# Huda khanam.docx

NUSRL, Ranchi

## Document Details

Submission ID
trn:oid:::3618:141650553

Submission Date
Jun 4, 2026, 12:03 PM GMT+5:30

Download Date
Jun 4, 2026, 12:08 PM GMT+5:30

File Name
Huda khanam.docx

File Size
213.3 KB

30 Pages

10,384 Words

63,250 Characters

# 4% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

## Filtered from the Report

- Bibliography
- Quoted Text
- Cited Text

## Match Groups

**43** Not Cited or Quoted 4%
Matches with neither in-text citation nor quotation marks

**0** Missing Quotations 0%
Matches that are still very similar to source material

**0** Missing Citation 0%
Matches that have quotation marks, but no in-text citation

**0** Cited and Quoted 0%
Matches with in-text citation present, but no quotation marks

## Top Sources

2% Internet sources
2% Publications
2% Submitted works (Student Papers)

## Integrity Flags

**0 Integrity Flags for Review**

No suspicious text manipulations found.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

## Match Groups

- **43** Not Cited or Quoted 4%
  Matches with neither in-text citation nor quotation marks
- **0** Missing Quotations 0%
  Matches that are still very similar to source material
- **0** Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- **0** Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

## Top Sources

2% Internet sources
2% Publications
2% Submitted works (Student Papers)

## Top Sources

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

| | | |
|---|---|---|
| 1 | Internet | v3r.esp.org <1% |
| 2 | Publication | Neeraj Mishra, Sumel Ashique, Mithun Bhowmick. "Biotics and the Gut–Brain Axis... <1% |
| 3 | Internet | www.frontiersin.org <1% |
| 4 | Internet | investingnews.com <1% |
| 5 | Publication | Vishal Ahuja, Preeti Solanki, Harmail Singh. "The Gut-Brain Axis in Neurological Di... <1% |
| 6 | Internet | www.touchneurology.com <1% |
| 7 | Publication | Jiaming Jin, Chaoliang Wen, Junying Li, Chunning Mai et al. "Collaboration of the s... <1% |
| 8 | Internet | discovery.researcher.life <1% |
| 9 | Submitted works | J S S Academy of Higher Education & Research on 2025-09-10 <1% |
| 10 | Internet | www.genengnews.com <1% |

11 Internet
www.preprints.org <1%

12 Internet
mdpi-res.com <1%

13 Internet
www.biorxiv.org <1%

14 Publication
Amr Ali Mohamed Abdelgawwad El-Sehrawy, Hamed Soleimani samarkhazan. "Th... <1%

15 Publication
Satish K. Guttikonda, Pradeep Marri, Jafar Mammadov, Liang Ye et al. "Molecular ... <1%

16 Submitted works
University of Lancaster on 2021-09-10 <1%

17 Submitted works
University of Southampton on 2025-03-28 <1%

18 Submitted works
Vrije Universiteit Amsterdam on 2026-06-01 <1%

19 Internet
busqueda.bvsalud.org <1%

20 Submitted works
Queensland University of Technology on 2024-11-05 <1%

21 Submitted works
University of Science and Technology on 2016-11-24 <1%

22 Internet
www.mdpi.com <1%

23 Submitted works
Barry University on 2025-05-29 <1%

24 Publication
Sachchida Nand Rai. "Vitamins and Neurodegeneration - Mechanisms and Therap... <1%

25 Publication

Wentao Yang, Hua Cui, Chaojie Wang, Xuan Wang, Ciai Yan, Weiping Cheng. "A re... <1%

26 Publication

Young, A., Stice S.L.. "Chapter 20 Dopaminergic Neurons Derived from Human E... <1%

27 Internet

ses.library.usyd.edu.au <1%

28 Internet

www.matscisci.com <1%

29 Internet

www.nucleicacidresearch.com <1%

30 Publication

Ali Keshavarzian, Phillip Engen, Salvatore Bonvegna, Roberto Cilia. "The gut micr... <1%

31 Submitted works

J S S Academy of Higher Education & Research on 2025-07-23 <1%

32 Publication

Tahira Farooqui. "The contribution of gut microbiota in the pathogenesis of Parki... <1%

