circulating α-synuclein levels. Intestinal microbial ring-fission metabolites of EGCG are found in both free and conjugated forms in blood plasma, and *in vitro* data suggests that these metabolites can cross the blood brain barrier where they are in contact with potential neurotoxic oligomers [78,79]. A previous study demonstrated that EGCG strongly inhibited α-synuclein aggregation and prevented toxicity in PC12 cells [80]. Additionally, EGCG was found to bind to the native, unfolded α-synuclein polypeptide chains, preventing the formation of toxic β structures by promoting the formation of unstructured oligomers. This suggests a potential mechanism by which TC metabolites could exert neuroprotective effects, offering a link between their presence in plasma and their ability to impact brain function through the mitigation of circulating α-synuclein.

Hemizygous A53T display typical age-related behavior at 5 months, however between 7 and 9 months the mice display mild hyperactivity that progresses to wobbling movement and decreased activity. Severe motor impairment can be seen anywhere from 9 – 16 months of age [56,81], and these mice age, there is a noticeable decrease in anxiety levels, possibly attributed to alterations in neurotransmitter systems, particularly dopamine and gamma-aminobutyric acid (GABA) [56,82]. These changes are likely impacted by the advancement of disease pathology, involving modifications in neuronal circuits and neurotransmitter concentrations. In the open field test, which assesses locomotor activity and spatial behavior, no significant differences were observed in distance traveled or average speed between treatment groups at any time points. By day 90, mice in the high-dose group displayed a preference for the periphery of the open field, spending significantly more time in this zone compared to controls, which spent more time in the center of the open field. This behavioral shift in the high-dose group suggests heightened anxiety levels, a

factor that could be further supported by examining aberrant grooming behaviors, known to be influenced by stress and anxiety in rodents [83]. A53T mice have been reported to display impaired grooming observed as early as two months old [48,83]. High-dose mice exhibited increased grooming behavior compared to controls which implies alterations in stress response or anxiety-related behaviors. The trend towards significance in grooming behavior observed in low-dose mice also suggests a dose-dependent effect of GTP treatment on anxiety-related behaviors that are downregulated in A53T phenotypes. These findings underscore the need for further investigation into the potential anxiolytic effects of long-term TC treatment in PD. The rotarod test revealed where, while all groups demonstrated a decrease in maximum duration achieved on the rotarod by day 45 compared to baseline, control mice exhibited significant reductions in duration on the rod at both day 45 and day 90. This decline in motor coordination and balance over time in control mice highlights the progressive nature motor impairments in PD. This progressive decline in motor performance observed in control mice underscores the importance of therapeutic interventions, as evidenced by a 2017 study where MPTP mice treated with EGCG had a significantly improved duration on the rotarod compared to the MPTP controls [84]. Additionally, a related study examining the neuroprotective potential of EGCG in a chronic PD mouse model induced by α-synuclein preformed fibrils reported EGCG's capacity to ameliorate the motor impairment symptoms of α-synuclein on the rotarod performance test [85]. A 2015 study provided evidence that tea polyphenols were effective in mitigating motor impairments, reducing dopaminergic neuronal injury, and inhibiting α-synuclein aggregation in monkeys with MPTP induced PD [81]. Taken together, this data suggests that GTP may have therapeutic potential in addressing key aspects of PD pathology, including motor dysfunction and the abnormal accumulation of α-synuclein. Our observations indicate a potential association between the A53T α-synuclein mutation and behavioral deficits in the hemizygous A53T PD mouse model, suggesting a comorbidity between these behaviors and the disease.

5. Conclusion

This study demonstrates that sustained administration of TCs significantly reduces circulating α-synuclein levels and improves motor coordination in a dose-dependent manner in a hemizygous transgenic A53T mouse model of PD. The safety outcomes from the present animal study lay the groundwork for determining a safe maximum daily dosage for prolonged consumption of TC supplements for the prevention of PD symptoms. Additionally, investigations into the relationship between urinary TC levels and clinical endpoints may provide valuable insights into the potential therapeutic efficacy of TC interventions. The observed improvement in motor function, as evidenced by enhanced performance in rotarod tests and changes in anxiety-related behavior, underscores the potential of TCs as a therapeutic agent for PD symptoms. Our results add evidence to the claim that TC treatment may have protective effects on locomotor activity, motor coordination, and α-synuclein levels in the A53T mouse model of PD. These findings highlight the promise of TCs in mitigating key aspects of PD pathology and warrant further research to clarify their underlying mechanisms of action and assess their potential therapeutic utility in preventing PD-related symptoms and pathology. Further comprehensive studies are needed to investigate the *in vivo* dynamics of TC metabolites, their metabolism, and the interactions with α-synuclein species.

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References

1. Musial C, Kuban-Jankowska A, Gorska-Ponikowska M. Beneficial Properties of Green Tea Catechins. Int J Mol Sci. 2020 Mar 4;21(5).
2. Botten D, Fugallo G, Fraternali F, et al. Structural Properties of Green Tea Catechins. J Phys Chem B. 2015 Oct 8;119(40):12860-7.
3. Babu PV, Liu D. Green tea catechins and cardiovascular health: an update. Curr Med Chem. 2008;15(18):1840-50.
4. Sun K, Wang L, Ma Q, et al. Association between tea consumption and osteoporosis: A meta-analysis. Medicine (Baltimore). 2017 Dec;96(49):e9034.
5. Lee DB, Song HJ, Paek YJ, et al. Relationship between Regular Green Tea Intake and Osteoporosis in Korean Postmenopausal Women: A Nationwide Study. Nutrients. 2021 Dec 26;14(1).
6. Shen CL, Yeh JK, Cao JJ, et al. Green tea and bone metabolism. Nutr Res. 2009 Jul;29(7):437-56.
7. Sharifi-Rad M, Pezzani R, Redaelli M, et al. Preclinical Pharmacological Activities of Epigallocatechin-3-gallate in Signaling Pathways: An Update on Cancer. Molecules. 2020 Jan 22;25(3).
8. Bonuccelli G, Sotgia F, Lisanti MP. Matcha green tea (MGT) inhibits the propagation of cancer stem cells (CSCs), by targeting mitochondrial metabolism, glycolysis and multiple cell signalling pathways. Aging (Albany NY). 2018 Aug 23;10(8):1867-1883.
9. Fujiki H, Watanabe T, Sueoka E, et al. Cancer Prevention with Green Tea and Its Principal Constituent, EGCG: from Early Investigations to Current Focus on Human Cancer Stem Cells. Mol Cells. 2018 Feb 28;41(2):73-82.

10. Trabalzini L, Ercoli J, Trezza A, et al. Pharmacological and In Silico Analysis of Oat Avenanthramides as EGFR Inhibitors: Effects on EGF-Induced Lung Cancer Cell Growth and Migration. Int J Mol Sci. 2022 Aug 1;23(15).
11. Filippini T, Malavolti M, Borrelli F, et al. Green tea (Camellia sinensis) for the prevention of cancer. Cochrane Database Syst Rev. 2020 Mar 2;3(3):CD005004.
12. Zhang Y, Yu W, Zhang L, et al. The Interaction of Polyphenols and the Gut Microbiota in Neurodegenerative Diseases. Nutrients. 2022 Dec 17;14(24).
13. Seidl SE, Santiago JA, Bilyk H, et al. The emerging role of nutrition in Parkinson's disease. Front Aging Neurosci. 2014;6:36.
14. Riegelman E, Xue KS, Wang JS, et al. Gut-Brain Axis in Focus: Polyphenols, Microbiota, and Their Influence on alpha-Synuclein in Parkinson's Disease. Nutrients. 2024 Jun 27;16(13).
15. Group GBDNDC. Global, regional, and national burden of neurological disorders during 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Neurol. 2017 Nov;16(11):877-897.
16. Kouli A, Torsney KM, Kuan WL. Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis. In: Stoker TB, Greenland JC, editors. Parkinson's Disease: Pathogenesis and Clinical Aspects. Brisbane (AU)2018.
17. Baldini F, Hertel J, Sandt E, et al. Parkinson's disease-associated alterations of the gut microbiome predict disease-relevant changes in metabolic functions. BMC Biol. 2020 Jun 9;18(1):62.
18. Ghiglieri V, Calabrese V, Calabresi P. Alpha-Synuclein: From Early Synaptic Dysfunction to Neurodegeneration. Front Neurol. 2018;9:295.
19. Calabresi P, Di Lazzaro G, Marino G, et al. Advances in understanding the function of alpha-synuclein: implications for Parkinson's disease. Brain. 2023 Sep 1;146(9):3587-3597.

20. Blauwendraat C, Nalls MA, Singleton AB. The genetic architecture of Parkinson's disease. Lancet Neurol. 2020 Feb;19(2):170-178.

21. Lubbe S, Morris HR. Recent advances in Parkinson's disease genetics. J Neurol. 2014 Feb;261(2):259-66.

22. Zagare A, Preciat G, Nickels SL, et al. Omics data integration suggests a potential idiopathic Parkinson's disease signature. Commun Biol. 2023 Nov 20;6(1):1179.

23. De Miranda BR, Goldman SM, Miller GW, et al. Preventing Parkinson's Disease: An Environmental Agenda. J Parkinsons Dis. 2022;12(1):45-68.

24. Grenn FP, Kim JJ, Makarious MB, et al. The Parkinson's Disease Genome-Wide Association Study Locus Browser. Mov Disord. 2020 Nov;35(11):2056-2067.

25. Narayan S, Liew Z, Bronstein JM, et al. Occupational pesticide use and Parkinson's disease in the Parkinson Environment Gene (PEG) study. Environ Int. 2017 Oct;107:266-273.

26. Mostafalou S, Abdollahi M. The susceptibility of humans to neurodegenerative and neurodevelopmental toxicities caused by organophosphorus pesticides. Arch Toxicol. 2023 Dec;97(12):3037-3060.

27. Pyatha S, Kim H, Lee D, et al. Association between Heavy Metal Exposure and Parkinson's Disease: A Review of the Mechanisms Related to Oxidative Stress. Antioxidants (Basel). 2022 Dec 15;11(12).

28. Doroszkiewicz J, Farhan JA, Mroczko J, et al. Common and Trace Metals in Alzheimer's and Parkinson's Diseases. Int J Mol Sci. 2023 Oct 29;24(21).

29. Hantikainen E, Roos E, Bellocco R, et al. Dietary fat intake and risk of Parkinson disease: results from the Swedish National March Cohort. Eur J Epidemiol. 2022 Jun;37(6):603-613.

30. Shokri-Mashhadi N, Ghiasvand R, Feizi A, et al. Association between major dietary patterns and Parkinson's disease risk: a case-control study. Neurol Sci. 2024 May;45(5):2003-2010.

31. Pajares M, A IR, Manda G, et al. Inflammation in Parkinson's Disease: Mechanisms and Therapeutic Implications. Cells. 2020 Jul 14;9(7).

32. Li C, Lin J, Yang T, et al. Green Tea Intake and Parkinson's Disease Progression: A Mendelian Randomization Study. Front Nutr. 2022;9:848223.

33. Qi H, Li S. Dose-response meta-analysis on coffee, tea and caffeine consumption with risk of Parkinson's disease. Geriatr Gerontol Int. 2014 Apr;14(2):430-9.

34. Li FJ, Ji HF, Shen L. A meta-analysis of tea drinking and risk of Parkinson's disease. ScientificWorldJournal. 2012;2012:923464.

35. Chen Y, Sun X, Lin Y, et al. Non-Genetic Risk Factors for Parkinson's Disease: An Overview of 46 Systematic Reviews. J Parkinsons Dis. 2021;11(3):919-935.

36. Akbarialiabad H, Dahroud MD, Khazaei MM, et al. Green Tea, A Medicinal Food with Promising Neurological Benefits. Curr Neuropharmacol. 2021;19(3):349-359.

37. Belvisi D, Pellicciari R, Fabbrini G, et al. Modifiable risk and protective factors in disease development, progression and clinical subtypes of Parkinson's disease: What do prospective studies suggest? Neurobiol Dis. 2020 Feb;134:104671.

38. Malar DS, Prasanth MI, Brimson JM, et al. Neuroprotective Properties of Green Tea (Camellia sinensis) in Parkinson's Disease: A Review. Molecules. 2020 Aug 27;25(17).

39. Yang Z, Yao Y, Zhou Y, et al. EGCG attenuates alpha-synuclein protofibril-membrane interactions and disrupts the protofibril. Int J Biol Macromol. 2023 Mar 1;230:123194.

40. Gronnemose AL, Osterlund EC, Otzen DE, et al. EGCG has Dual and Opposing Effects on the N-terminal Region of Self-associating alpha-synuclein Oligomers. J Mol Biol. 2022 Dec 15;434(23):167855.

41. Zhao J, Liang Q, Sun Q, et al. (−)-Epigallocatechin-3-gallate (EGCG) inhibits fibrillation, disaggregates amyloid fibrils of α-synuclein, and protects PC12 cells against α-synuclein-induced toxicity. RSC advances. 2017;7(52):32508-32517.

42. Bieschke J, Russ J, Friedrich RP, et al. EGCG remodels mature alpha-synuclein and amyloid-beta fibrils and reduces cellular toxicity. Proc Natl Acad Sci U S A. 2010 Apr 27;107(17):7710-5.

43. Zhou T, Zhu M, Liang Z. (-)-Epigallocatechin-3-gallate modulates peripheral immunity in the MPTP-induced mouse model of Parkinson's disease. Mol Med Rep. 2018 Apr;17(4):4883-4888.

44. Kim JS, Kim JM, O JJ, et al. Inhibition of inducible nitric oxide synthase expression and cell death by (-)-epigallocatechin-3-gallate, a green tea catechin, in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. J Clin Neurosci. 2010 Sep;17(9):1165-8.

45. Levites Y, Weinreb O, Maor G, et al. Green tea polyphenol (-)-epigallocatechin-3-gallate prevents N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurodegeneration. J Neurochem. 2001 Sep;78(5):1073-82.

46. Choi JY, Park CS, Kim DJ, et al. Prevention of nitric oxide-mediated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease in mice by tea phenolic epigallocatechin 3-gallate. Neurotoxicology. 2002 Sep;23(3):367-74.

47. Mustapha M, Mat Taib CN. MPTP-induced mouse model of Parkinson's disease: A promising direction of therapeutic strategies. Bosn J Basic Med Sci. 2021 Aug 1;21(4):422-433.

48. Paumier KL, Sukoff Rizzo SJ, Berger Z, et al. Behavioral characterization of A53T mice reveals early and late stage deficits related to Parkinson's disease. PLoS One. 2013;8(8):e70274.

49. FDA US. Food Additives Permitted For Direct Addition To Food For Human Consumption. In: Services DoHaH, editor. 2023.

50. La-Vu M, Tobias BC, Schuette PJ, et al. To Approach or Avoid: An Introductory Overview of the Study of Anxiety Using Rodent Assays. Front Behav Neurosci. 2020;14:145.

51. Sakayori N, Kato S, Sugawara M, et al. Motor skills mediated through cerebellothalamic tracts projecting to the central lateral nucleus. Mol Brain. 2019 Feb 8;12(1):13.

52. Campos FL, Carvalho MM, Cristovao AC, et al. Rodent models of Parkinson's disease: beyond the motor symptomatology. Front Behav Neurosci. 2013;7:175.

53. Wang JS, Luo H, Wang P, et al. Validation of green tea polyphenol biomarkers in a phase II human intervention trial. Food Chem Toxicol. 2008 Jan;46(1):232-40.

54. Zhao X, He H, Xiong X, et al. Lewy Body-Associated Proteins A-Synuclein (a-syn) as a Plasma-Based Biomarker for Parkinson's Disease. Front Aging Neurosci. 2022;14:869797.

55. Chang CW, Yang SY, Yang CC, et al. Plasma and Serum Alpha-Synuclein as a Biomarker of Diagnosis in Patients With Parkinson's Disease. Front Neurol. 2019;10:1388.

56. Graham DR, Sidhu A. Mice expressing the A53T mutant form of human alpha-synuclein exhibit hyperactivity and reduced anxiety-like behavior. J Neurosci Res. 2010 Jun;88(8):1777-83.

57. Unger EL, Eve DJ, Perez XA, et al. Locomotor hyperactivity and alterations in dopamine neurotransmission are associated with overexpression of A53T mutant human alpha-synuclein in mice. Neurobiol Dis. 2006 Feb;21(2):431-43.

58. Yang W, Hamilton JL, Kopil C, et al. Current and projected future economic burden of Parkinson's disease in the U.S. NPJ Parkinsons Dis. 2020;6:15.

59. Gandhi KR, Saadabadi A. Levodopa (L-Dopa). StatPearls. Treasure Island (FL) ineligible companies. Disclosure: Abdolreza Saadabadi declares no relevant financial relationships with ineligible companies.2024.

60. Cryan JF, O'Riordan KJ, Sandhu K, et al. The gut microbiome in neurological disorders. Lancet Neurol. 2020 Feb;19(2):179-194.

61. Elfil M, Kamel S, Kandil M, et al. Implications of the Gut Microbiome in Parkinson's Disease. Mov Disord. 2020 Jun;35(6):921-933.

62. Nie S, Ge Y. The link between the gut microbiome, inflammation, and Parkinson's disease. Appl Microbiol Biotechnol. 2023 Sep 22.

63. Romano S, Savva GM, Bedarf JR, et al. Meta-analysis of the Parkinson's disease gut microbiome suggests alterations linked to intestinal inflammation. NPJ Parkinsons Dis. 2021 Mar 10;7(1):27.

64. Perez-Burillo S, Navajas-Porras B, Lopez-Maldonado A, et al. Green Tea and Its Relation to Human Gut Microbiome. Molecules. 2021 Jun 26;26(13).

65. Shen CL, Brackee G, Song X, et al. Safety Evaluation of Green Tea Polyphenols Consumption in Middle-aged Ovariectomized Rat Model. J Food Sci. 2017 Sep;82(9):2192-2205.

66. Chan PC, Ramot Y, Malarkey DE, et al. Fourteen-week toxicity study of green tea extract in rats and mice. Toxicol Pathol. 2010 Dec;38(7):1070-84.