## ABSTRACT

An aging population is predicted to increase the global burden of Parkinson's disease (PD), a neurodegenerative disorder that progresses over time and manifests in both motor and non-motor ways. There is mounting evidence that the gut-brain axis (GBA) mediates PD development from sites other than the central nervous system (CNS), including the gastrointestinal tract. This review compiles the most recent findings on the function of gut microbiome dysbiosis in Parkinson's disease, including its impact on neurodegenerative processes related to the immune system, neuroendocrine systems, and metabolism. Patients with PD typically exhibit changes in the composition of their gut microbiome, including elevated pro-inflammatory taxa and decreased numbers of bacteria that produce short-chain fatty acids (SCFAs). These changes are linked with disruption of gut barrier function, intestinal inflammation and disturbance of neurotransmitter metabolism. When looking at it from a scientific perspective, dysbiosis can cause α-synuclein misfolding and propagation, inflammation in the nervous system, and problems with the kynurenine pathway's metabolism of the amino acid tryptophan. Thanks to developments in shotgun metagenomics, which have increased our understanding of disease-associated pathways, high-resolution functional profiling of microbial communities is now feasible. Furthermore, prodromal symptoms, such as REM sleep behavior disorder, were similarly associated with microbiome changes, suggesting a potential for early diagnosis. Competing, though still in their infancy, therapies that target the microbiome include fecal microbiota transplantation, probiotics, and prebiotics. More longitudinal and mechanistic research into the gut microbiome is necessary since it may one day serve as a biomarker for Parkinson's disease and a target for treatments.


**Keywords :** Parkinson’s Disease , Gut-Brain Axis , Gut Microbiome Dysbiosis , α-Synuclein Pathology , Short-Chain Fatty Acids (SCFAs)

## INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder in the world and is expected to double by 2040 with aging populations overall affecting about 10 million people globally [1]. PD is a disorder with intraneuronal accumulation of misfolded α-synuclein protein aggregates (Lewy bodies and Lewy neurites) and the cardinal motor features of PD are bradykinesia, resting tremor, rigidity, and postural instability [2]. The disease load is far beyond its motor appearance, however, as are non-motor symptoms such as rapid eye movement (REM) sleep behaviour disorder, constipation, anosmia, depression, anxiety, cognitive impairment and autonomic dysfunction, which are often seen in patients for decades before the beginning of motor symptoms and account for a significant proportion of disability.

This prevailing neuropathological staging model by Braak and colleagues helped redefine the pathogenesis of PD, indicating that the processing of α-synuclein pathology does not start in the substantia nigra, but rather first occurs in the peripheral nervous system in the ENS and olfactory bulb and then travels in a caudal-to-rostral fashion through the brainstem via the vagus nerve and ultimately to the midbrain [3].

This "body-first" hypothesis of PD, which was places the gastrointestinal tract and its microbial ecosystem as possible starting points for neurodegeneration and has sparked an explosion of research on gut microbiota as a pathogenic factor in, and target for, PD. This present review encompasses the available evidence reinforcing the gut-brain axis as one of the key mechanisms by which gut microbiome dysbiosis contributes to PD pathogenesis, highlights some of the mechanistic pathways involved, reviews methodological approaches used in gut microbiome research and provides thoughts on potential translation into biomarker development and treatment [4].

**Objective**

1. To study changes in gut microbiota in PD patients. Know which taxa of bacteria are reduced (producers of SCFAs) and increased (pro-inflammatory bacteria).
2. To assess effect of microbial metabolites on neurodegeneration. Pay attention to short-chain fatty acids (SCFAs), neurotransmitter precursors and their role in inflammation and neuronal survival.
3. To investigate the mechanisms that connect gut dysbiosis to progressive PD. Research on aggregation of α-synuclein, neuroinflammation, and dysfunction of the intestinal barrier.
4. To investigate the gut-brain axis in PD Pathophysiology. Analyze neural (vagus nerve), immune, endocrine and metabolic communication. The interaction between gut microbiota and dopamine metabolism is explored to determine its relationship.
5. Explore the effect of microbial changes on vitamin synthesis (B6, B1, folate) and dopamine production. With the aim of assessing gut permeability and inflammatory markers in PD Discuss the correlation between disease severity, “leaky gut,” LPS and cytokines.
6. In search for early biomarkers of PD Investigate microbiome alterations in prodromal state to identify early diagnosis. To review the new therapeutic approaches that arise in the microbiome.
7. Discuss the future of probiotics, prebiotics and fecal microbiota transplantation (FMT) in the treatment of PD.

## 2. Gut-Brain Axis: Architecture and Communication Pathways

The gut-brain axis is a multifaceted bidirectional communication network between the central nervous system (CNS), enteric nervous system, ANS, hypothalamic-pituitary-adrenal (HPA) axis, and the immune and endocrine system in the intestine [5]. This network allows for bidirectional communication from gut to brain and back again via at least four different anatomically and functionally distinct pathways, with each pathway showing relevance to PD pathogenesis. The vagus nerve is the major afferent pathway linking gut-to-brain communication, consisting of ~80–90% sensory fibres from the gastrointestinal tract to the nucleus tractus solitarius in the brainstem [6] .

Vagal afferents innervate the enteroendocrine cells, enterochromaffin cells and the enteric neurons and are profoundly sensitive to luminal microbial metabolites, such as short-chain fatty acids, secondary bile acids and neuropeptides produced by the gut microbiome [7] .

The epidemiological support for the vagal route of α-synuclein propagation from gut to brain is overwhelming, with the near 15,000 patients from the Danish registry study showing a significantly reduced long-term risk of developing PD in patients with truncal vagotomy [8] .

The neuroendocrine route refers to the gut microbiota synthesis of neuroactive metabolites and/or neurotransmitter precursors that either cross the blood-brain barrier or are delivered peripherally to sensory neurons. In the body, about 90% of the serotonin is synthesized in the gut by enterochromaffin cells, which is regulated by microbial metabolites such as short chain fatty acids and secondary bile acids [9] . Many aspects of the disruption of serotonergic signalling in PD have been found and relate to non-motor symptoms such as depression and constipation [10] .

**Microbial Metabolites and Immune Dysregulation in Parkinson's Disease's GBA**

The gut microbial enzyme activity is well-known to strongly regulate the kynurenine pathway of tryptophan catabolism that produces both neuroprotective (kynurenic acid) and neurotoxic (quinolinic acid) metabolites, the ratio of which is altered in PD [11]. Moreover, the short-chain fatty acids such as butyrate and propionate, and the dopamine precursors, as well as GABA produced by microbes, regulate blood-brain barrier integrity and neuroinflammation pathways that are relevant to survival of dopaminergic neurons [12] .

The role of immunology in the pathogenesis of PD is gaining more and more significance. Intestinal barrier dysfunction (leaky gut) leads to the entry of bacterial products such as LPS and flagellin into the systemic circulation resulting in peripheral and neuroinflammation [13]. Plasma levels of LPS, gut permeabilization markers (zonulin and fatty acid binding protein-2), and pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) have been consistently found in PD patients compared to controls, and such inflammatory markers have been shown to be correlated with disease severity and duration [14].

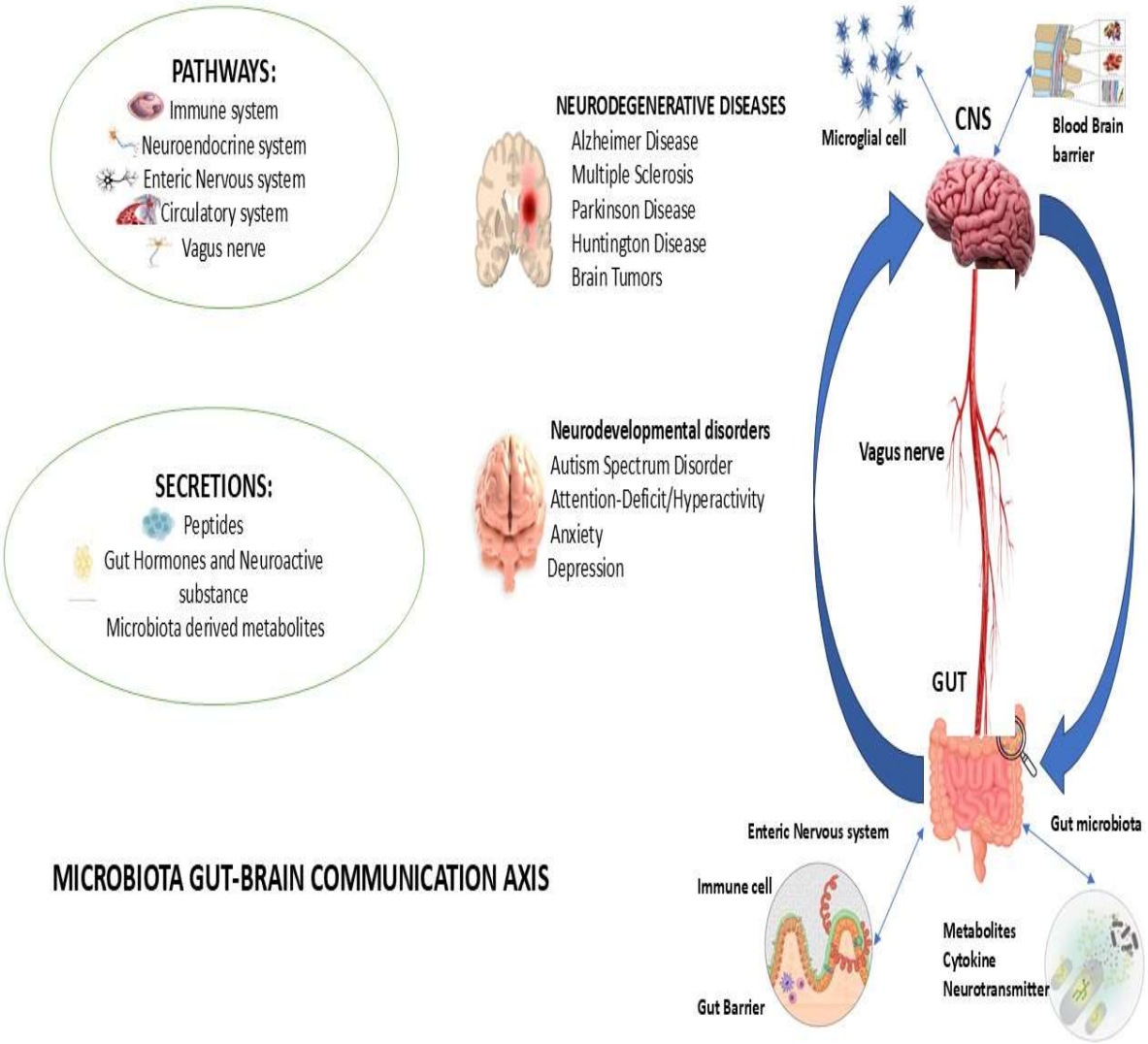
In PD, microglia in the substantia nigra are activated and peripheral LPS exposure primes them to be activated, implicating a mechanistic relationship between gut barrier dysfunction, systemic LPS/endotoxaemia and neuroinflammatory dopaminergic cell death [15].