67. Otto GP, Rathkolb B, Oestereicher MA, et al. Clinical Chemistry Reference Intervals for C57BL/6J, C57BL/6N, and C3HeB/FeJ Mice (Mus musculus). J Am Assoc Lab Anim Sci. 2016;55(4):375-86.

68. Wang J, Tang L, Zhou H, et al. Long-term treatment with green tea polyphenols modifies the gut microbiome of female sprague-dawley rats. J Nutr Biochem. 2018 Jun;56:55-64.

69. Li F, Gao C, Yan P, et al. EGCG Reduces Obesity and White Adipose Tissue Gain Partly Through AMPK Activation in Mice. Front Pharmacol. 2018;9:1366.

70. Song WY, Aihara Y, Hashimoto T, et al. (-)-Epigallocatechin-3-gallate induces secretion of anorexigenic gut hormones. J Clin Biochem Nutr. 2015 Sep;57(2):164-9.

71. Ushiroda C, Naito Y, Takagi T, et al. Green tea polyphenol (epigallocatechin-3-gallate) improves gut dysbiosis and serum bile acids dysregulation in high-fat diet-fed mice. J Clin Biochem Nutr. 2019 Jul;65(1):34-46.

72. Huang J, Feng S, Liu A, et al. Green Tea Polyphenol EGCG Alleviates Metabolic Abnormality and Fatty Liver by Decreasing Bile Acid and Lipid Absorption in Mice. Mol Nutr Food Res. 2018 Feb;62(4).

73. Hursel R, Viechtbauer W, Westerterp-Plantenga MS. The effects of green tea on weight loss and weight maintenance: a meta-analysis. Int J Obes (Lond). 2009 Sep;33(9):956-61.

74. Lambert JD, Lee MJ, Lu H, et al. Epigallocatechin-3-gallate is absorbed but extensively glucuronidated following oral administration to mice. J Nutr. 2003 Dec;133(12):4172-7.

75. Liu AB, Tao S, Lee MJ, et al. Effects of gut microbiota and time of treatment on tissue levels of green tea polyphenols in mice. Biofactors. 2018 May 8.

76. Magalhaes P, Lashuel HA. Opportunities and challenges of alpha-synuclein as a potential biomarker for Parkinson's disease and other synucleinopathies. NPJ Parkinsons Dis. 2022 Jul 22;8(1):93.

77. Shu H, Zhang P, Gu L. Alpha-synuclein in peripheral body fluid as a biomarker for Parkinson's disease. Acta Neurol Belg. 2024 Jun;124(3):831-842.

78. Pervin M, Unno K, Takagaki A, et al. Function of Green Tea Catechins in the Brain: Epigallocatechin Gallate and its Metabolites. Int J Mol Sci. 2019 Jul 25;20(15).

79. Unno K, Pervin M, Nakagawa A, et al. Blood-Brain Barrier Permeability of Green Tea Catechin Metabolites and their Neuritogenic Activity in Human Neuroblastoma SH-SY5Y Cells. Mol Nutr Food Res. 2017 Dec;61(12).

80. Ehrnhoefer DE, Bieschke J, Boeddrich A, et al. EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers. Nat Struct Mol Biol. 2008 Jun;15(6):558-66.

81. AlzForum. Research Models: α-synuclein A53T Mouse (Tg) AlzForum.org2019 [updated February 8, 2019; March 1, 2024].

82. Lai TT, Gericke B, Feja M, et al. Anxiety in synucleinopathies: neuronal circuitry, underlying pathomechanisms and current therapeutic strategies. NPJ Parkinsons Dis. 2023 Jun 22;9(1):97.

83. Kalueff AV, Stewart AM, Song C, et al. Neurobiology of rodent self-grooming and its value for translational neuroscience. Nat Rev Neurosci. 2016 Jan;17(1):45-59.

84. Xu Q, Langley M, Kanthasamy AG, et al. Epigallocatechin Gallate Has a Neurorescue Effect in a Mouse Model of Parkinson Disease. J Nutr. 2017 Oct;147(10):1926-1931.

85. Shen J, Xie J, Ye L, et al. Neuroprotective effect of green tea extract (-)-epigallocatechin-3-gallate in a preformed fibril-induced mouse model of Parkinson's disease. Neuroreport. 2024 Apr 3;35(6):421-430.

CHAPTER 4

GREEN TEA CATECHINS MODULATE GUT MICROBIOTA COMPOSITION AND ALTER KEGG PATHWAYS IN A TRANSGENIC A53T MOUSE MODEL OF PARKINSON'S DISEASE[1]

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[1] Riegelman, E.; Xue, K.S.; Wang, J.-S.; Tang, L. Green Tea Catechins Modulate Gut Microbiota Composition and Alter Kegg Pathways in a Transgenic A53T Mouse Model of Parkinson's Disease.

To be submitted to a peer-reviewed journal

Abstract

Green tea polyphenols (GTPs) have been demonstrated to provide a range of health benefits in both animals and humans, likely stemming from their interaction with the gut microbiota. As only about 5-10% of GTPs are metabolized by the human body, the rest are in direct contact with the gut microbes present in the large intestine, where they can be biotransformed into potent antioxidant and anti-inflammatory metabolites. In the past decade, numerous studies have illuminated the intricate relationship between microbial dysbiosis and Parkinson's disease (PD), paving the way for new biomarkers and therapeutic approaches. With advances in high-throughput sequencing, the role of the gut microbiome has gained significant attention in the context of PD prevention. GTPs and their interaction with gut microbes could offer promising therapeutic potential for microbial dysbiosis. This study investigated the effects of 90-day supplementation with green tea catechins (TC) via drinking water in 6-month-old male A53T transgenic mice, focusing on compositional shifts and functional outcomes of the gut microbiome. Our findings revealed significant changes in microbial α-diversity, with TC-treated groups exhibiting higher diversity compared to control mice. In addition to changes in microbial composition, we observed alterations in key metabolic pathways, including enhanced cAMP signaling and a reduction in nicotinamide metabolism, lipopolysaccharide biosynthesis, and biofilm formation in TC-treated groups. These results support the hypothesis that TCs may mitigate gut microbiome dysbiosis and promote neuroprotection through modulation of the microbial metabolome. Our study highlights the therapeutic potential of TCs in influencing gut microbiota and their metabolic pathways.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, following Alzheimer's disease, and is pathologically characterized by the progressive degeneration of dopaminergic neurons in *substantia nigra pars compacta* and accumulation of misfolded α-synuclein protein [1]. The prevalence of PD has more than doubled over the last 25 years [2], highlighting not only the impact of an aging global population but also potential environmental and genetic factors that have contributed to the rise in cases. About 10% of PD cases can be linked to inherited genetic mutations, however most cases are idiopathic in origin [3], pointing to a complex interplay between age, the environment, and genetic factors as disease promoters. Advances in genomic sequencing have revolutionized our understanding of PD, uncovering the crucial role of the gut microbiome in PD development and progression, particularly through its ability to modulate inflammatory signals and interact with other organs [4]. Age-related alterations in the gut microbiota can be linked to aging-related changes in gut physiology, such as mucosal thinning, barrier disruption, reduced gut motility, and immune senescence [5]. Additionally, changes in lifestyle factors, including diet, environmental/living conditions, and physical activity, as well as changes in health status contribute to gut perturbation.

Since the publication of the first study on gut dysbiosis in PD patients in 2015 [6], a myriad of studies have demonstrated where patients with PD have a significantly altered gut microbial composition compared to age-matched healthy controls [7-20], which has led to an increased interest in uncovering the alterations in gut-derived microbial products and immune pathways promoting PD pathology. The most consistent findings across gut microbiome studies in the context of PD have described an increased abundance of bacteria in the *Akkermansia* genus in the *Verrucomicrobiaceae* family, along with increased abundance of Bifidobacterium and *Lactobacillus* genera and reduced abundance of *Roseburia*, *Faecalibacterium*, and *Blautia* genera [5]. *Bifidobacterium* and *Lactobacillus*, commonly regarded as beneficial bacteria and frequently

found in probiotic supplements [21], may exhibit increased abundance in PD due to their adaptability to the altered gut environment. However, the underlying reasons for this shift remain unclear. Decreased levels of the fecal short-chain fatty acids (SCFAs) butyrate, acetate, and propionate have been consistently observed in PD [11,22,23]. SCFAs play a crucial role in microbiota-gut-brain communication, partly by regulating the integrity of the gut and blood-brain barriers (BBB), modulating inflammatory responses, influencing endocrine signaling, and promoting neuronal survival [24,25]. Colonic biopsies from PD patients have revealed an accumulation of α-synuclein in the GI tract before onset of motor symptoms [7,26]. This finding suggests that α-synuclein may begin to aggregate in the enteric nervous system (ENS) in the early stages of PD, potentially spreading to the brain in later stages. Additionally, increased gut permeability has been documented in early stages of PD, which is a significant trigger for inflammation and may contribute to the heightened aggregation of α-synuclein and increased oxidative stress [27]. From a systemic perspective, increased intestinal inflammation and permeability can lead to systemic inflammation, which in turn may heighten the permeability of the BBB, ultimately activating microglia in the brain. Microglial activation can result in increased systemic oxidative stress, further exacerbating neuroinflammation and potential aggregation of α-synuclein, resulting in the progression of PD.

Green tea polyphenols (GTP) are a mixture of natural flavonoids that offer a spectrum of health benefits largely due to their anti-oxidative and anti-inflammatory properties [28,29]. The oral bioavailability of major green tea constituents, namely tea catechins (TC), is generally low [30]. Of the four main TCs found in green tea, epicatechin (EC), epicatechin-gallate (ECG), epigallocatechin (EGC), and epigallocatechin-gallate (EGCG), EGCG is the most abundant and most studied. EGCG has garnered attention due to its beneficial health effects, including, but not limited to, antioxidant properties [31,32], anti-inflammatory properties [33,34], anti-carcinogenic [32,35] and anti-protein aggregation [36,37] properties, and the regulation in the production of

SCFAs [38,39]. It is known that EGCG has potential benefits for PD patients, but whether this protection involves remodeling gut microbiota remains unclear despite proposed mechanisms. Certain studies have suggested where green tea administration can influence the gut microbiome by either stimulating the growth of beneficial bacteria species or inhibiting the growth of adverse species [40-42]. Further, EGCG has been proposed as a therapeutic for diseases with an inflammatory component through the modulation of local intestinal inflammation and the improvement of intestinal barrier integrity. As such, the goal of this study was to assess whether 90 days of TC administration altered the microbial composition and resulting microbial functional pathways in transgenic A53T mouse model of PD.

2. Experimental

2.1 Animals, Chemicals, and Experimental Design

This study was conducted alongside a recently published article that evaluated the therapeutic potential of TCs in alleviating symptoms in an A53T transgenic PD mouse model. Figure 1 illustrates the study design and sample collection.

As described previously, six-month-old, male B6.Cg-2310039L15Rik$^{Tg(Prnp\text{-}SNCA*A53T)23Mkle}$/J mice (Strain #006823, Hualpha-Syn(A53T), Hemizygous transgenic line G2-3) (Jackson Laboratories), were housed under a 12-12h light-dark cycle with free access to pelleted PicoLab Rodent Diet 5053 (LabDiet, Fort Worth, TX) and distilled drinking water during the study period. Mice (n=30) were randomly assigned to one of three groups (n=10/group): control, low-dose supplementation (0.5% wt/vol GTP), or high-dose supplementation (1.5% wt/vol GTP). Decaffeinated GTP (Lot # 2597074) was purchased from LKT Laboratories, Inc. (St. Paul, MN). Distilled and GTP extract water was prepared fresh daily while the amount of water drank (mL) and the animal's body weight (g) were recorded. Mice were transferred to Nalgene metabolic cage systems (Rochester, NY, USA) on days 0, 30, 60, and 90. Feces were collected at two-time points over 24 hours into

sterilized tubes, which were then stored at -80°C until further analysis. At the conclusion of the study, animals were humanely euthanized and then subjected to a gross necropsy. All experiments were reviewed, approved, and carried out under the University of Georgia Animal Use and Care Committee.

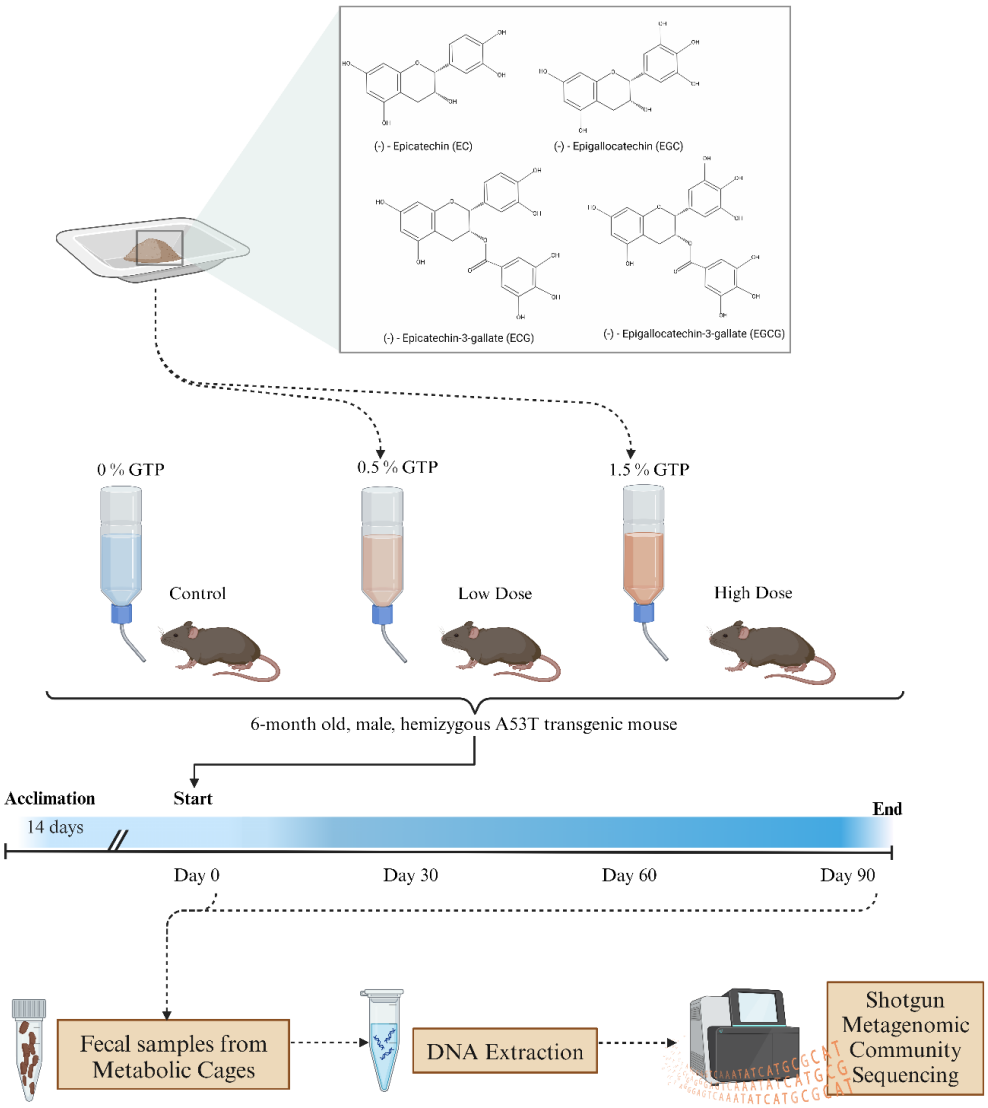

Figure 4.1. Study design of 90-day TC administration and sample collection for SMC sequencing of murine gut microbial communities.

2.2 DNA Extraction

Total DNA was isolated using ZymoBiomics DNA Fecal Microbiome extraction mini kit (Zymo Research, USA). Frozen fecal samples were thawed at room temperature and an aliquot of fecal materials (150mg) was lysed and homogenized in a bead beating tube containing lysis buffer and 0.1 mm and 0.5 mm beads using Omni's Bead Ruptor 96 (Perkin Elmer, USA) following manufacturer instruction, then centrifuged at ≥10,000 x g for 1 minute. Up to 400 µl of the supernatant was transferred to a Zymo-Spin™ III-F Filter in a collection tube and centrifuged at 8,000 x g for 1 minute. The filtrate was combined with 1,200 µl of Genomic Lysis Buffer, mixed, and 800 µl of the mixture was transferred to a Zymo-Spin™ IICR Column. After centrifugation at 10,000 x g for 1 minute, the flow-through was discarded, and the step was repeated. The column was washed with 200 µl of DNA Pre-Wash Buffer and 500 µl of g-DNA Wash Buffer, centrifuging after each step. DNA was eluted with 100 µl of DNA Elution Buffer by centrifugation at 10,000 x g for 30 seconds. The eluted DNA was further purified using a Zymo-Spin™ III-HRC Filter, prepped with 600 µl of Prep Solution, and centrifuged at 16,000 x g for 3 minutes. Double stranded DNA (dsDNA). dsDNA was measured using Qubit® dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) on an Invitrogen Qubit® 4.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.3 Sequencing Data Preparation

The profiling process started by surveying the potential presence of bacterial species for each raw metagenomic sample read by using Kraken2 and a pre-built core gene database containing k-mers (k=35) of reference genomes obtained from the EzBioCloud database (Version 2023.08.23). After acquiring a list of candidate species, a custom bowtie2 database [43] was built utilizing the core genes and genomes from the species found during the first step. The raw sample was then mapped against the bowtie2 database using the -very-sensitive option and a phredd33 quality score of 30. Samtools was used to cover and sort the output bam file. Coverage of the mapped

reads against the bam file was obtained using Bedtools (Version 2.31.0) [44]. To avoid false positives, we used an in-house script to quantify all the reads that mapped to a given species only if the total coverage of their core genes was ≥ 25%. Finally, species abundance was calculated using the total number of reads counted and normalized species abundance was calculated by using their total length of their reference.