The enteric nervous system is considered a fourth, local acting arm of the GBA. The ENS has 200-600 million neurons, similar to the number of neurons in the spinal cord, and regulates gastrointestinal motility, secretion and immune function on its own to a large extent without input from the CNS [16].

It has been proven that Parkinson's disease patients have Lewy body pathology in the extraspinal nervous system (ENS), which includes phosphorylated α-SYN deposits in neurons of the myenteric and submucosal plexuses, even before the substantia nigra is involved. In fact, some studies have shown that ENS Lewy body pathology can occur in patients up to 20 years before motor symptoms begin. Colonic biopsy of submucosal neurons

may serve as a biomarker for the prodromal stage of PD, before clinical motor manifestation, according to some of these arguments [17].

**Figure 1. The Microbiota-Gut-Brain Axis**: Its Bidirectional Nature and Clinical Applications. The figure demonstrates how the gut microbiome interacts with the central nervous system through different mechanisms, including vagus nerve stimulation, circulation, and immune responses.



## Table 1: Pathways Associated with Parkinson's Disease and Gut Dysbiosis

| Pathway | Key Components | Mechanism | Impact on PD |
|---|---|---|---|
| Neural Pathway | Vagus nerve, enteric nervous system (ENS) | Misfolded α-synuclein passes via vagal channels from the gut to the brain. | Lewy body spread and progressive neurodegeneration |
| Immune Pathway | LPS, cytokines (TNF-α, IL-1β, IL-6), microglia | As a result of gut permeability, bacterial endotoxins can enter the bloodstream and cause neuroinflammation. | Dopaminergic neuronal death |
| Neuroendocrine Pathway | Serotonin, dopamine precursors, GABA | Neurotransmitter production and signaling are regulated by gut microorganisms. | Non-motor symptoms like constipation and depression |
| SCFA Signaling | Butyrate, acetate, propionate | Microglial activation, neuroprotection, and BBB integrity are all regulated by SCFAs. | Inflammation is a result of decreased SCFA levels. |
| Kynurenine Pathway | Kynurenic acid, quinolinic acid | The balance is shifted toward neurotoxic metabolites by altered tryptophan metabolism. | Neuronal injury and excitotoxicity |

### 2.1 Description of Gut-Brain Axis (GBA)

- The gut-brain axis is a multifaceted bidirectional communication network between the central nervous system (CNS), enteric nervous system, ANS, hypothalamic-pituitary-adrenal (HPA) axis, and immune and endocrine systems in the intestine [18]
- This network allows bidirectional communication between the gut and brain.
- Communication occurs via at least four anatomically and functionally distinct pathways.
- Each pathway is relevant to the pathogenesis of Parkinson’s disease (PD).