2.4 Statistical Analysis

Data is presented as mean ± SEM. Statistical analyses were conducted within the GraphPad Prism Software environment (Version 10.2.3) and EZBioCloud interface (Version 2023.08.23). To understand the impact on gut microbiota composition, α-diversity, β-diversity, and Linear discriminant analysis Effect Size (LEfSe) were conducted. The Bray–Curtis distance, which assesses the shared microbial communities in each sample, was used to create the distance matrix, followed by subsequently conducted Principal Coordinate Analysis (PCoA) to determine clustering patterns based on the Bray–Curtis distance matrix. Normality was assessed using the Shapiro-Wilk test while homogeneity was evaluated using Levene's test. Data that was homogeneous and normally distributed was evaluated through a one-way ANOVA, while data that did not meet normality and homogeneity assumptions was evaluated through Kruskal-Wallis parameters. Post hoc analysis in multi-group tests was performed using Tukey's test in ANOVA and Games-Howell test in the Kruskal-Wallis test. A significance threshold of 0.05 was set for the test among groups, and the threshold for discriminative features was set at a logarithmic LDA score of 3.0.

3. Results

3.1 Relative abundance

Following bacterial taxonomy annotation using the EZBioCloud gene database, Table 1 demonstrates the phyla composition between the three groups on day 90. The control and

supplemented groups exhibited a consistent pattern in phylum-level composition, with Bacteroidetes and Firmicutes remaining the two most abundant phyla throughout the study. There was a dose-dependent shift characterized by an increase in the relative abundance of Bacteroidetes and a corresponding decrease in Firmicutes. Table 2 lists the relative abundance of the top 12 genera on day 90. The genera *Duncaniella* and *Fusimonas* had dose-depended decreases over the 90-day period, while *RIAY_g* had a dose-depended increase over 90-day period.

When considering genera implicated in PD, we observed that control mice had a significantly increased abundance of *Lactobacillus* ($p = 0.0074$) (F: 25.41 (2.000, 3.614)), and *Akkermansia* ($p = 0.0347$) (F: 7.093 (2.000, 4.995)) compared to the supplemented groups (Figure 2). The genera *Bacteroides*, *Clostridium*, and *Pseudoflavonifactor* decreased slightly with dosing, while *Bifidobacterium* increased slightly with dosing. These findings highlight notable dose-dependent shifts with potential implications for the modulation of microbial communities through TC supplementation.

Table 4.1. Relative abundance of top 8 phyla measured on day 90.

	Day 90			
Phyla	Control (100%)	Low Dose (100%)	High Dose (100%)	*p*-value
Bacteroidetes	48.19	50.28	51.59	0.7624
Firmicutes	48.35	44.84	44.51	0.7272
Tenericutes	0.92	1.18	0.84	0.8389
Verrucomicrobia	0.61	1.09	0.92	0.4969
Actinobacteria	1.06	1.59	1.31	0.4510
Proteobacteria	0.77	0.38	0.63	0.0608
Desulfobacterota	0.02	0.45	0.08	0.0693
Saccharibacteria	0.10	0.18	0.12	0.9493

Table 4.2. Relative abundance of top 12 genera measured on day 90.

	Day 90			
Genus	Control (100%)	Low Dose (100%)	High Dose (100%)	*p*-value
Duncaniella	33.43	31.74	29.49	0.7224
Fusimonas	11.13	6.18	5.80	0.3427
Paramuribaculum	5.04	5.65	5.30	0.8248
RIAY_g	1.72	4.43	7.67	0.3406
g__CAG-873	3.75	3.94	3.03	0.6484
KE159571_g	3.49	2.44	3.51	0.6620
g__Coproplasma	0.69	2.74	0.94	0.7224
Akkermansia	1.49	0.31	0.41	0.4969
Ligilactobacillus	4.32	3.41	3.37	0.8195
Bacteroides	1.04	1.31	1.31	0.7224
Oscillibacter	1.08	1.81	1.30	0.1333
PAC001524_g	1.219	2.09	1.42	0.8860
Other	31.60	33.95	36.45	--

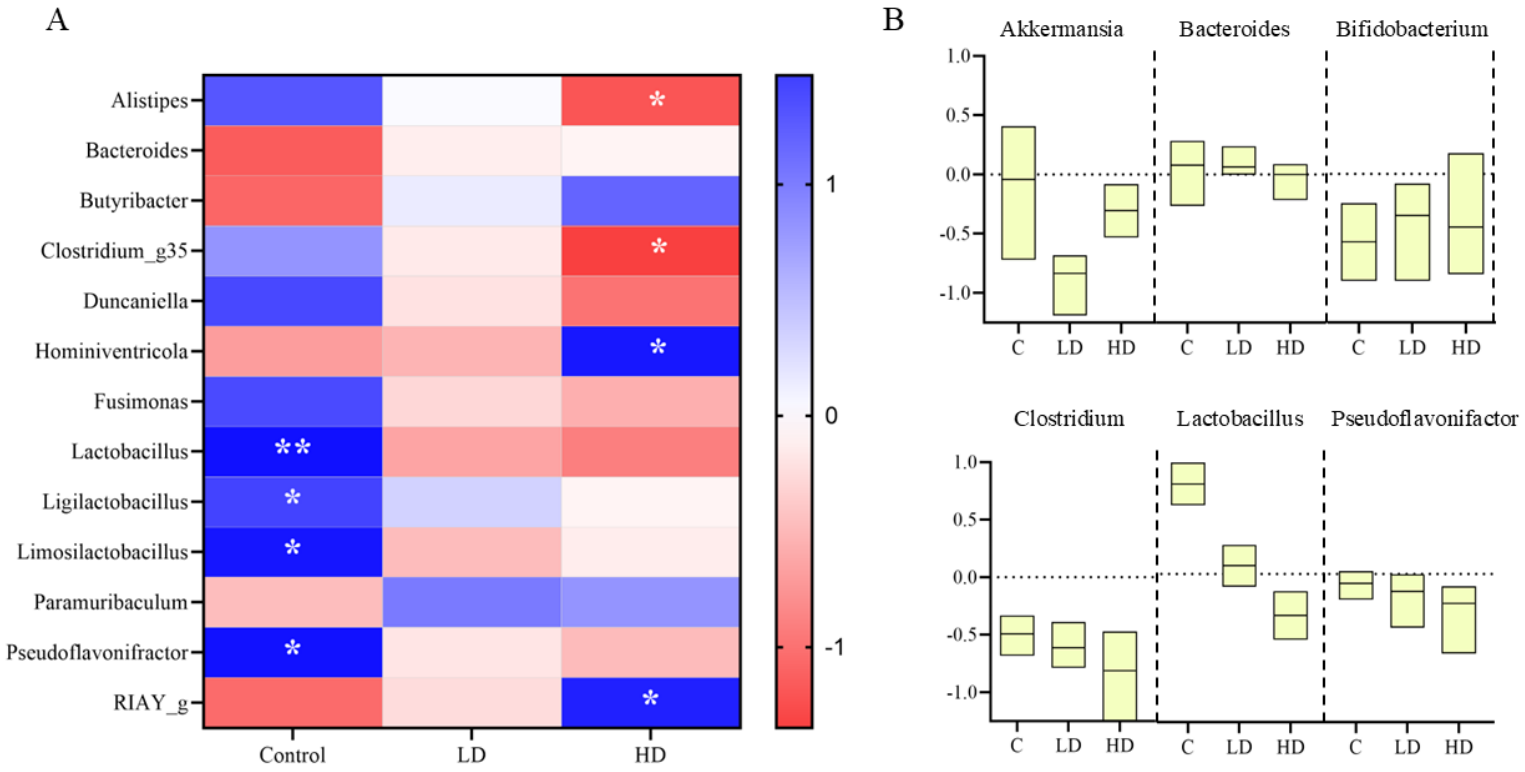

Figure 4.2. (A) Genus level heatmap showing z-score relative abundance of genera in treatment groups genera measured on day 90 (relative abundance >0.5% among three treatment groups).

(B) Log transformation of the relative abundance of specific genera implicated in gut microbiome of human patients with PD. Data are shown as min-max.

3.2 α-diversity

α-diversity analysis includes multiple aspects of microbial composition, such as richness, evenness, dominance, rare or low abundance species, and coverage. In our study, we evaluated several indices to capture these characteristics. We used two indices highlighting species richness and evenness, including Shannon index (Figure 3A) and Fisher index (Figure 3B), as well as the Chao1 index (Figure 3C) for observing community richness. The control group exhibited minimal changes in average α-diversity from baseline to day 90, whereas the low-dose group showed an increase in average α-diversity. All three of the measured indices showed a significant increase in α-diversity in the high-dose group compared to the control, with the Shannon index also indicating a significant increase from the baseline to day 90 in the high-dose group. The increase in α-diversity from TC administration indicates a more balanced and robust microbial community which may contribute to improved gut health and functionality.

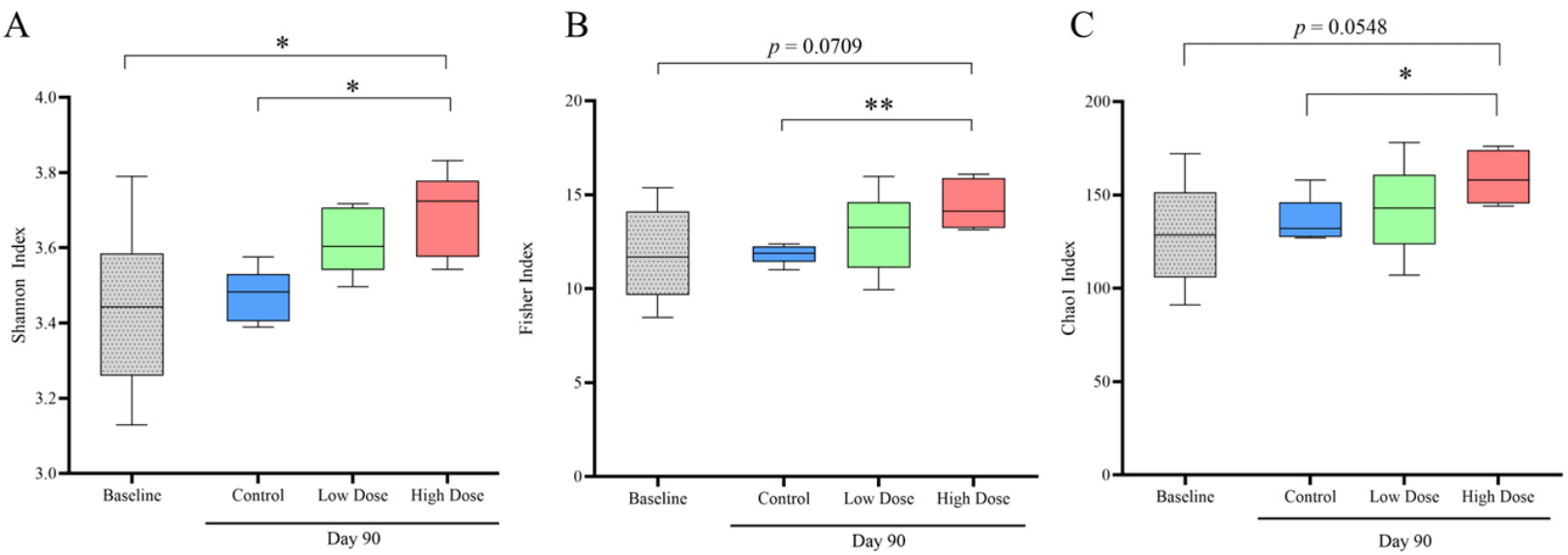

Figure 4.3. Shannon index, Fisher index, and Chao1 index as measures of α-diversity.

(A) Shannon index measures species evenness in a community F = 3.302 (6.000, 13.24) (p = 0.0304, p = 0.0275).

(B) Fisher index quantifies the species diversity within a community based on the richness and evenness of their distribution F = 1.425 (6.000, 10.58) (p = 0.0079).

(C) Chao1 index estimates species richness by taking both the number of observed species and the number of rare taxa into account F = 1.531 (6.000, 10.31) (p = 0.0433).

3.3 β-diversity

B-diversity, in contrast to α-diversity, focuses on the specific composition of the microbial community rather than its quantity. This distinction allows for differentiation between subjects that share identical α-diversity scores, as their β-diversity scores can vary widely. A common method for analyzing β-diversity involves creating a distance matrix for Principal Coordinate Analysis (PCoA). The Bray–Curtis distance, which ranges from 0 to 1, reflects the degree of shared species between two samples, with values closer to 1 indicating greater compositional dissimilarity. As illustrated in Figure 4, following the 90-day intervention, PERMANOVA indicated a small dissimilarity between the groups (F: 1.366, r2 = 0.12, p = 0.129). which can be visualized from where the low-dose and high-dose treatment groups ellipses intersect. The control group showed relatively increased similarity compared to the dissimilarity observed in the low-dose and high-dose groups.

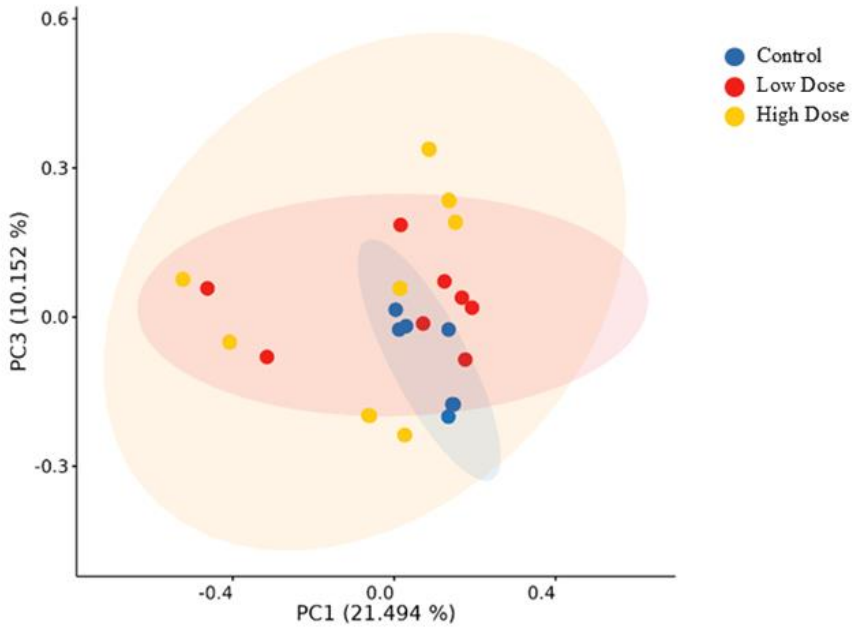

Figure 4.4. Linear discriminant analysis Effect Size (LEfSe) Principal Coordinate Analysis (PCoA) of β-diversity. Each dot represents one subject in its respective group, and the three colors represent the treatment groups. The elliptical area around the dots in the same color represents the uncertainty inside the group. PC1 and PC3 are two mathematical dimensions which explain most of the variation without biological characteristics, and the percentage shown in parenthesis is the percent of total variation explained by that axis. PERMANOVA was used to examine the dissimilarity between the groups (F = 1.366, r2 = 0.12, p = 0.129).

3.4 LEfSe analysis of biomarker bacteria

LEfSe, an algorithm tailored for high-dimensional data, was used to identify and interpret biomarker taxa by detecting genomic features, such as genes, pathways, or taxa, that distinguish between various biological conditions. In our study, we utilized LEfSe to investigate microbial differences between the control and treatment groups on day 90. As depicted in Figure 5, the control group exhibited significantly higher compositions of *Lactobacillus*, *Limosilactobacillus*, and *Parasutterella*. In contrast, the low-dose group showed an increased presence of *Acutalibacter*, *Adlercreutzia*, and *KE159628_g*, while the high-dose group was characterized by elevated levels of *Hominiventricola*, *Eubacterium*, and *Butyribacter*. These findings suggest distinct microbial shifts corresponding to the dosage, with specific taxa enriched in both low- and high-dose groups, highlighting the potential for dose-dependent modulation of the gut microbiome in response to treatment.

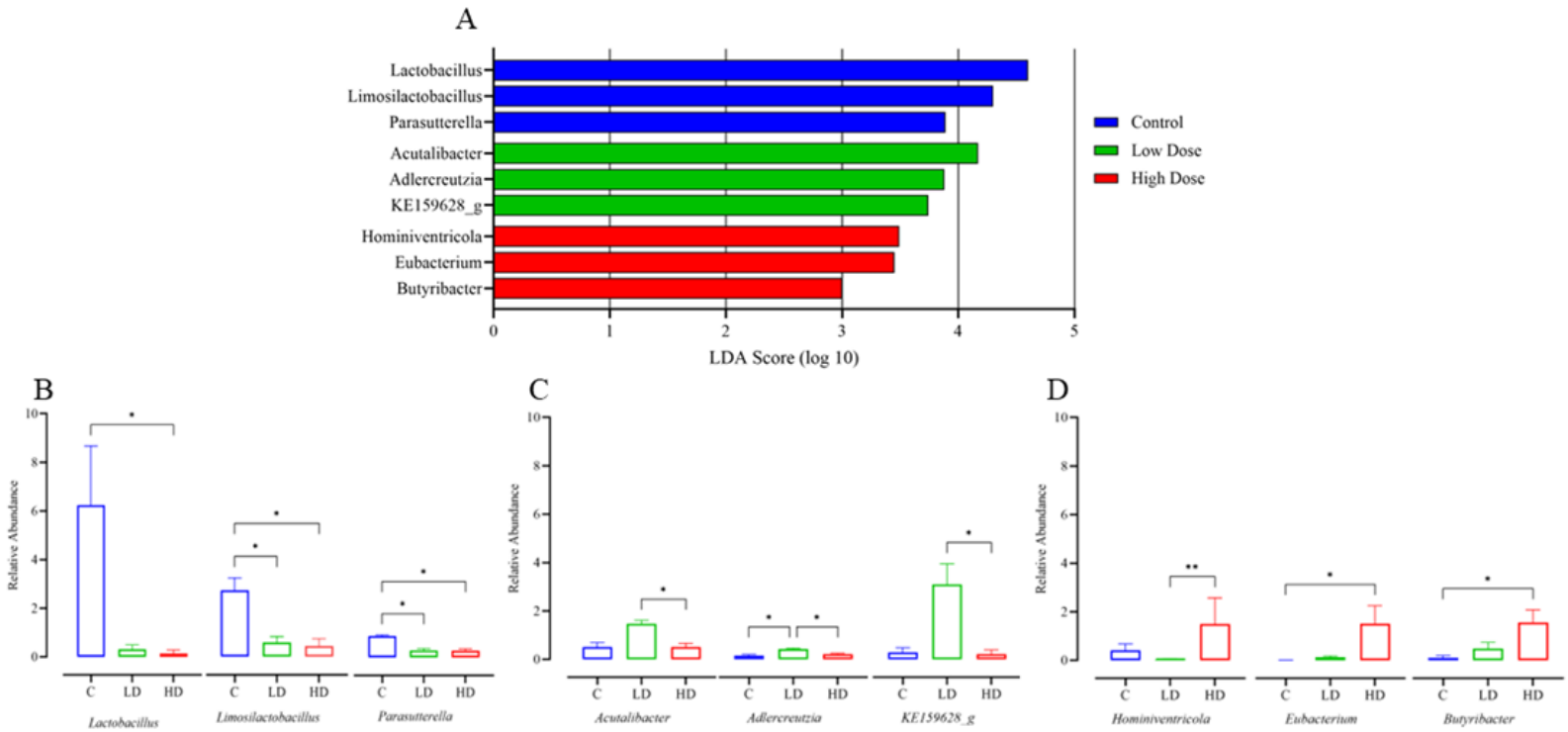

Figure 4.5. (A) Linear discriminant analysis Effect Size (LefSe)(log10) Genus level LEfSe showing the biomarker taxa with an LDA score >2 and a significance of p > 0.05 determined by the Wilcoxon ranked sum-test for C and HD groups. The relative abundance of genera in the C, LD, and HD (groups shown as B, C, and D). Data are plotted as mean ± SEM.