### 2.1 Neural Pathway: Vagus Nerve

**Role of the Vagus Nerve**

- The vagus nerve is the major afferent pathway linking gut-brain communication.
- It consists of approximately 80–90% sensory fibers from the gastrointestinal tract to the nucleus tractus solitarius in the brainstem [19].

**Innervation and Sensitivity**

- Vagal afferents innervate the

1. Enteroendocrine cells
2. Enterochromaffin cells
3. Enteric neurons

- These fibers are profoundly sensitive to luminal microbial metabolites, such as:
    1. Short-chain fatty acids
    2. Secondary bile acids
    3. Neuropeptides produced by the gut microbiome

**Evidence for α-Synuclein Propagation**

- Epidemiological support for the vagal route of α-synuclein propagation from the gut to the brain is overwhelming.
- The Danish registry study involving nearly 15,000 patients showed that:
    - Significantly reduced long-term risk of developing PD disease in patients with truncal vagotomy.
    - Hazard Ratio: 0.53

**2.2 Neuroendocrine Pathway**

**Microbial Production of Neuroactive Compounds**

- The neuroendocrine route refers to the gut microbiota synthesis of
    - Neuroactive metabolites
    - Neurotransmitter precursors
- These compounds either:
    - Cross the blood-brain barrier
    - They are delivered peripherally to sensory neurons. [20]

**Serotonin Production**

- Approximately 90% of serotonin is synthesized in the gut by enterochromaffin cells.
- Serotonin synthesis is regulated by microbial metabolites, such as:
    - Short-chain fatty acids
    - Secondary bile acids [21]

**Serotonergic Dysfunction in PD**

- Disruption of serotonergic signalling in PD is related to non-motor symptoms such as:
    - Depression

- Constipation [22]

**Kynurenine Pathway**

- Gut microbial enzyme activity strongly regulates the kynurenine pathway of tryptophan catabolism.
- The pathway produces:
  1. Neuroprotective metabolite: kynurenic acid
  2. Neurotoxic metabolite: quinolinic acid
- The ratios of these metabolites are altered in patients with PD [23].

**Other Microbial Metabolites**

- Short-chain fatty acids, such as
  1. Butyrate
  2. Propionate
- Dopamine precursors and GABA production by microbes
  1. Regulate blood-brain barrier integrity
  2. Regulate neuroinflammation pathways
  3. Influence survival of dopaminergic neurons [24]

**2.3 Immune Pathway and Neuroinflammation**

**Intestinal Barrier Dysfunction**

- Intestinal barrier dysfunction ("leaky gut") leads to the entry of bacterial products into the systemic circulation.
- Important bacterial products include:
  1. Lipopolysaccharide (LPS)
  2. Flagellin
- This results in:
  1. Peripheral inflammation
  2. Neuroinflammation

**Inflammatory Markers in PD**

- Increased plasma levels have been observed in patients with PD:
  1. LPS
  2. Zonulin

3. Fatty acid binding protein-2
4. TNF-α
5. IL-1β
6. IL-6

- These inflammatory markers correlate with:
    1. Disease severity
    2. Disease duration

**Microglial Activation**

- Microglia in the substantia nigra are activated in patients with PD.
- Peripheral LPS exposure primes microglial activation.
- This suggests a mechanistic relationship between the following:
    1. Gut barrier dysfunction
    2. Systemic LPS/endotoxaemia
    3. Neuroinflammatory dopaminergic cell death [25]

**2.4 Enteric Nervous System (ENS)**

**Characteristics of ENS**

- The ENS is considered the fourth, locally acting arm of the GBA.
- The ENS contains 200–600 million neurons.
- This number is similar to the number of neurons present in the spinal cord [26]

**Functions of ENS**

- Regulates:
    1. Gastrointestinal motility
    2. Secretion
    3. Immune function
- Functions are largely independent of CNS input.

**Lewy Body Pathology in ENS**

- Lewy body pathology in the ENS includes:
    - Phosphorylated α-SYN deposits
    - Deposits in neurons of:

    - Myenteric plexus
    - Submucosal plexus
- Confirmed in patients with PD before substantia nigra involvement.