3.5 Analysis of KEGG Pathways

Results from Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis are summarized, revealing that many constituents of the gut microbiome have been identified through metagenomics but remain uncharacterized, particularly in murine mammals. Therefore, the conclusions drawn here can be considered carefully due to the current knowledge and available resources. 2681 KEGG ortholog groups and 526 metabolic pathways were identified in the study group. While most of the altered pathways between control and treatment mice likely indicate dysbiosis, several features within the A53T metagenome correspond with inflammatory pathological markers of PD. Figure 6(A) illustrates KEGG data with functional relevance to PD, while Figure 6(B) illustrates MiniPath (Minimal set of Pathways) data for relevant PD mouse model inflammatory pathways.

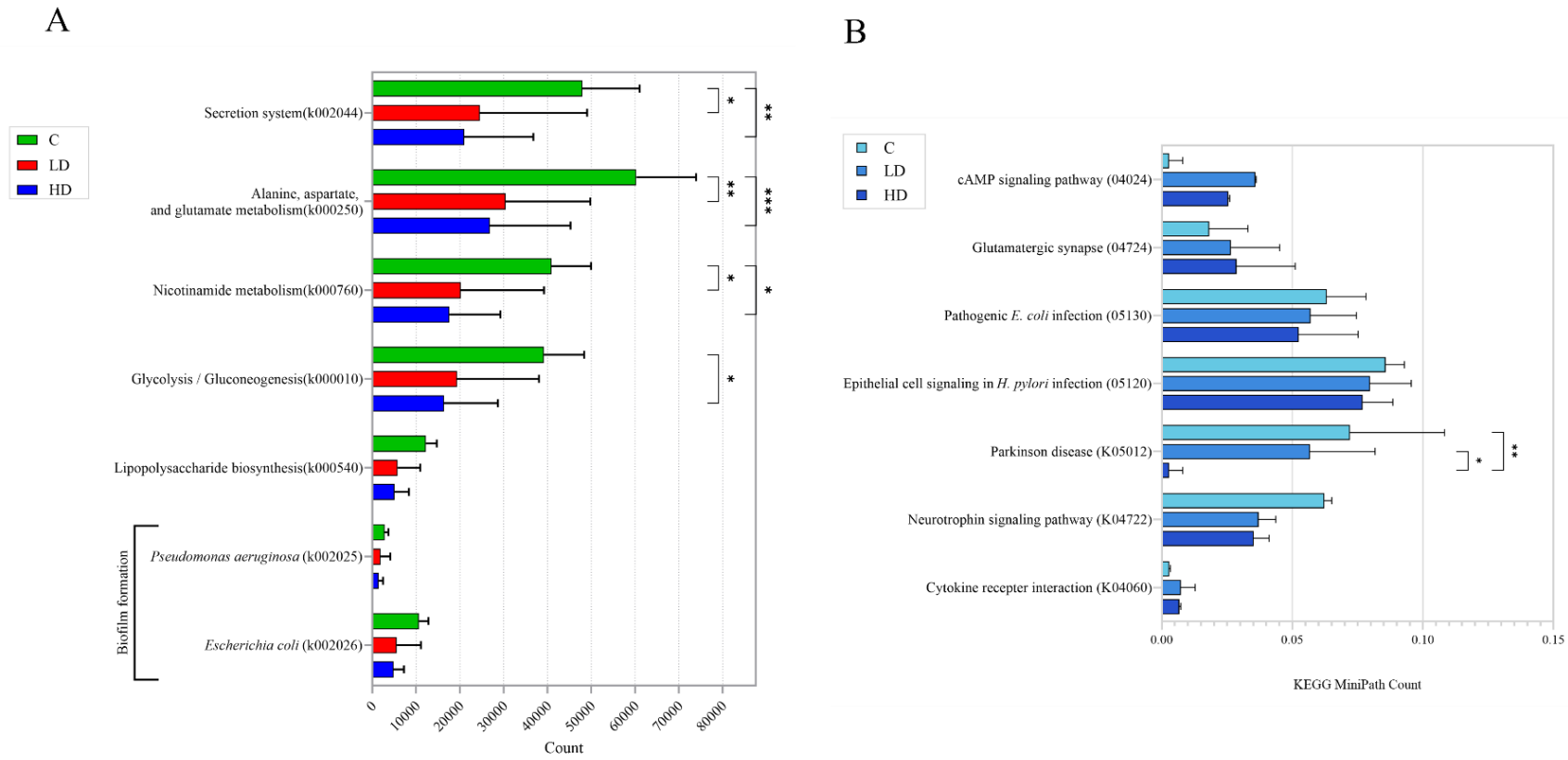

Figure 4.6. (A) Alterations cellular processes and metabolism KEGG pathways measured after 90 days intervention with tea catechins. The control group had significantly upregulated pathways on day 90, including Ko00250 (p = 0.0032, p = 0.0010), Ko00760 (p = 0.0462, p = 0.0232), and Ko00010 (p = 0.0347). (B) MiniPath KEGG data of inflammatory pathways relevant to PD. Pathways for PD were significantly lower in the high-dose and low-dose groups compared to the control group (p = 0.0055, p = 0.0399)

Pathway analysis revealed significant differences between the control, low-dose, and high-dose treatment groups. The control group exhibited a significant up-regulation of alanine, aspartate, and glutamate metabolism compared to both low-dose and high-dose groups ($p = 0.0032$, $p = 0.0010$). Additionally, nicotinamide metabolism was significantly up regulated in the control group relative to the treated groups ($p = 0.0462$, $p = 0.0232$). A notable increase in the regulation of glycolysis/gluconeogenesis pathways was also observed in the control group ($p = 0.0347$). A dose-dependent decrease in lipopolysaccharide biosynthesis was detected across treatment groups. In terms of microbial metabolism, the control group showed increased activity in P. aeruginosa and E. coli biofilm formation metabolism compared to the low- and high-dose groups. MiniPath analysis revealed where pathways for PD were significantly lower in the high-dose and low-dose groups compared to the control group ($p = 0.0055$, $p = 0.0399$). MiniPath also described elevated counts of glutamatergic synapse and cytokine receptor interaction pathways with increasing TC doses. Notably, the cAMP signaling pathway was elevated across all treatment groups, however the low-dose group showed higher counts of this pathway compared to the high-dose group.

4. Discussion

Growing evidence suggests that the gut microbiome plays a significant role in neurodegenerative diseases, with increasing links between gut microbes and the development and symptoms of PD. This study, focusing on the gut microbiome's compositional shifts and functions, evaluated the effects of 90-day TC supplementation via drinking water in 6-month-old male A53T transgenic mice. Green tea has been evidenced by epidemiological studies to reduce risk of developing PD and potentially alleviate PD progression [45,46], though clinical trial data is insufficient. We found that 90 days of repeated dosing with 1.5% TC in drinking water induced significant changes in the α-diversity of gut microbiota but not β-diversity. In many human studies, α-diversity has been reported as unchanged in PD patients or decreased in PD patients [47,48]. In

the transgenic A53T control group, we saw significantly lower α-diversity compared to the high-dose TC group. In a human intervention study where volunteers were given green tea, α-diversity was increased and associated with 2 weeks of green tea drinking [49]. However, this study also found a significant increase in β-diversity of tea-drinking volunteers, while our study did not report a significant shift. The consistent administration of TCs may act as an external input, promoting the growth of beneficial species and enhancing microbial functions. At the same time, it may reduce the abundance of potentially pathogenic species, thereby compensating for any negative effects associated with reduced biodiversity.

Several microbial species and functional gene pathways were altered in accordance with TC dosing. In the control group, there was an increase in the genera *Alistipes* within the *Rickenellaceae* family, *Lactobacillus*, *Ligilactobacillus*, and *Limosilactobacillus* in the *Lactobacillaceae* family, and *Pseudoflavonifractor* in the *Ruminococcaceae* family. Members of the *Lactobacillaceae* family are commonly regarded as beneficial probiotics that contribute to gut homeostasis by fermenting carbohydrates into lactic acid, lowering gut pH, and inhibiting the growth of pathogenic bacteria. Interestingly, while *Lactobacillus* species are generally seen as favorable, their enrichment has also been reported in studies of PD patient microbiomes [20]. This dual role suggests that while they may offer protective effects in a healthy gut, their overrepresentation or altered functionality in PD could be symptomatic of gut dysbiosis, potentially contributing to disease progression through undenounced mechanisms. In the low-dose mice, we observed an increase in the genera *Acutalibacter*, *Adlercreutzia*, *Flintibacter,* and *KE159628_g*, indicating a shift in microbial composition at this dosage level. In the high-dose group, there was an increase in *Hominiventricola*, *Sporofaciens,* and *PAC001043* within the *Lachnospiraceae* family, as well as *RIAY_g* in the *Muribaculaceae* family. The observed increase in *Lachnospiraceae*-related genera in the high-dose group could suggest a compensatory response aimed at restoring butyrate production. Similarly, the rise in *RIAY_g* from the *Muribaculaceae*

family, which is involved in polysaccharide breakdown and SCFA production, might reflect alterations in microbial metabolism. The genera *Bifidobacterium* showed increased enrichment as TC doses were elevated. Effects of green tea consumption in humans have demonstrated the tendency for increase of *Bifidobacterium* species [50,51]. The genera *Bacteroides*, *Clostridium*, and *Pseudoflavonifractor* showed a slight decline with increasing TC doses, suggesting that TC administration may exert an inhibitory effect on these bacteria. EGCG has been reported to have an inhibitory effect on the growth of *Bacteroides* and *Clostridium* groups [52]. While moderate doses of TCs are known to promote the growth of *Akkermansia* and *Lactobacillus* due to their prebiotic properties and ability to enhance gut mucin production [53,54], higher doses might create a different microbial environment, especially in the context of PD. Excessive catechins may inhibit certain microbial pathways or shift the gut ecosystem in favor of other beneficial species. Consequently, the shifts in microbial community structure observed between the low- and high-dose groups may underscore the intricate balance of microbial interactions, where excessive TC levels could favor the dominance of certain species over others. These differences in microbial community structure between low- and high-dose groups may reflect dose-dependent effects on gut microbiota, potentially influencing the metabolic and functional outcomes associated with each dosage.

We observed that the metagenome of control A53T mice more closely reflected that of the PD dysbiosis metagenome, with an enrichment of immunogenic components, dysregulated neuroactive signaling, and an overproduction of cellular-derived toxicants. Specifically, control mice exhibited markedly higher alanine, aspartate, and glutamate metabolism compared to both the low-dose and high-dose groups. These amino acids are key neurotransmitters, and their dysregulated metabolism is linked to excitotoxicity and neurodegeneration in PD [55]. Decreased glutamatergic synapses in the striatum occurs in early-stage PD [56], and we saw where control mice had lower counts of glutamatergic synapse compared to treatment groups. There could be

many reasons for these interrelated findings, including heightened metabolic activity as a result of the brain compensating for the disruption of balance in synaptic activity, the loss of glutamatergic neurons or changes in synaptic plasticity, or overstimulation from excess glutamate leading to excitotoxicity [57]. Conversely, we saw increases in cAMP signaling pathways in our TC treated groups. Previous studies have established the role of the cAMP/PKA signaling pathway in controlling lipid metabolism, specifically by regulating lipid accumulation and breakdown. This pathway is also crucial in the regulation of mitochondrial functions. Given these roles, it is plausible that TCs may promote fat decomposition through modulation of the cAMP/PKA pathway [58]. By activating adenylate cyclase, TCs could elevate intracellular cAMP levels, which in turn may enhance cAMP signaling. Given the protective role of cAMP in neurodegenerative conditions, cAMP signaling presents itself as a promising therapeutic target for PD.

While cAMP signaling may offer therapeutic potential through lipid metabolism and mitochondrial regulation, our analysis also revealed another important pathway involved in PD: nicotinamide metabolism, a pathway involved in NAD+ production, which is critical for cellular energy homeostasis and mitochondrial function—processes that are often impaired in PD [59]. A recent clinical trial on PD using nicotinamide supplementation demonstrated a significant improvement in pathological markers, including reduced inflammation [60]. In our study, we observed an increase in nicotinamidase degradation with control mice, suggesting that the gut microbiome may be breaking down this neuroprotective compound. The control group also had increased glycolysis/gluconeogenesis, indicative of heightened glucose metabolism, which can reflect shifts in microbial energy utilization that may influence neurodegenerative processes. PD patients typically exhibit glucose hypometabolism in the posterior temporoparietal, occipital, and occasionally frontal regions, along with glucose hypermetabolism in the putamen, sensorimotor cortex, and cerebellum [61]. Though the exact mechanisms remain unclear, the impact of elevated

glucose levels on lipid metabolism in PD and how this may contribute to disease progression warrants further investigation. There was also a decrease in lipopolysaccharide (LPS) biosynthesis in our TC treatment groups, which is notable given that LPS can trigger systemic inflammation and has been associated with gut dysbiosis and PD pathogenesis through the gut-brain axis. Lastly, the TC treated groups showed down regulation of biofilm formation, which could reflect microbial community changes that affect gut permeability and inflammation, both of which are critical factors in the progression of PD.

5. Conclusion

This study provides insights into the effects of TC supplementation on gut microbiome composition and function in a transgenic A53T mouse model of PD. Notably, we observed significant increases in α-diversity in TC-treated groups, contrasting with the lower α-diversity seen in control mice. The higher α-diversity in the TC groups suggests that repeated TC dosing may promote a more resilient and balanced gut microbiome, potentially enhancing microbial functions linked to neuroprotection. TC supplementation led to notable shifts in microbial composition. Furthermore, we observed dose-dependent effects on microbial species, with low-dose and high-dose groups showing distinct compositional profiles, underscoring the complex interaction between catechin levels and microbial ecosystems. Our findings also highlight key metabolic pathways influenced by TC supplementation, particularly cAMP signaling and nicotinamide metabolism, which may play pivotal roles in mitochondrial regulation, fat metabolism, and inflammation reduction. Additionally, the reduction in LPS biosynthesis and biofilm formation in the TC-treated groups suggests potential anti-inflammatory and gut-protective effects. Collectively, these results indicate that TCs, through modulating gut microbial diversity, composition, and metabolic pathways, should garner more attention in the context of therapeutic strategy for the prevention of PD. However, further research, including extensive

clinical trials with robust populations, is needed to fully understand these mechanisms and their clinical relevance.

References

1. Kouli, A., K.M. Torsney, and W.L. Kuan, Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis, in Parkinson's Disease: Pathogenesis and Clinical Aspects, T.B. Stoker and J.C. Greenland, Editors. 2018: Brisbane (AU).
2. World Health Organization. Parkinson’s disease. 2023
3. Zhang, P.L., et al., Genetics of Parkinson's disease and related disorders. J Med Genet, 2018. 55(2): p. 73-80.
4. Calabrese, V., et al., Aging and Parkinson's Disease: Inflammaging, neuroinflammation and biological remodeling as key factors in pathogenesis. Free Radic Biol Med, 2018. 115: p. 80-91.
5. Tan, A.H., S.Y. Lim, and A.E. Lang, The microbiome-gut-brain axis in Parkinson disease - from basic research to the clinic. Nat Rev Neurol, 2022. 18(8): p. 476-495.
6. Scheperjans, F., et al., Gut microbiota are related to Parkinson's disease and clinical phenotype. Mov Disord, 2015. 30(3): p. 350-8.
7. Hilton, D., et al., Accumulation of alpha-synuclein in the bowel of patients in the pre-clinical phase of Parkinson's disease. Acta Neuropathol, 2014. 127(2): p. 235-41.
8. Petrov, V.A., et al., Analysis of Gut Microbiota in Patients with Parkinson's Disease. Bull Exp Biol Med, 2017. 162(6): p. 734-737.
9. Aho, V.T.E., et al., Relationships of gut microbiota, short-chain fatty acids, inflammation, and the gut barrier in Parkinson's disease. Mol Neurodegener, 2021. 16(1): p. 6.
10. Nishiwaki, H., et al., Meta-Analysis of Gut Dysbiosis in Parkinson's Disease. Mov Disord, 2020. 35(9): p. 1626-1635.
11. Unger, M.M., et al., Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. Parkinsonism Relat Disord, 2016. 32: p. 66-72.