**Early Appearance of ENS Pathology**

Some studies have reported ENS Lewy body pathology occurring in:

1. Up to 20 years before motor symptom onset [27].
2. Biomarker Potential

- Colonic biopsies of submucosal neurons may serve as:
    1. A biomarker for PD prodromal stage
    2. Detection before clinical motor manifestation [28]

**3. Evidence of gut microbiome dysbiosis in PD.**

This is a cross-sectional study of compositional changes. The hypothesis of gut microbiome alteration in PD started to be supported by the first major study of faecal microbiota composition using 454 pyrosequencing of the V1–V3 hypervariable region of the 16S rRNA gene, on a total of 72 PD patients and 72 healthy controls. They found a relative decrease in Prevotellaceae, a family that synthesizes folate and thiamine, which are metabolites involved in dopamine synthesis, in PD patients (77% decrease), and that abundance of Enterobacteriaceae was positively correlated with postural instability and pace difficulty [29]

**3.1 Coherent Microbial Patterns Across Studies**

Almost all subsequent research on the PD microbiome cites this study, which laid the groundwork for GBA studies on PD. Over the next decade, a plethora of cross-sectional 16S rRNA and shotgun metagenomic studies came out, confirming microbiome changes in a wide variety of cohorts from different parts of the world and different ethnic backgrounds. However, there were notable differences in methodology across studies due to differences in DNA extraction, hypervariable region selection, bioinformatic pipelines, and cohort makeup.

Reduced relative abundance of SCFA-producing bacteria (e.g., Faecalibacterium prausnitzii, Roseburia intestinalis, and Blautia spp. from the Lachnospiraceae and Ruminococcaceae families) and increased relative abundance of pro-inflammatory Gram-negative bacteria (e.g., Enterobacteriaceae, Akkermansia muciniphila, and Ralstonia spp.) is the most consistently replicated finding across separate cohorts. [30]

**3.2 Practical Significance of SCFA-Producing Bacteria**

Especially, the decrease in Faecalibacterium prausnitzii, which has been shown to have anti-inflammatory effects, should be emphasized. This strictly anaerobic butyrate-producing species can make up as much as 5% of the healthy adult gut microbiome, and has been shown to have strong antilipolytic properties through inhibition of NF-κB signalling using butyrate,

the induction of differentiation of regulatory T cells, and maintaining the integrity of the intestinal epithelial barrier [31].

Butyrate has also been shown to have neuroprotective effects in animal models of dopaminergic neurodegeneration and is known to reduce motor impairments and neuroinflammation in germ-free mouse models of PD resulting from α-synuclein. This persistent decline in populations of taxa that produce butyrate in the PD cohorts therefore suggests that reduced gut-derived butyrate is a mechanistic link to intestinal barrier dysfunction and neuroinflammation in PD [32]- [34].

### 3.3 Confirming Clinical and Metabolic Evidence

Supporting evidence from studies indicated that sigmoid colon biopsies of 38 PD patients compared to 34 controls had significantly higher levels of α-synuclein and significantly increased colonic mucosal permeability. In contrast, PD patients had significantly lower abundances of the bacteria that produce SCFAs (Firmicutes, Blautia, Coprococcus, and Roseburia). Specifically, these microbial alterations in the gut were not linked to levodopa medication or constipation, suggesting that they are not likely caused by the drug or PD symptoms—a finding that has cast doubt on the validity of numerous subsequent research. Not all subsequent cohorts have seen the decrease in Prevotellaceae. The enzymes needed to synthesize dopamine from dopa and to decarboxylate dopa into dopamine are produced in large part by Prevotella copri in the intestines. The vitamins B6, B1, and folate are also important components of this process. More than just a taxonomic relationship, the functional relevance of Prevotellaceae loss for dopamine bioavailability in PD implies a metabolic pathway of interest for disease pathogenesis [35].

### 3.2 Akkermansia muciniphila's role in health and disease

One of the most repeated, but counter intuitive, observations in the GBA-PD research field is the enrichment of Akkermansia muciniphila in PD patients. A. muciniphila is typically regarded as a beneficial, mucosal commensal bacterium of metabolic health and gut barrier integrity which is consistently elevated in PD within a variety of cohorts including European and North American patient populations [36]-[38]. The interpretation of the mechanism is still debated: the enrichment of A. muciniphila may be a secondary compensatory mechanism to compensate for gut barrier dysfunction, or the hypersecretion of mucolytic may have the opposite effect and increase LPS translocation and gut barrier dysfunction. A third interpretation – one that has not been confirmed – is that A. muciniphila affects dopaminergic signalling by its ability to produce propionate that can cross the blood–brain barrier and exert a complex neuroactive effect [39].