12. Wallen, Z.D., et al., Characterizing dysbiosis of gut microbiome in PD: evidence for overabundance of opportunistic pathogens. NPJ Parkinsons Dis, 2020. 6: p. 11.
13. Heintz-Buschart, A., et al., The nasal and gut microbiome in Parkinson's disease and idiopathic rapid eye movement sleep behavior disorder. Mov Disord, 2018. 33(1): p. 88-98.
14. Cirstea, M.S., et al., Microbiota Composition and Metabolism Are Associated With Gut Function in Parkinson's Disease. Mov Disord, 2020. 35(7): p. 1208-1217.
15. Barichella, M., et al., Unraveling gut microbiota in Parkinson's disease and atypical parkinsonism. Mov Disord, 2019. 34(3): p. 396-405.
16. Mehanna, M., et al., Study of the gut microbiome in Egyptian patients with Parkinson's Disease. BMC Microbiol, 2023. 23(1): p. 196.
17. Qian, Y., et al., Gut metagenomics-derived genes as potential biomarkers of Parkinson's disease. Brain, 2020. 143(8): p. 2474-2489.
18. Kwon, D., et al., Diet and the gut microbiome in patients with Parkinson's disease. NPJ Parkinsons Dis, 2024. 10(1): p. 89.
19. Li, Z., et al., Gut bacterial profiles in Parkinson's disease: A systematic review. CNS Neurosci Ther, 2023. 29(1): p. 140-157.
20. Romano, S., et al., Meta-analysis of the Parkinson's disease gut microbiome suggests alterations linked to intestinal inflammation. NPJ Parkinsons Dis, 2021. 7(1): p. 27.
21. (NIH), N.I.o.H. Probiotics: Fact Sheet for Health Profesionals. Dietary Supplement Fact Sheets 2023 [cited 2024; Available from: https://ods.od.nih.gov/factsheets/Probiotics-HealthProfessional/.
22. Wu, G., et al., Serum short-chain fatty acids and its correlation with motor and non-motor symptoms in Parkinson's disease patients. BMC Neurol, 2022. 22(1): p. 13.
23. Metzdorf, J. and L. Tonges, Short-chain fatty acids in the context of Parkinson's disease. Neural Regen Res, 2021. 16(10): p. 2015-2016.

24. Fock, E. and R. Parnova, Mechanisms of Blood-Brain Barrier Protection by Microbiota-Derived Short-Chain Fatty Acids. Cells, 2023. 12(4).

25. O'Riordan, K.J., et al., Short chain fatty acids: Microbial metabolites for gut-brain axis signalling. Mol Cell Endocrinol, 2022. 546: p. 111572.

26. Fricova, D., J. Harsanyiova, and A. Kralova Trancikova, Alpha-Synuclein in the Gastrointestinal Tract as a Potential Biomarker for Early Detection of Parkinson's Disease. Int J Mol Sci, 2020. 21(22).

27. Salim, S., et al., Gut microbiome and Parkinson's disease: Perspective on pathogenesis and treatment. J Adv Res, 2023. 50: p. 83-105.

28. Musial, C., A. Kuban-Jankowska, and M. Gorska-Ponikowska, Beneficial Properties of Green Tea Catechins. Int J Mol Sci, 2020. 21(5).

29. Wang, S., et al., Immunomodulatory Effects of Green Tea Polyphenols. Molecules, 2021. 26(12).

30. Cai, Z.Y., et al., Bioavailability of Tea Catechins and Its Improvement. Molecules, 2018. 23(9).

31. Yiannakopoulou, E., Targeting oxidative stress response by green tea polyphenols: clinical implications. Free Radic Res, 2013. 47(9): p. 667-71.

32. Lambert, J.D. and R.J. Elias, The antioxidant and pro-oxidant activities of green tea polyphenols: a role in cancer prevention. Arch Biochem Biophys, 2010. 501(1): p. 65-72.

33. Li, S., et al., Neurodegenerative diseases and catechins: (-)-epigallocatechin-3-gallate is a modulator of chronic neuroinflammation and oxidative stress. Front Nutr, 2024. 11: p. 1425839.

34. Jorgenson, M.C., et al., Effects of green tea polyphenols on inflammation and iron status. J Nutr Sci, 2023. 12: p. e119.

35. Ferrari, E., S. Bettuzzi, and V. Naponelli, The Potential of Epigallocatechin Gallate (EGCG) in Targeting Autophagy for Cancer Treatment: A Narrative Review. Int J Mol Sci, 2022. 23(11).

36. Goncalves, P.B., A.C.R. Sodero, and Y. Cordeiro, Green Tea Epigallocatechin-3-gallate (EGCG) Targeting Protein Misfolding in Drug Discovery for Neurodegenerative Diseases. Biomolecules, 2021. 11(5).

37. Payne, A., et al., Epigallocatechin-3-Gallate (EGCG): New Therapeutic Perspectives for Neuroprotection, Aging, and Neuroinflammation for the Modern Age. Biomolecules, 2022. 12(3).

38. Wu, Z., et al., Epigallocatechin-3-Gallate Improves Intestinal Gut Microbiota Homeostasis and Ameliorates Clostridioides difficile Infection. Nutrients, 2022. 14(18).

39. Che, S., et al., EGCG drives gut microbial remodeling-induced epithelial GPR43 activation to lessen Th1 polarization in colitis. Redox Biol, 2024. 75: p. 103291.

40. Perez-Burillo, S., et al., Green Tea and Its Relation to Human Gut Microbiome. Molecules, 2021. 26(13).

41. Liu, Z., et al., Green and Black Tea Phenolics: Bioavailability, Transformation by Colonic Microbiota, and Modulation of Colonic Microbiota. J Agric Food Chem, 2018. 66(32): p. 8469-8477.

42. Riegelman, E., et al., Gut-Brain Axis in Focus: Polyphenols, Microbiota, and Their Influence on alpha-Synuclein in Parkinson's Disease. Nutrients, 2024. 16(13).

43. Yoon, S. H., Ha, S. M., Kwon, S., Lim, J., Kim, Y., Seo, H. and Chun, J. (2017). Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. Int J Syst Evol Microbiol. 67:1613-1617.

44. Quinlan AR and Hall IM, 2010. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics. 26, 6, pp. 841–842

45. Li, C., et al., Green Tea Intake and Parkinson's Disease Progression: A Mendelian Randomization Study. Front Nutr, 2022. 9: p. 848223.

46. Chen, H., et al., Parkinson's disease research in a prospective cohort in China. Parkinsonism Relat Disord, 2015. 21(10): p. 1200-4.

47. Li, Z., et al., Differences in Alpha Diversity of Gut Microbiota in Neurological Diseases. Front Neurosci, 2022. 16: p. 879318.

48. Plassais, J., et al., Gut microbiome alpha-diversity is not a marker of Parkinson's disease and multiple sclerosis. Brain Commun, 2021. 3(2): p. fcab113.

49. Yuan, X., et al., Green Tea Liquid Consumption Alters the Human Intestinal and Oral Microbiome. Mol Nutr Food Res, 2018. 62(12): p. e1800178.

50. Jin, J.S., et al., Effects of green tea consumption on human fecal microbiota with special reference to Bifidobacterium species. Microbiol Immunol, 2012. 56(11): p. 729-39.

51. Jung, E.S., et al., Seven-day Green Tea Supplementation Revamps Gut Microbiome and Caecum/Skin Metabolome in Mice from Stress. Sci Rep, 2019. 9(1): p. 18418.

52. Xin Zhang, X.Z., Yongkang Sun, Bing Hu, Yi Sun, Saqib Jabbar, Xiaoxiong Zeng, Fermentation in vitro of EGCG, GCG and EGCG3"Me isolated from Oolong tea by human intestinal microbiota. Food Research International, 2013. 54(2): p. 1589-1595.

53. Jeong, H.W., et al., Green Tea Encourages Growth of Akkermansia muciniphila. J Med Food, 2020. 23(8): p. 841-851.

54. Axling, U., et al., Green tea powder and Lactobacillus plantarum affect gut microbiota, lipid metabolism and inflammation in high-fat fed C57BL/6J mice. Nutr Metab (Lond), 2012. 9(1): p. 105.

55. Abdik, E. and T. Cakir, Transcriptome-based biomarker prediction for Parkinson's disease using genome-scale metabolic modeling. Sci Rep, 2024. 14(1): p. 585.

56. Gcwensa, N.Z., et al., Molecular Mechanisms Underlying Synaptic and Axon Degeneration in Parkinson's Disease. Front Cell Neurosci, 2021. 15: p. 626128.

57. Andersen, J.V., et al., Glutamate metabolism and recycling at the excitatory synapse in health and neurodegeneration. Neuropharmacology, 2021. 196: p. 108719.

58. Yang Jiang, S.D., Feng Li, Chen Zhang, Dongxiao Sun-Waterhouse, Yilun Chen, Dapeng Li, Effects of (+)-catechin on the differentiation and lipid metabolism of 3T3-L1 adipocytes. Journal of Functional Foods, 2019. 62.

59. Wallen, Z.D., et al., Metagenomics of Parkinson's disease implicates the gut microbiome in multiple disease mechanisms. Nat Commun, 2022. 13(1): p. 6958.

60. Brakedal, B., et al., The NADPARK study: A randomized phase I trial of nicotinamide riboside supplementation in Parkinson's disease. Cell Metab, 2022. 34(3): p. 396-407 e6.

61. Dai, C., et al., Glucose metabolism impairment in Parkinson's disease. Brain Res Bull, 2023. 199: p. 110672.

CHAPTER 5

INTEGRATIVE INSIGHTS OF BEHAVIORAL SYMPTOMS AND MICROBIOME ALTERATIONS ASSOCIATED WITH TEA CATECHIN ADMINISTRATION IN THE A53T MOUSE MODEL

1. Introduction to the Integrative Approach

1.1 Overview of Tea Catechins and Parkinson's Disease

Polyphenols are secondary metabolites of plants that have an array of highly specific biological roles for plant protection. Thousands of polyphenols have been identified to date, the majority of which belong to the flavonoids classification. All flavonoids contain two benzene A and B rings and are classified further based on heterocyclic C ring variations. Within the flavonoid class, there is a subclass of flavanols referred to as tea catechins (TC). TCs are derived from the tea plant, *Camellia sinesis*, and can be consumed as fermented black tea, semifermented oolong tea, and unfermented (dried) green tea. There are four major TC compounds found in tea leaves, (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG), where EGCG is the most abundant and the most bioactive. TCs are metabolized in the intestine via glucuronidation and sulfation, followed by metabolism in the liver via glucuronidation, sulfation, and methylation metabolism, which improves TC elimination through urine [1]. TCs have low oral bioavailability, therefore it has been estimated that up to 90-95% of TCs travel to the large intestine and come in direct contact with gut microbes. There are a variety of TC metabolites that depend largely on the diversity of gut microbial populations [2]. Metabolites of TCs have been identified in their ability to cross the blood brain barrier (BBB)

after ingestion, resulting in an increased activity of antioxidants, iron chelation, anti-inflammatory, and antimutagenic effects [3].

Accumulating evidence has demonstrated that the gut-brain axis has an important role in Parkinson's disease (PD) pathogenesis, including key events such as microbial dysbiosis, increased gut inflammation and permeability, and seeding of α-synuclein in the enteric nervous system (ENS) [4]. α-synuclein is theorized to propagate from the gut to the brain through multiple pathways. One theory suggests α-synuclein may travel bound to plasma proteins, facilitating its movement in the bloodstream [5]. Another potential route involves erythrocytic extracellular vesicles, which could carry α-synuclein across the BBB through membrane fusion, allowing direct transmission to the brain [6]. Additionally, α-synuclein could move from the gut to the brain through retrograde transport along the vagus nerve, connecting peripheral sites directly to central nervous tissue [7]. The brain's lymphatic system may also play a role, as it can transport cellular waste, including proteins, through drainage pathways that could potentially introduce α-synuclein into neural tissues [8]. Data also suggests that circulating α-synuclein may influence levels of conglomerated protein levels in the brain [9]. Extracellular forms of α-synuclein are thought to facilitate the prion-like cell-to-cell transmission of α-synuclein pathology within the brain. Emerging evidence suggests that average levels of "total α-synuclein" are lower in cerebrospinal fluid (CSF) samples from PD patients compared to those from healthy control groups. Some research has examined α-synuclein as a potential biomarker in the more accessible peripheral blood, with early findings indicating increased α-synuclein levels in plasma from PD patients relative to healthy controls [10]. Together, these mechanisms underscore the complex ways in which α-synuclein might propagate through the body to the brain, contributing to neurodegenerative processes.

While α-synuclein plays a central role in PD pathology, its use as a clinical biomarker remains limited. Meanwhile, a range of gut disorders, including microbial dysbiosis and dietary factors,

have been associated with an increased risk of developing PD [4]. Within the last 15 years, clinical and preclinical evaluations have provided evidence of increased intestinal permeability and gut dysbiosis in PD patients. Though studies have demonstrated such evidence, the alterations within PD patient gut microbiomes are often only evident in a subset of patients and have high heterogeneity [11].

While these studies highlight gut microbiome alterations in PD patients, this variability suggests that additional factors may influence disease progression and response to therapeutic interventions. Among these factors, diet plays a pivotal role in shaping microbial composition, potentially modulating the microbiome's influence on disease progression and therapeutic outcomes. In PD, protective actions of TCs include preventing toxic protein aggregate formation, alleviation of mitochondrial dysfunction, reduction of antioxidative stress and neuro-inflammation, and the activation of neurotrophic factor and signaling pathways. Although clinical trials on the health effects of dietary tea consumption remain limited, existing evidence suggests that tea drinking can positively alter gut microbiome composition and may reduce the risk of PD outcomes [12-14].

1.2 Methodology

As stated previously, six-month-old male B6.Cg-2310039L15Rik$^{Tg(Prnp\text{-}SNCA*A53T)23Mkle}$/J mice (Strain #006823, Hualpha-Syn (A53T), Hemizygous transgenic line G2-3) from Jackson Laboratories were used in this study. Mice were housed individually under a 12-hour light/dark cycle with unrestricted access to PicoLab Rodent Diet 5053 and distilled water. Mice (n=30) were randomly assigned to three groups (n=10/group): control, low-dose (0.5% weight/volume GTP), or high-dose (1.5% weight/volume GTP). Distilled water and GTP solutions were freshly prepared each day, and water intake (mL) and body weight (g) were recorded. Animals were monitored daily for clinical signs of mortality or morbidity.

Behavioral Analyses

As detailed in Chapter 3, the open field maze and rotarod behavioral analyses were conducted on days 0, 45, and 90. ANY-maze video tracking software (Version 7.35, Stoelting Co.) was employed to analyze and quantify behavioral occurrences in the open field maze. Data exploration and statistical analyses were conducted using the GraphPad Prism software (Version 10.2.3).

Biological Sample Collection

As detailed in Chapters 3 and 4, biological sample collection involved transferring mice to Nalgene metabolic cage systems on days 0, 30, 60, and 90, where urine and feces were collected twice over a 24-hour period, then stored at -20°C and - 80°C until further analyses. On day 90, mice were anesthetized with isoflurane and euthanized via cardiac puncture and CO_2 inhalation. Major organs were dissected, trimmed, weighed, and stored in 4% formalin solution at 4°C. Blood collected through cardiac puncture was placed in EDTA-treated tubes, centrifuged at 2,000 g for 10 minutes in a refrigerated centrifuge, and the plasma supernatant was stored at -20°C for later analyses. Total DNA was extracted from fecal pellets using the ZymoBiomics DNA Fecal Microbiome Extraction Mini Kit, then sequenced via Illumina NextSeq for metagenomic analysis. Metagenomic data exploration was conducted in the EZBioCloud Pro interface [15] and GraphPad Prism.

1.3 Importance of an Integrative Analysis

An integrative approach that combines metagenomic and behavioral data is essential for a more robust understanding of the gut-brain axis in PD because it allows researchers to connect microbial changes with functional outcomes. Metagenomic data provides a detailed view of gut microbial composition and gene functions, potentially identifying bacteria that may produce metabolites impacting neurological health. Behavioral data helps to reveal the observable

symptoms and neurological changes associated with PD progression. In order to analyze the association between behavioral outcomes and TC administration, we must investigate the simultaneous effects of several variables, in this case bacterial compositions on the resulting phenotypic expression. By linking shifts in the gut microbiome to behavioral manifestations, an integrative approach can help clarify causal relationships, illuminate pathways by which gut health impacts brain function, and identify potential therapeutic targets to mitigate PD symptoms.

2. Effects of 90-Day TC Treatment on Microbiome and Metabolome Profiles

2.1 Key Findings in from Metagenomic Analysis

We observed dose-dependent changes in microbial composition at the genus level after 90 days of TC supplementation. Both control and supplemented groups maintained a consistent dominance of the Bacteroidetes and Firmicutes phyla, with supplementation driving an increase in Bacteroidetes and a reduction in Firmicutes. Genus level analysis revealed dose-dependent declines in *Duncaniella* and *Fusimonas* and an increase in *RIAY_g*. In PD-associated genera, *Lactobacillus* and *Akkermansia* were significantly higher in control mice than in the supplemented groups, whereas *Bacteroides*, *Clostridium*, and *Pseudoflavonifactor* slightly decreased, and *Bifidobacterium* showed a mild increase with dosing. For α-diversity, metrics including the Shannon, Fisher, and Chao1 indices demonstrated that the diversity of the control group remained stable and both low- and high-dose groups showed increased diversity over time. The high-dose group, in particular, exhibited a significant rise across all indices, indicating a more enriched microbial community. β-diversity analysis, based on the Bray–Curtis distance Principal Coordinate Analysis (PCoA), showed that control samples were relatively similar, whereas the low- and high-dose groups had greater compositional dissimilarity, especially notable in the high-dose group. LEfSe analysis on day 90 identified specific taxa enriched by treatment dosage. The control group had higher levels of *Lactobacillus*, *Limosilactobacillus*, and *Parasutterella*. The low-dose group showed increased *Acutalibacter*, *Adlercreutzia*, and

KE159628_g, whereas the high-dose group had higher *Hominiventricola*, *Eubacterium*, and *Butyribacter* levels. These shifts suggest that treatment modulates the gut microbiome in a dose-responsive manner, with distinct taxa enriched at different dosage levels.