### 3.3 Shotgun Metagenomics and Functional Evidence

Most of the GBA-PD early studies have used 16S rRNA sequencing, but the shift to whole genome shotgun metagenomics has yielded significantly greater mechanistic insights. The first study using whole-metagenome shotgun sequencing (WMS) to investigate the PD gut microbiota in 31 PD patients and 28 healthy controls, using functional profiling with HUMAnN2.

They validated species-level changes in composition such as a reduction in the abundance of Prevotellaceae and an increase in the abundance of Enterobacteriaceae, while also revealing substantial functional differences at the species level, such as a decrease in SCFA biosynthesis pathways, a decrease in mucin degradation pathways, and an increase in LPS biosynthesis pathways, with the latter representing the first functional genomic evidence of linkage between gut microbiome alteration and inflammatory pathway dysregulation in PD. [40]

More importantly, this study also revealed that antibiotic exposure history was a significant factor confounder for microbiome composition in PD cohorts, highlighting the critical importance of detailed medication recording in the design of studies.

**Large-Scale Metagenomic Insights and Functional Variations**

To date, the largest and most comprehensively phenotyped shotgun metagenomics study of the microbiome of PD microbiota sequenced 490 PD patients and 234 controls to a mean sequencing depth of 7.5 Gb per sample from the Parkinson's Foundation PD GENEration study and BioFIND biorepository. [41]

Using two tools, MetaPhlAn3 and HUMAnN3, they were able to identify 55 microbial species that had undergone significant changes. Among these changes were changes to functional pathways, such as a reduced pathway for indole-3-propionic acid, a decreased capacity to convert secondary bile acid, an increased capacity to produce lipopolysaccharide, and a decreased capacity for butyrate production (bacterial gene reduction). The pathophysiology of the microbiome can be better understood at a systems level with the help of network analysis, which revealed that PD is associated with changes in functionally coherent microbial modules rather than specific species. Additionally, this work demonstrated that microbiome variance was substantially impacted by PD drug use, constipation, and BMI, underscoring the necessity for stringent adjusters in GBA-PD trials [42].

**Prodromal Microbiome Changes and Early Disease Signs**

The question of whether dysbiosis of gut microbiome is a precursor to the onset of motor symptoms in PD is particularly exciting and clinically relevant, and may potentially allow for development of early biomarkers for the prodromal stage of the disease. There is a number of evidence to support this possibility [43].

Phosphorylated α-synuclein deposits have been found in the submucosal plexus in colonoscopic biopsy studies of people with idiopathic REM sleep behaviour disorder (iRBD), a prodromal PD syndrome with about 80% conversion to PD or related synucleinopathies over 10 years [44] .

Dysbiosis of the iRBD cohorts has shown similarities with the dysbiosis previously observed in established PD, such as decrease in Prevotellaceae and reduction in Lachnospiraceae, suggesting a dysbiosis preceding motor symptom onset. The temporal relationship of constipation and PD further supports [45].

**Role of Gastrointestinal Dysfunction in Early PD Pathogenesis**

One of the earliest and most common prodromal symptoms of PD is constipation, which is a sign of ENS dysfunction and a decrease in gut motility, and is linked to a 2- to 4-fold higher risk of motor diagnosis of PD in prospective cohort studies.

Enteral transit time is a major determinant of the composition of the gut microbiome; in PD, a slow enteral transit may favour dysbiosis, through a mechanism of selection of saccharolytic and proteolytic fermenters, and of a loss of the competitive advantage of the butyrate-producing bacteria, which could trigger a vicious circle of dysbiosis, intestinal barrier dysfunction and consequent neuroinflammation before clinical disease [46].

**Mechanistic Pathways: From Gut Dysbiosis to Dopaminergic Neurodegeneration.**

**4.1 α-Synuclein Misfolding and Prion-Like Propagation**

The most direct mechanistic pathway proposed for the link between gut microbiome dysbiosis and PD neuropathology is the process of α-synuclein misfolding in enteric neurons that then spread to dorsal motor nucleus of the vagus nerve via retrograde flow along vagal efferent and afferent fibres and eventually to the brainstem, including the substantia nigra compelling experimental evidence that misfolded α-synuclein can be transferred from the gut to the brain through the vagus nerve, causing progressive dopaminergic neurodegeneration with a temporal progression similar to that observed in PD.

**The big question in the research of GBA-PD remains: Can gut microbiome dysbiosis lead to the initiation or progression of α-synuclein misfolding?**

There are a number of suggested mechanisms. Structural homology exists between bacterial amyloid proteins, such as curli fibres of E. coli and other Enterobacteriaceae, which are enriched in PD, and α-synuclein, and they have been demonstrated to induce cross-seeding of α-synuclein aggregation [47].