2.2 Functional Metagenomic Insights

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis highlighted both characterized and uncharacterized microbial constituents in the murine gut microbiome, revealing potential limitations in current knowledge. Within the study group, 2,681 KEGG orthologs and 526 metabolic pathways were identified. Pathway shifts between control and treatment groups generally indicated dysbiosis, with several pathways in the A53T metagenome associated with pathways linked to PD. The treatment groups showed regulated metabolic pathways in alanine, aspartate, and glutamate metabolism, nicotinamide metabolism, and glycolysis/gluconeogenesis, whereas the control group displayed heightened expression of these pathways. Further, lipopolysaccharide (LPS) biosynthesis decreased with increasing treatment doses, while biofilm formation linked to *P. aeruginosa* and *E. coli* was higher in control mice. MiniPath analysis indicated lower PD-related pathway activity in both treatment groups versus controls, alongside dose-dependent increases in glutamatergic synapse and cytokine receptor interaction pathways. The cAMP signaling pathway was elevated across all groups, notably highest in the low-dose group.

These findings suggest potential mechanisms by which the gut microbiome and its metabolic pathways could influence α-synuclein pathology in PD pathogenesis. The key pathways altered in the control versus treatment groups highlight several connections. Upregulation of amino acid pathways like alanine, aspartate, and glutamate metabolism in the control group may influence excitotoxicity and oxidative stress, both implicated in α-synuclein misfolding and aggregation. Dysregulated glutamate levels can lead to excitotoxicity, potentially triggering α-synuclein aggregation. Increased biofilm formation activity in the control group may indicate a higher

prevalence of potentially pathogenic bacteria that can produce neurotoxic compounds or endotoxins, promoting an environment conducive to α-synuclein pathology. The elevated cAMP signaling observed across treatment groups, especially in the low-dose group, may have neuroprotective effects by modulating inflammation and cellular stress responses, potentially mitigating α-synuclein accumulation. However, precise effects would depend on pathway regulation and crosstalk with other signaling cascades. A dose-dependent decrease in LPS biosynthesis may lower LPS-induced inflammation, which is known to exacerbate α-synuclein pathology through heightened immune responses in the brain-gut axis.

3. Impact of TCs on PD Behavioral Symptoms and α-synuclein

Hemizygous A53T mice displayed an age-dependent progression of motor and anxiety-related behaviors. While typical behaviors were noted at 5 months, mild hyperactivity developed between 7-9 months, followed by wobbling movements and reduced activity. Severe motor impairments generally emerge between 9-16 months, alongside reduced anxiety levels, possibly due to neurotransmitter system changes, particularly in dopamine and Gamma-aminobutyric acid (GABA). In the open field test, no significant differences in locomotion were observed across treatment groups. However, by day 90, high-dose mice spent more time in the field's periphery (thigmotaxic behavior) than controls, indicating possible heightened anxiety. Increased grooming in high-dose mice and a trend in low-dose mice suggest a dose-dependent impact of TC treatment on anxiety-like behaviors, potentially attenuating anxiety levels typical of A53T phenotypes. The rotarod test revealed progressive motor decline in all groups, particularly in controls, which showed marked decreases in performance by day 45 and 90. These results align with previous studies showing that TCs, such as EGCG, improve motor function in PD models by reducing α-synuclein aggregation and dopaminergic neuronal injury [16-18].

Increased levels of α-synuclein in the bloodstream are commonly associated with PD, reflecting the pathological accumulation of the protein and contributing to neurodegenerative processes

characteristic of the disease. Using the sandwich ELISA, we quantified recombinant full-length mouse SNCα protein in the blood plasma across three groups: control, low-dose, and high-dose. The α-synuclein concentrations were measured at 189.9 ± 20.05 pg/mL for controls, 154.9 ± 9.376 pg/mL for the low-dose group, and 123.0 ± 7.090 pg/mL for the high-dose group. Analysis with the Brown-Forsythe ANOVA revealed significant differences between groups. Subsequent Dunnett's *post hoc* test indicated that the high-dose group had significantly lower levels of circulating α-synuclein than both the control and low-dose groups on day 90. Collectively, these findings suggest that TCs may hold therapeutic promise for alleviating key PD pathologies, including motor dysfunction and α-synuclein accumulation, emphasizing the mutation's association with behavioral deficits in the A53T model.

4. Integrative Analysis

4.1 Linear Regression for Genera Implications in Integrative Analysis

A linear regression analysis was conducted to investigate the relationship between gut microbial genera and rotarod motor performance in the transgenic PD model. This analysis aimed to determine which genera among those present could be linked with the primary experimental variables, namely genera relative abundance, treatment dose, animal weight, and performance on the rotarod. By estimating the strength of these associations, linear regression helped to clarify how each factor may interactively or independently contribute to variations in rotarod performance. Further, the regression provides a quantitative means to assess the significance of each genus, highlighting those with potential relevance to neurobehavioral outcomes (Figure 5.1). Ten genera were identified as candidates for further integrative metagenomic and behavioral analyses, given their statistical association within the respective treatment groups. The results indicated that six genera—*Acutalibacter, Bacteroides, Clostridium, Fusimonas, Lactobacillus,* and *Ligilactobacillus*—had significance ($p \leq 0.05$), suggesting meaningful associations with the variables of the model. This insight suggests potential involvement in gut-brain interactions that

could impact neurobehavioral health. Conversely, other genera, such as *Akkermansia*, *Butyribacter*, *RIAY_G,* and *Schaedlerella*, did not meet the threshold for statistical significance. Although these genera were not significantly associated in this specific analysis, they may still warrant consideration in future studies or in exploratory models given their established roles in gut microbiota function.

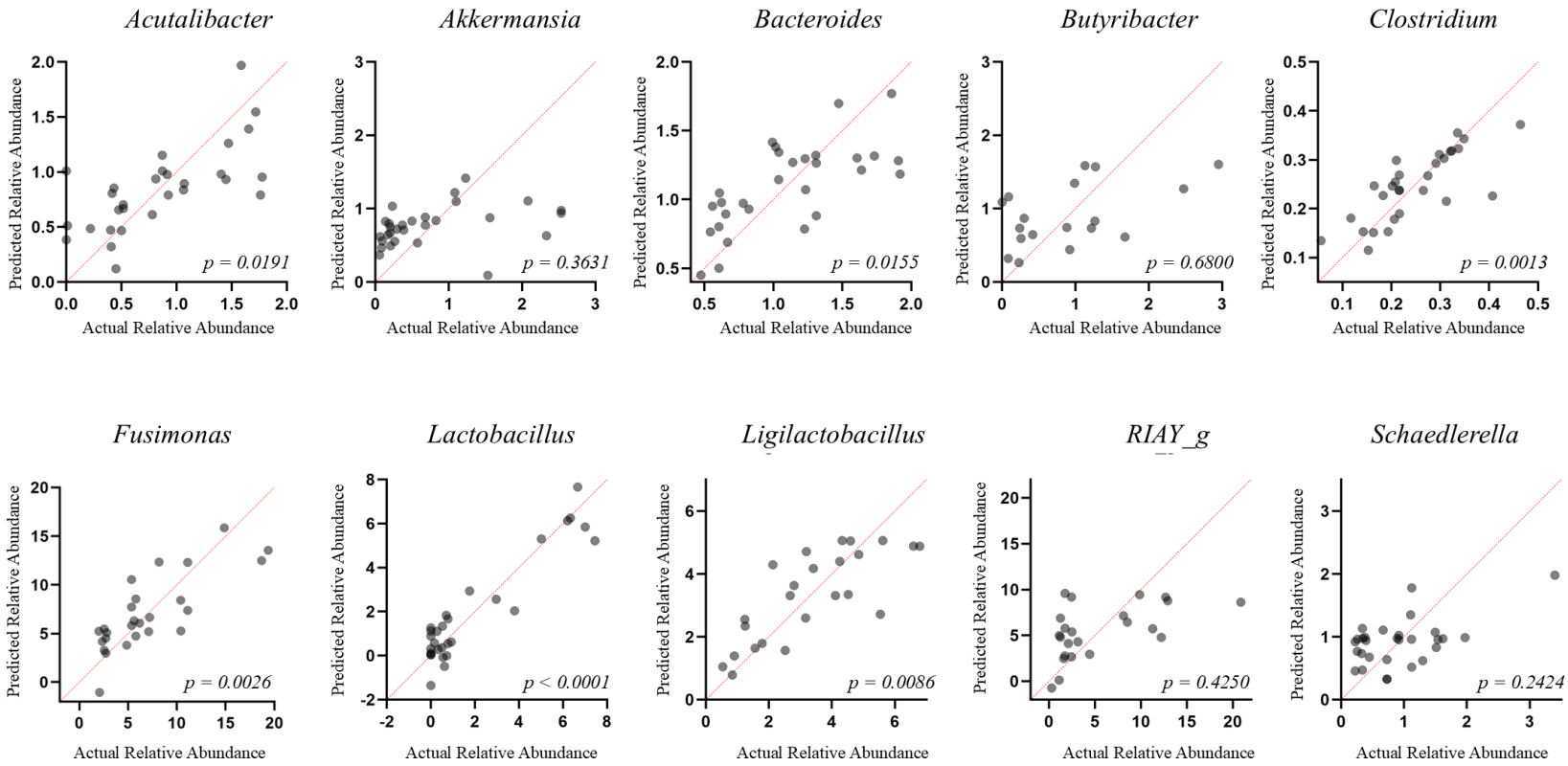

Figure 5.1 Linear Regression Analysis of Major Genera Influenced by Treatment Group at Day 90.

Each graph represents best fit line comparing the actual relative abundance of bacterial genera in relation to predicted relative abundance based on dose group, weight, and neurobehavioral measurements (rotarod duration, time in center of open field maze, average speed, and distance traveled).

4.2 Linking Behavioral Outcomes with Microbiome Changes and α-synuclein Concentrations

We aimed to evaluate the relationship between abundant microbial genera and behavioral outcomes in the A53T model after 90 days of TC supplementation by using a Spearman correlation matrix to assess the strength and direction of associations between these ranked variables. Spearman's rank correlation does not assume a linear relationship or normally distributed data, making it ideal for exploring complex, potentially non-linear interactions within biological data. By computing a correlation coefficient for each pair of variables, the correlation matrix provides insight into the degree to which changes in microbial genera abundance may be associated with shifts in behavioral outcomes. Microbiota composition showed distinct correlations with behavioral performance metrics in rotarod and open field maze tests (Figure 5.2). Specifically, higher levels of *Acutalibacter*, *Bacteroides*, and *RIAY_g* were positively associated with increased rotarod times, suggesting a potential link between these genera and improved motor coordination. The genera *Butyribacter* and *Schaedlerella* also exhibited a slight positive correlation with rotarod time. In contrast, *Clostridium*, *Fusimonas*, *Lactobacillus*, and *Ligilactobacillus* were negatively correlated with rotarod performance, while *Akkermansia* demonstrated a slight negative association. Behavioral correlations extended to grooming behavior in the open field maze, where *Lactobacillus*, *Clostridium*, *Fusimonas*, and *Ligilactobacillus* demonstrated negative correlations with grooming frequency, indicating a potential link between these microbes and decreased grooming activity. Additionally, both *Bacteroides* and *Butyribacter* negatively correlated with average speed in the open field maze, whereas *Fusimonas* and *Lactobacillus* were positively correlated with average speed, though average speed was not a statistically significant metric between control and treatment groups.

The positive correlations between *Acutalibacter*, *Bacteroides*, *RIAY_g*, *Butyribacter*, and *Schaedlerella* with rotarod performance suggest that these bacterial genera might promote an anti-inflammatory environment, potentially enhancing neuromuscular coordination. Butyrate-

producing bacteria, such as *Butyribacter,* could support gut barrier integrity and reduce systemic inflammation, which could positively impact motor performance and resilience [19]. On the contrary, the negative correlation of *Clostridium*, *Fusimonas*, *Lactobacillus*, and *Ligilactobacillus* with rotarod time might indicate that these bacteria contribute to pro-inflammatory pathways that impede motor coordination. Similarly, *Lactobacillus*, *Clostridium*, *Fusimonas*, and *Ligilactobacillus* were associated with reduced grooming in the open field maze, which could reflect increased anxiety-like behavior or a dysregulated stress response, possibly driven by microbial metabolites affecting the hypothalamic-pituitary-adrenal (HPA) axis. The negative correlation of *Bacteroides* and *Butyribacter* with average speed in the open field maze suggests that, while some bacteria may support coordination, they may also modulate energy metabolism or stress response in ways that reduce exploratory behavior. Shifts in these microbial populations could influence behavioral outcomes by modulating systemic inflammation, neuroinflammatory signaling, or even neurotransmitter synthesis, further highlighting the complexity of gut-brain interactions in behavioral regulation.

Plasma α-synuclein concentrations were negatively correlated with *Acutalibacter*, *Bacteroides*, *RIAY_g*, and *Schaedlerella*, suggesting that these microbes may play a protective role in regulating α-synuclein levels (Figure 5.3). Lower α-synuclein levels could reduce neurotoxicity and improve behavioral performance. Conversely, higher levels of *Akkermansia*, *Butyribacter*, *Clostridium*, *Fusimonas*, *Lactobacillus*, and *Ligilactobacillus* were positively correlated with α-synuclein concentration, possibly indicating that these microbes are linked to pathways that elevate α-synuclein expression or aggregation. The increase in circulating α-synuclein likely contributes to processes that impair motor coordination and exploratory behaviors, highlighting the potential of microbiome-targeted interventions to modify disease-related biomarkers and behavioral outcomes.

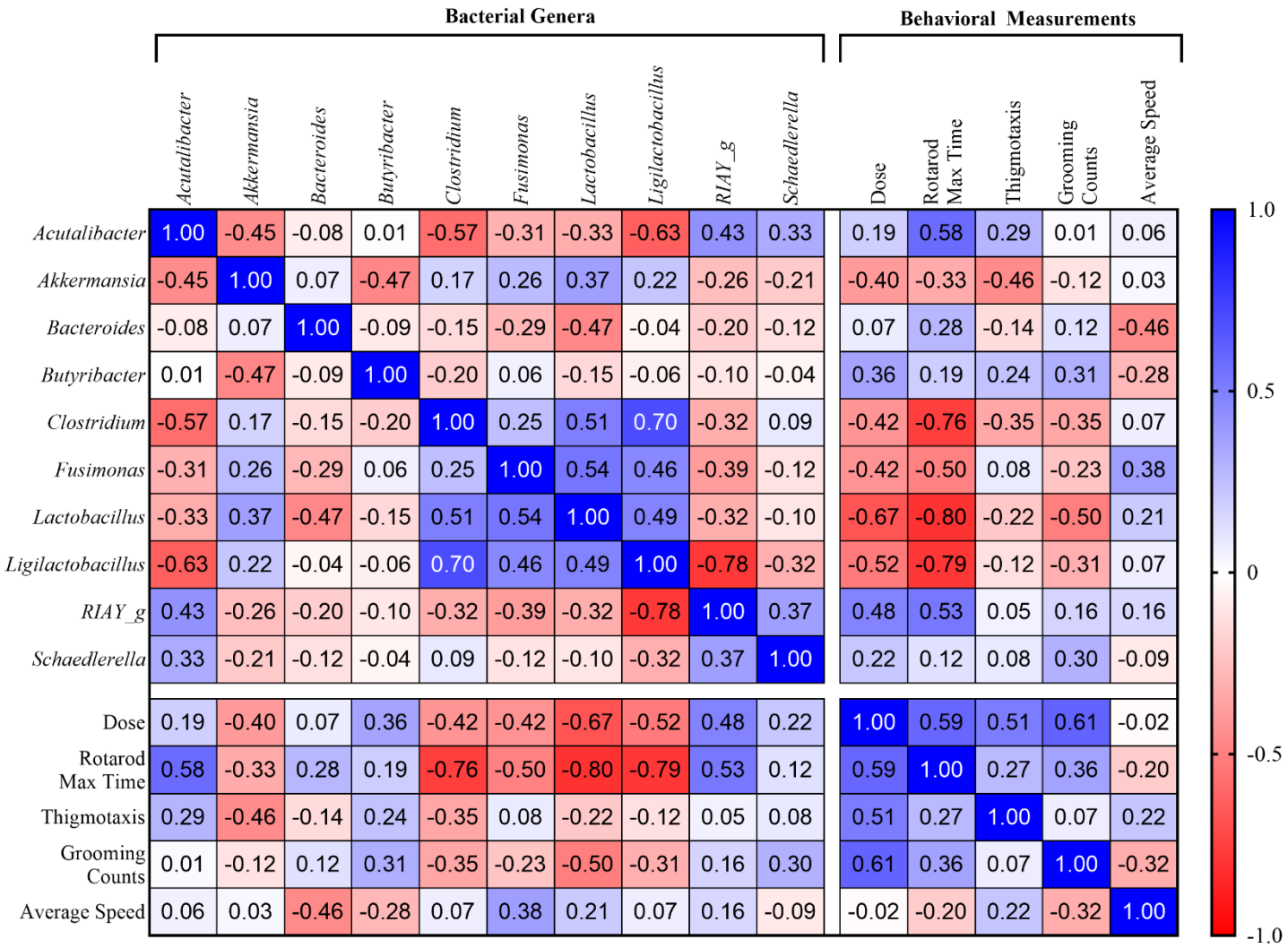

Figure 5.2 Spearman Correlation Matrix of microbial genera and behavioral outcomes measured on day 90. Statistically relevant bacterial genera were analyzed in correlation to dose and behavioral outcomes. The scale ranges from 1.0 to -1.0, where 1.0 (blue) indicates a positive correlation and -1.0 (red) indicates a negative correlation. Each value within the respective box indicates the level of correlation between the horizontal and vertical columns.

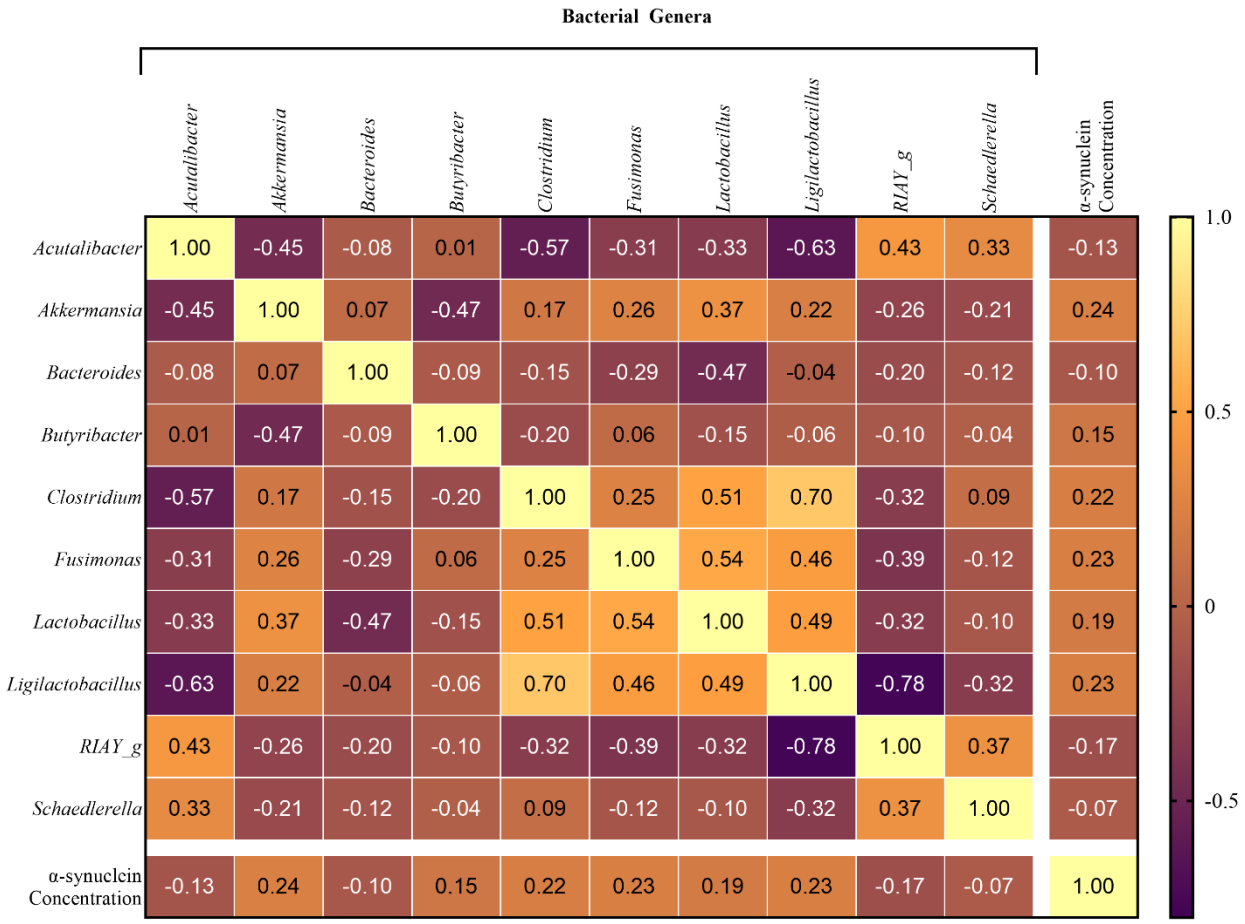

Figure 5.3 Spearman Correlation Matrix of microbial genera and α-synuclein concentration in blood plasma measured on day 90. Statistically relevant bacterial genera were analyzed in correlation to α-synuclein measurements. The scale ranges from 1.0 to -1.0, where 1.0 (yellow) indicates a correlation with increased α-synuclein and -1.0 (burgundy) indicates a correlation with decreased α-synuclein. Each value within the respective box indicates the level of correlation between the horizontal and vertical columns.

4.3 Linking KEGG Pathways with Microbiome Changes

As depicted in Figure 5.4, certain microbial families exhibited distinct correlations with key metabolic pathways, highlighting how microbiome composition may influence metabolic functions. The families *Erysipelotrichaceae*, *Lachnospiraceae*, *Clostridiaceae*, *Eggerthellaceae*, *Bacteroidaceae*, *Lactobacillaceae*, and *Christenellaceae* showed positive correlations with metabolic pathways including LPS biosynthesis, glycolysis/gluconeogenesis, nicotinate and nicotinamide metabolism, alanine, aspartate, and glutamate metabolism. These pathways were notably upregulated in control mice compared to the TC-treated groups, suggesting that the presence of these families may support higher metabolic activity in these pathways under control conditions. In contrast, the families *Bifidobacteriaceae*, *Muribaculaceae*, *Mogibacterium*, *Saccharimondaceae*, *Peptostreptococcaceae*, *Coprobacillaceae*, and *Turicibacteraceae* were negatively correlated with the same metabolic pathways, implying that their abundance may contribute to a reduction in pathway expression. This opposing trend between bacterial families underscores the complex role of microbial diversity in modulating host metabolism. For example, families such as *Lachnospiraceae*, *Bacteroidaceae*, and *Christenellaceae* were positively correlated with pathways involved in LPS biosynthesis, glycolysis/gluconeogenesis, and amino acid metabolism, indicating their presence may enhance the expression of these pathways in control mice. Conversely, families like *Bifidobacteriaceae*, *Muribaculaceae*, and *Mogibacterium* demonstrated negative correlations with these same metabolic pathways suggesting their presence could downregulate these same processes in the TC-treated group. This nuanced relationship demonstrates the importance of microbial diversity in influencing metabolic expression, as certain bacterial families may create a conducive environment for specific metabolic pathways while simultaneously inhibiting others, which is demonstrated by the inverse relationship between *Muribaculaceae* and *Lachnospiraceae* families. Such findings emphasize the importance of microbial diversity and balance in influencing metabolic processes, where specific bacteria not

only facilitate metabolic activities but may also create an environment that inhibits competing pathways, ultimately contributing to the metabolic health and resilience of the host to dietary interventions.

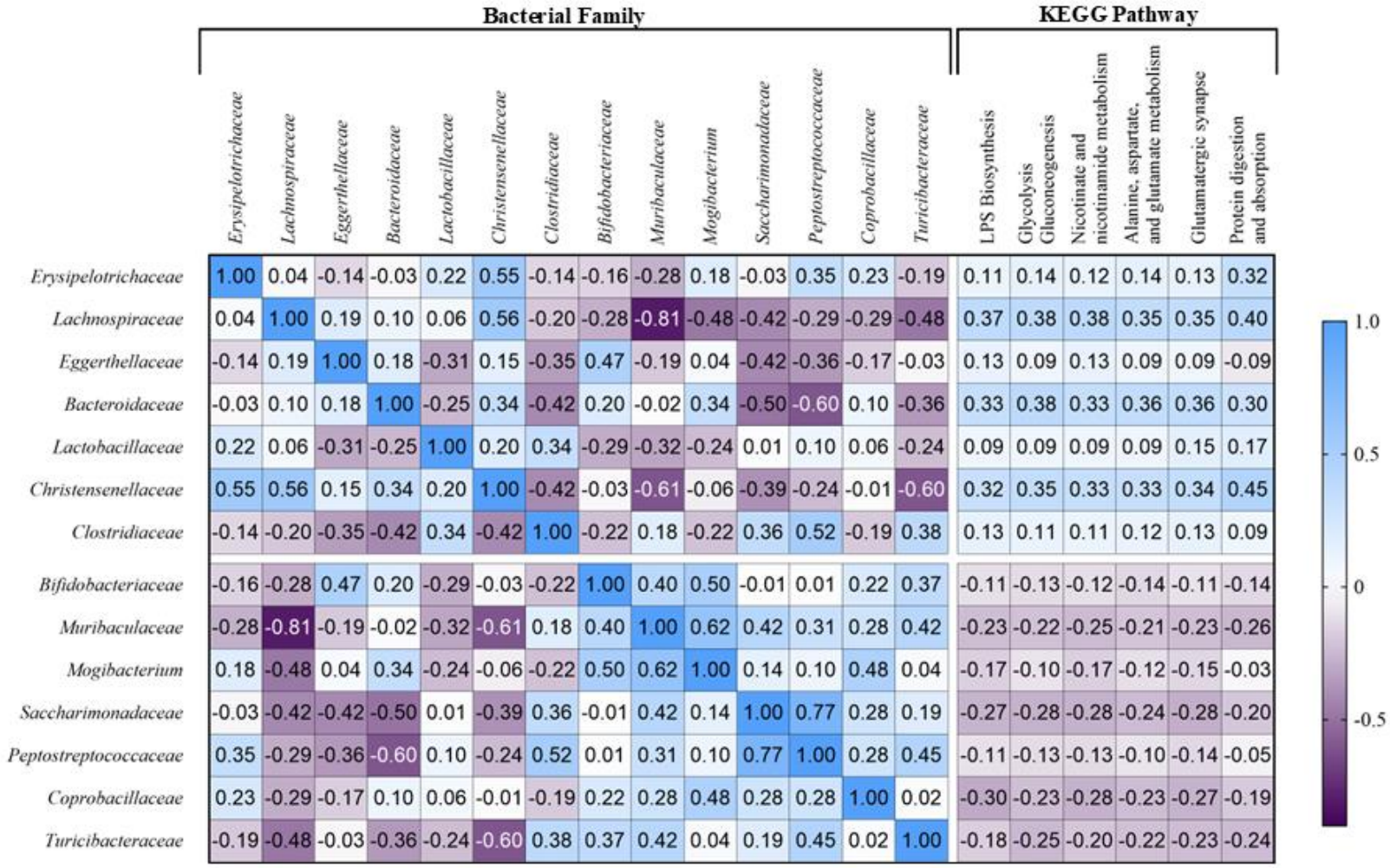

Figure 5.4 Spearman Correlation Matrix of microbial families and KEGG Pathways implicated in PD measured on day 90. Microbial families associated with the increased expression of KEGG pathways are on the top half of the figure, while families associated with lower expression are on the bottom half. The scale ranges from 1.0 to -1.0, where 1.0 (blue) indicates a positive correlation and -1.0 (purple) indicates a negative correlation. Each value within the respective box indicates the level of correlation between the horizontal and vertical columns.

5. Implications for Therapeutic Interventions

TCs, particularly EGCG, possess potent antioxidant and anti-inflammatory properties, which are beneficial in combating oxidative stress and neuroinflammation - two critical factors in PD progression. Beyond their systemic effects, TCs also demonstrate selective modulation of gut microbiota composition, suggesting their potential role as prebiotics. By promoting the growth of beneficial bacterial genera and inhibiting pathogenic strains, TCs could enhance gut health and support microbiome homeostasis, thus reinforcing gut-brain axis integrity. Recent studies indicate that gut microbiota dysbiosis in PD patients is associated with increased intestinal permeability and a pro-inflammatory state, which may exacerbate neurodegenerative processes [20-22]. By encouraging the proliferation of beneficial microbes that produce neuroprotective metabolites, TCs may help restore microbial balance, reduce gut inflammation, and improve barrier function. This effect could mitigate the pro-inflammatory signals traveling along the GBA, potentially alleviating PD symptoms and slowing disease progression. Moreover, TCs appear to influence neurochemicals relevant to PD pathology, such as dopamine and glutamate, by impacting both gut microbial composition and host neurotransmitter levels. TCs may serve as a therapeutic approach that bridges dietary intervention with direct gut-brain axis modulation.

The rotarod behavioral performance results support these findings by highlighting specific bacterial genera associated with improved motor coordination and resilience. Positive correlations were observed between genera such as *Acutalibacter*, *Bacteroides*, *RIAY_g*, *Butyribacter*, and *Schaedlerella* and enhanced rotarod performance, suggesting that these genera may contribute to an anti-inflammatory environment conducive to neuromuscular function. A 2020 study evaluating the anti-inflammatory effects of acupuncture on a PD mouse model also documented where *Acutalibacter* was positively associated with increased performance on rotarod [23], however, the other genera mentioned were either absent from our samples or lacked sufficient statistical significance to warrant inclusion in this analysis. In contrast, the genera *Clostridium* and

Fusimonas were negatively correlated with rotarod time, indicating a role in pro-inflammatory processes [24,25] that may impair motor skills. Interestingly, *Lactobacillus* and *Ligilactobacillus* were also correlated with decreased rotarod time, however both genera are known to be associated with anti-inflammatory properties and intestinal barrier integrity [26,27]. Both *Lactobacillus* and *Ligilactobacillus* are known to produce a variety of metabolic byproducts, including lactic acid [28], which can lower gut pH and create an environment that favors their growth over other microbial species. This dominance, while occasionally beneficial in maintaining a robust gut barrier under healthy conditions, could reduce microbial diversity and interfere with the balance of bacteria that produce other beneficial metabolites. Consequently, the competitive inhibition posed by *Lactobacillus* and *Ligilactobacillus* could indirectly increase inflammation or oxidative stress, both of which are detrimental to neuromuscular coordination and motor performance.

Assessments made regarding the open field maze reveal additional connections between microbiome composition and behavioral responses. Negative correlations of *Lactobacillus*, *Clostridium*, *Fusimonas*, and *Ligilactobacillus* with grooming behavior may suggest that these genera, through metabolites influencing the HPA axis, could drive anxiety-like or dysregulated stress responses. The genera *Bacteroides* and *Butyribacter* were negatively correlated with average speed in the maze, indicating potential influences on energy metabolism or stress response, which could dampen exploratory behavior. Prior research has indicated that various species of *Bacteroides* significantly enhance the risk of depression [29], which is a comorbidity of PD [30], and evidence suggests that mouse models of secondary depression exhibit decreased exploratory behavior [31]. These results highlight the potential impact of microbial shifts on neurobehavioral outcomes, emphasizing the complex and often unknown dynamics between bacterial gut composition and mechanisms such as systemic inflammation, neuroinflammatory signaling, and neurotransmitter synthesis.

Plasma α-synuclein levels were negatively correlated with *Acutalibacter*, *Bacteroides*, *RIAY_g*, and *Schaedlerella*, suggesting that these genera may play protective roles by reducing neurotoxic α-synuclein aggregation. Lower α-synuclein levels could alleviate neurotoxicity and improve motor and exploratory behaviors. Conversely, elevated levels of *Akkermansia*, *Butyribacter*, *Clostridium*, *Fusimonas*, *Lactobacillus*, and *Ligilactobacillus* were positively correlated with α-synuclein concentration, indicating these genera may be associated with pathways that enhance α-synuclein expression or aggregation. Higher circulating α-synuclein likely contributes to motor impairment and exploratory behavior deficits, underscoring the potential of microbiome-targeted therapies, such as TCs, to influence both neurodegenerative biomarkers and behavior.

The current analysis suggests that TCs may act as a prebiotic intervention, promoting beneficial shifts in the gut microbiome that support neuroprotective pathways. This hypothesis is supported by the observed microbiome alterations in TC-treated mice, which showed a downregulation of key metabolic pathways including LPS biosynthesis, glycolysis/gluconeogenesis, nicotinate and nicotinamide metabolism, alanine, aspartate, and glutamate metabolism compared to control groups. Families such as *Erysipelotrichaceae*, *Lachnospiraceae*, *Clostridiaceae*, *Eggerthellaceae*, *Bacteroidaceae*, *Lactobacillaceae*, and *Christenellaceae* positively correlated with these pathways in control mice, suggesting that TC intervention reduces the activity of pathways associated with inflammation and metabolic stress. By contrast, families like *Bifidobacteriaceae*, *Muribaculaceae*, *Mogibacterium*, *Saccharimondaceae*, *Peptostreptococcaceae*, *Coprobacillaceae*, and *Turicibacteraceae* showed negative correlations with these same pathways, indicating that TCs may help suppress metabolic processes that contribute to an inflammatory state, thereby offering neuroprotection.

6. Conclusion

These findings suggest that TCs, particularly EGCG, may provide a promising intervention for mitigating PD progression through multifaceted mechanisms along the gut-brain axis. TCs'

antioxidant and anti-inflammatory effects address core challenges in PD, namely oxidative stress and neuroinflammation, while their prebiotic properties appear to selectively modulate gut microbiota. By promoting beneficial bacterial genera and suppressing potentially pathogenic strains, TCs could enhance gut health and restore microbial homeostasis, which is crucial for gut-brain axis integrity. This microbial shift may alleviate neuroinflammatory signaling and reinforce intestinal barrier function, potentially reducing pro-inflammatory signals that exacerbate PD pathology. Behavioral correlations further reinforce the impact of microbiome modulation. Positive associations between beneficial genera (e.g., *Acutalibacter*, *Bacteroides*, *RIAY_g*) and improved motor function in the rotarod test imply a protective role against motor impairment. Conversely, negative correlations with *Clostridium* and *Fusimonas* indicate that specific taxa may hinder motor abilities through pro-inflammatory mechanisms. Moreover, genera associated with stress responses (*Lactobacillus*, *Ligilactobacillus*) correlated with altered grooming and exploratory behaviors, highlighting the impact of TCs on behavioral symptoms relevant to PD, such as anxiety and depression. At the biochemical level, the inverse relationship between protective genera and plasma α-synuclein levels suggests that TCs may mitigate neurotoxic aggregation, a hallmark of PD neurodegeneration. By decreasing metabolic pathways linked to inflammation and oxidative stress, TCs foster a microbiome profile that may protect enteric tissues and support motor and exploratory behaviors. Together, these findings highlight the potential of TCs as a holistic dietary approach for PD prevention and potentially management, targeting both microbiome composition and neuroinflammatory pathways. This study emphasizes the role of TCs in supporting neuroprotective microbial ecosystems, which could ultimately contribute to slowing PD progression and alleviating its symptoms.

References

1. Schantz, M., T. Erk, and E. Richling, Metabolism of green tea catechins by the human small intestine. Biotechnol J, 2010. 5(10): p. 1050-9.
2. Sidhu, D., M. Vasundhara, and P. Dey, The intestinal-level metabolic benefits of green tea catechins: Mechanistic insights from pre-clinical and clinical studies. Phytomedicine, 2024. 123: p. 155207.
3. Ohishi, T., et al., Anti-inflammatory Action of Green Tea. Antiinflamm Antiallergy Agents Med Chem, 2016. 15(2): p. 74-90.
4. Tan, A.H., S.Y. Lim, and A.E. Lang, The microbiome-gut-brain axis in Parkinson disease - from basic research to the clinic. Nat Rev Neurol, 2022. 18(8): p. 476-495.
5. Iyer, A., et al., The Impact of N-terminal Acetylation of alpha-Synuclein on Phospholipid Membrane Binding and Fibril Structure. J Biol Chem, 2016. 291(40): p. 21110-21122.
6. Matsumoto, J., et al., Transmission of alpha-synuclein-containing erythrocyte-derived extracellular vesicles across the blood-brain barrier via adsorptive mediated transcytosis: another mechanism for initiation and progression of Parkinson's disease? Acta Neuropathol Commun, 2017. 5(1): p. 71.
7. Kim, S., et al., Transneuronal Propagation of Pathologic alpha-Synuclein from the Gut to the Brain Models Parkinson's Disease. Neuron, 2019. 103(4): p. 627-641 e7.
8. Fujita, K., et al., Mutant alpha-synuclein propagates via the lymphatic system of the brain in the monomeric state. Cell Rep, 2023. 42(8): p. 112962.
9. Bogale, T.A., et al., Alpha-Synuclein in the Regulation of Brain Endothelial and Perivascular Cells: Gaps and Future Perspectives. Front Immunol, 2021. 12: p. 611761.
10. Foulds, P.G., et al., A longitudinal study on alpha-synuclein in blood plasma as a biomarker for Parkinson's disease. Sci Rep, 2013. 3: p. 2540.

11. Clairembault, T., et al., Structural alterations of the intestinal epithelial barrier in Parkinson's disease. Acta Neuropathol Commun, 2015. 3: p. 12.

12. Li, F.J., H.F. Ji, and L. Shen, A meta-analysis of tea drinking and risk of Parkinson's disease. ScientificWorldJournal, 2012. 2012: p. 923464.

13. Qi, H. and S. Li, Dose-response meta-analysis on coffee, tea and caffeine consumption with risk of Parkinson's disease. Geriatr Gerontol Int, 2014. 14(2): p. 430-9.

14. Chen, Y., et al., Non-Genetic Risk Factors for Parkinson's Disease: An Overview of 46 Systematic Reviews. J Parkinsons Dis, 2021. 11(3): p. 919-935.

15. Yoon SH, Ha SM, Kwon S, et al. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol. 2017;67(5):1613-1617. doi:10.1099/ijsem.0.001755.

16. Chen, M., et al., Tea polyphenols alleviate motor impairments, dopaminergic neuronal injury, and cerebral alpha-synuclein aggregation in MPTP-intoxicated parkinsonian monkeys. Neuroscience, 2015. 286: p. 383-92.

17. Bitu Pinto, N., et al., Neuroprotective Properties of the Standardized Extract from Camellia sinensis (Green Tea) and Its Main Bioactive Components, Epicatechin and Epigallocatechin Gallate, in the 6-OHDA Model of Parkinson's Disease. Evid Based Complement Alternat Med, 2015. 2015: p. 161092.

18. Malar, D.S., et al., Neuroprotective Properties of Green Tea (Camellia sinensis) in Parkinson's Disease: A Review. Molecules, 2020. 25(17).

19. Zou, Y., et al., Butyribacter intestini gen. nov., sp. nov., a butyric acid-producing bacterium of the family *Lachnospiraceae* isolated from human faeces, and reclassification of Acetivibrio ethanolgignens as Acetanaerobacter ethanolgignens gen. nov., comb. nov. Syst Appl Microbiol, 2021. 44(3): p. 126201.

20. van, I.S.C.D. and P. Derkinderen, The Intestinal Barrier in Parkinson's Disease: Current State of Knowledge. J Parkinsons Dis, 2019. 9(s2): p. S323-S329.

21. Gerhardt, S. and M.H. Mohajeri, Changes of Colonic Bacterial Composition in Parkinson's Disease and Other Neurodegenerative Diseases. Nutrients, 2018. 10(6).

22. Heravi, F.S., K. Naseri, and H. Hu, Gut Microbiota Composition in Patients with Neurodegenerative Disorders (Parkinson's and Alzheimer's) and Healthy Controls: A Systematic Review. Nutrients, 2023. 15(20).

23. Jang, J.H., et al., Acupuncture inhibits neuroinflammation and gut microbial dysbiosis in a mouse model of Parkinson's disease. Brain Behav Immun, 2020. 89: p. 641-655.

24. Boeriu, A., et al., The Current Knowledge on Clostridioides difficile Infection in Patients with Inflammatory Bowel Diseases. Pathogens, 2022. 11(7).

25. Kusada, H., et al., Fusimonas intestini gen. nov., sp. nov., a novel intestinal bacterium of the family *Lachnospiraceae* associated with diabetes in mice. Sci Rep, 2017. 7(1): p. 18087.

26. Yao, M., et al., Improved functionality of Ligilactobacillus salivarius Li01 in alleviating colonic inflammation by layer-by-layer microencapsulation. NPJ Biofilms Microbiomes, 2021. 7(1): p. 58.

27. Li, C., et al., The role of Lactobacillus in inflammatory bowel disease: from actualities to prospects. Cell Death Discov, 2023. 9(1): p. 361.

28. De Filippis, F., E. Pasolli, and D. Ercolini, The food-gut axis: lactic acid bacteria and their link to food, the gut microbiome and human health. FEMS Microbiol Rev, 2020. 44(4): p. 454-489.

29. Liu, L., et al., Gut microbiota and its metabolites in depression: from pathogenesis to treatment. EBioMedicine, 2023. 90: p. 104527.

30. Dalle, E. and M.V. Mabandla, Early Life Stress, Depression And Parkinson's Disease: A New Approach. Mol Brain, 2018. 11(1): p. 18.

31. Krishnan, V. and E.J. Nestler, Animal models of depression: molecular perspectives. Curr Top Behav Neurosci, 2011. 7: p. 121-47.

CHAPTER 6

CONCLUSION AND FUTURE PERSPECTIVES

This dissertation sheds light on the potential of tea catechins (TCs) as multifaceted neuroprotective agents against the pathological development of Parkinson's disease (PD). By examining the effects of TC administration on gut microbiome composition, metabolomic function, α-synuclein concentration, and neurobehavioral outcomes, our data reveals how TCs may contribute towards a healthier gut-brain axis in the context of PD progression. The mode of action of TC's effects are likely through the modulation of microbial populations, the enhancement of metabolic stability, and the modulation of key metabolic markers in PD. Moreover, by laying a foundation of evidence for the safety and efficacy of TCs in animal models, this research serves as a pathway for future studies aimed at defining optimal TC dosage ranges and safety profiles for therapeutic application. Establishing these guidelines will be essential for translating these findings to human trials, where the role of TC supplementation in PD prevention and symptom management can be more rigorously evaluated. While existing epidemiological studies primarily suggest associations between dietary polyphenol intake and reduced PD risk [1-3], our results add mechanistic evidence, highlighting TCs' ability to foster a gut microbiome profile that protects against neuroinflammatory pathways and supports motor and exploratory behaviors relevant to PD.

Given these results and in reflection of the specific aims of this dissertation work, we can conclude that:

1. We observed sustained TC metabolite circulation in the low-dose and high-dose treatment groups of the transgenic A53T mouse model;

2. Through sustained TC administration, there were significant reductions in circulating α-synuclein levels and dose-dependent improvements in motor coordination, as evidenced by enhanced rotarod performance and changes in anxiety-related behaviors evaluated from the open field maze;
3. TC supplementation influenced gut microbiome composition, significantly increasing α-diversity in treated groups compared to control mice, suggesting a more balanced gut microbial community;
4. TC treatment led to notable shifts in microbial composition, with dose-dependent differences in microbial profiles. This modulation of gut microbiota diversity, particularly through enhanced levels of beneficial genera and the suppression of potentially pathogenic bacteria, highlights TCs' potential as prebiotic agents in fostering a neuroprotective microbial ecosystem;
5. Behavioral correlations further reinforced these findings, as positive associations between beneficial taxa and improved motor function in the rotarod test suggested a protective effect against motor impairment, while negative correlations with inflammatory taxa indicated potential links to motor challenges;
6. At the metabolic level, TCs influenced key metabolic pathways such as cAMP signaling and nicotinamide metabolism, which are known to impact mitochondrial regulation, fat metabolism, and inflammation. TC treatment was associated with reduced lipopolysaccharide (LPS) biosynthesis and biofilm formation, supporting potential anti-inflammatory and gut-protective effects.

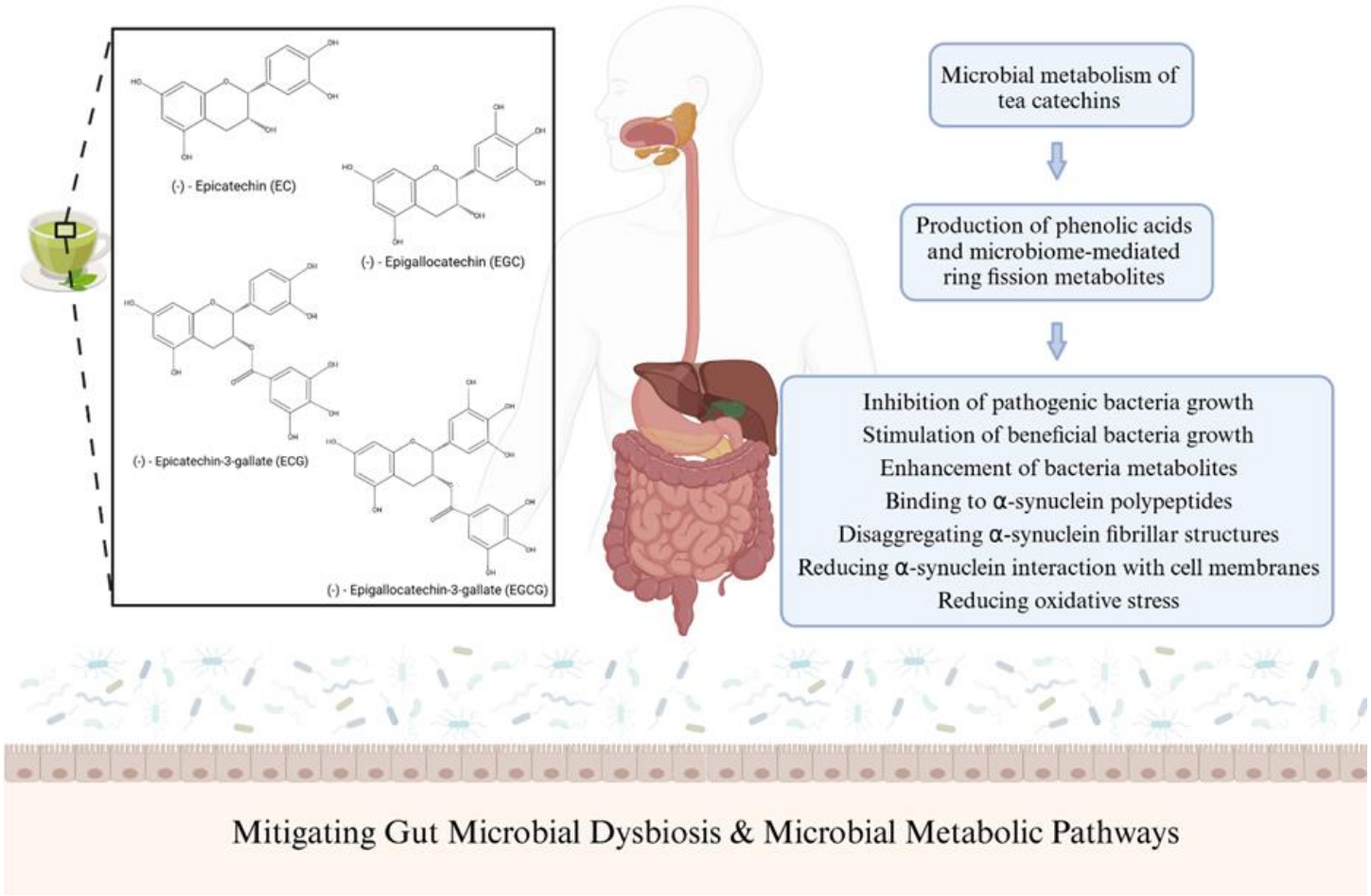

Figure 6.1 Graphical summary of findings, including how the microbial metabolism of TCs leads to the production of beneficial metabolites that mitigate gut microbial dysbiosis and alter metabolic pathways involved in PD.

Our findings contribute new insights to TCs as a dietary intervention capable of modulating gut-brain axis interactions through prebiotic mechanisms. By promoting a balanced microbial ecosystem that mitigates neuroinflammatory signaling and enhances intestinal barrier function, TCs may reduce pro-inflammatory stimuli that exacerbate PD pathology. Taken together, this study underscores the potential of TCs, especially EGCG, as a holistic approach for PD prevention and management, targeting both microbial composition and neuroinflammatory metabolic pathways. However, further clinical research is essential to fully understand these mechanisms and validate TCs' efficacy in human populations. By enhancing neuroprotective microbial ecosystems and modulating inflammatory processes along the gut-brain axis, TCs hold substantial potential to slow PD progression, alleviate symptoms, and contribute to future therapeutic strategies focused on dietary interventions for neurodegenerative diseases.

The data compiled from this study helps to underscore the potential of TCs and other plant polyphenols in mitigating PD symptoms and may serve as a reference for future therapeutic strategies aimed at targeting neurodegenerative diseases through dietary intervention. Future studies that build on these findings should consider several methodological enhancements to further elucidate the effects of TCs and other plant polyphenols on PD. One recommendation is to increase the frequency of TC administration via oral gavage to twice daily, which may allow for a more sustained therapeutic effect and better mimic potential clinical dosing regimens. While the mice in the high-dose treatment group eventually acclimated to the bitter taste of the dissolved decaffeinated GTP powder, increasing the frequency of administration may combat palatability challenges that could have an impact on consistent intake and stress levels. This sentiment further highlights the need for careful observation and potential adjustments to improve tolerance and keep animal stress to a minimum.

Also, extending the study duration could offer insights into the long-term impacts of TCs on disease progression and symptom management. However, ≥ 90 days of social isolation may have

an impact on behavioral and neurochemical consequences in mice [4]. To improve the welfare of animal models and reduce stress associated with single-housing, future studies should consider involving group housing with a suitable marking system to track individual's dosage, daily weight, and behavior. It should be considered that there is no clear conclusion for the practice of housing male laboratory mice as a group [5,6], so the welfare implications of housing protocols should be considered when determining a study timeline. Expanding the scope of this research to include other polyphenols, such as curcumin and resveratrol, would allow for comparative analysis of their unique and combined influences on the gut microbiome and disease symptoms. Exploring the effects of a mixture of tea catechins with other polyphenols could provide valuable insights into possible synergistic interactions, potentially amplifying neuroprotective benefits through multiple pathways.

While this study focused on urinary metabolites of TCs, future investigations could benefit from analyzing liver metabolites to provide a more comprehensive understanding of their metabolism and bioavailability. The liver plays a central role in the biotransformation of catechins [7], influencing their systemic circulation and physiological effects. Measuring liver metabolites could also facilitate the examination of circulating α-synuclein levels, offering a unique perspective on how catechin metabolism might modulate processes and protein circulation associated with PD. This approach may uncover additional bioactive metabolites and elucidate metabolic pathways that contribute to the observed effects on the gut-brain axis, providing deeper insights into the therapeutic potential of TCs. Furthermore, future studies could explore the integrity of the intestinal barrier by evaluating the gut epithelium and measuring α-synuclein concentrations in the GI tract in order to help clarify the relationship between gut-originating protein aggregation and systemic neurodegenerative processes. Investigating inflammatory markers in blood plasma would add further evidence to the systemic inflammatory response, offering a more holistic understanding of how TCs influence the gut-brain axis.

This study may serve as a reference point for future research into the neuroprotective potential of TCs that focuses on the exploration of optimized dosing regimens, mitigation of animal stress, and investigation of additional polyphenols. By introducing a framework to evaluate further critical biological parameters—such as gut epithelium integrity, α-synuclein concentrations in gut contents, and inflammatory markers in blood plasma—this work provides a strong foundation for advancing the assessment of TCs as dietary interventions. Here, we highlight the complex interplay between dietary polyphenols, gut health, and neurodegenerative disease processes, paving the way for future investigations to build on these findings. These findings not only underscore the promise of TCs as viable neuroprotective agents, but also illuminate pathways for future studies aimed at developing innovative, non-invasive strategies for preventing and managing PD and related neurodegenerative disorders.

References

1. Abbas MM, Xu Z, Tan LCS. Epidemiology of Parkinson's Disease-East Versus West. Mov Disord Clin Pract. 2018 Jan-Feb;5(1):14-28.
2. Li C, Lin J, Yang T, et al. Green Tea Intake and Parkinson's Disease Progression: A Mendelian Randomization Study. Front Nutr. 2022;9:848223.
3. Barranco Quintana JL, Allam MF, Del Castillo AS, et al. Parkinson's disease and tea: a quantitative review. J Am Coll Nutr. 2009 Feb;28(1):1-6.
4. Matsumoto K, Pinna G, Puia G, et al. Social isolation stress-induced aggression in mice: a model to study the pharmacology of neurosteroidogenesis. Stress. 2005 Jun;8(2):85-93.
5. Kappel S, Hawkins P, Mendl MT. To Group or Not to Group? Good Practice for Housing Male Laboratory Mice. Animals (Basel). 2017 Nov 24;7(12).
6. Zidar J, Weber EM, Ewaldsson B, et al. Group and Single Housing of Male Mice: Collected Experiences from Research Facilities in Sweden. Animals (Basel). 2019 Nov 21;9(12).
7. Chen, D., Wang, C., Lambert, D., Ai, N., Welsh, W., Yang, C. Inhibition of human liver catechol-O-methyltransferase by tea catechins and their metabolites: Structure–activity relationship and molecular-modeling studies, Biochemical Pharmacology, Volume 69, Issue 10, 2005, 1523-1531, https://doi.org/10.1016/j.bcp.2005.01.024.