**Experimental Evidences from Animal Models**

The gut microbiota play a crucial role in the full manifestation of motor impairments and neuroinflammation in transgenic mice that express α-synuclein through the Thy1 gene (Thy1-SNCA), according to a revolutionary study. The researchers found that GF mice exhibited far lower levels of α-synuclein aggregation, microglial activation, and motor impairments as contrasted with controls that had conventional colonization. This is the first experimental evidence linking dysbiosis associated with Parkinson's disease to motor neurodegeneration; microbiota from PD patients considerably reduced motor function in GF mice compared to healthy controls. Furthermore, the GF mice who were given SCFA as a supplement showed signs of microglial activation and α-synuclein pathology, which might be explained by specific microbial metabolites acting as intermediaries. [48]

**Dysfunction of Gut Barrier and Systemic Inflammation**

The dysfunction of the gut barrier (GB) and the associated increased permeability, which results from dysbiosis-mediated disruption of tight barrier proteins such as occludin, claudin-1 and ZO-1, is a consequence and is likely to be a perpetuating cause of GBA dysregulation in PD. Raised serum levels of markers of intestinal permeability (zonulin, fatty acid binding protein-2, LPS-binding protein) have been found in PD patients and are correlated with disease duration and burden of non-motor symptoms, respectively [49].

**Neuroinflammation and Selective Dopaminergic Neuron Vulnerability**

LPS and flagellin are translocated across a leaky epithelial barrier to activate lamina propria dendritic cells and macrophages by triggering TLR4 and TLR5 activation, pro-inflammatory cytokine release (TNF-α, IL-1β, IL-6, IL-17), and activation of the NLRP3 inflammasome [50].

LPS reaches the CNS through several mechanisms: pro-inflammatory cytokines disrupt the integrity of the blood brain barrier (BBB), they act on circumventricular organs that lack a complete BBB; activated monocytes and T lymphocytes transmigrate into the CNS parenchyma [51]. In the brain, LPS-priming of microglia is a particular vulnerability and chronic activation of these cells in PD creates a neuro-inflammatory milieu of reactive oxygen species, pro-inflammatory eicosanoids and glutamate excitotoxicity which drives the death of dopaminergic neurons [52]. Why substantia nigra dopaminergic neurons are more vulnerable to this inflammatory cascade, as compared to, for instance, cortical neurons, may have to do with their relative lack of antioxidant capacity, high basal metabolic rate, and comparatively low levels of trophic support [53].

**4.3 Short-Chain Fatty Acids as Neuromodulators**

Microbiologically derived SCFAs (especially acetate, propionate and butyrate) are an important mechanistic connection between gut microbiome composition and CNS function in PD. Butyrate is the main source of energy to the colonocytes and has several neuroprotective effects: it inhibits histone deacetylases, increasing the expression of neurotrophic factors such as BDNF and GDNF [54].

It triggers the release of the neuroprotective peptides YY (PYY) and glucagon-like peptide-1 (GLP-1) by activating the GPR41 and GPR43 on enteroendocrine cells [55]. Through GPR109a signaling, it inhibits microglial activation, and it modulates gene expression in neurons and astrocytes by directly crossing the blood-brain barrier [56]. Evidence from both metabolomic (faecal SCFA concentrations measured by gas chromatography-mass spectrometry, or GC-MS) and metagenomics (shotgun metagenomics functional profiling) approaches is consistent with lower concentrations of SCFA in the faeces of PD patients, particularly butyrate and propionate, and with decreased expression of butyrate-producing genes in the gut microbiome of PD patients. Bacteroides spp., Veillonella spp., and Akkermansia spp. all have complicated neuroactive qualities that are dose dependent, and propionate is mostly produced via the succinate and acrylate routes. At physiological doses, propionate has anti-inflammatory, intestinal gluconeogenesis-regulating, and free fatty acid receptor (FFAR3/GPR41) activating effects [57]. When administered intraventricularly,

propionate produces neuroinflammation and movement abnormalities in rats, as well as the social and behavioral deficits associated with autism and mitochondrial dysfunction at supraphysiological doses in rats. Future study within GBA-PD should focus on determining the overall impact on PD when adding Akkermansia changes propionate signaling (e.g., more propionate accessible in the colon) [58].

### 4.4 Tryptophan metabolism and the Kynurenine pathway

Tryptophan is an essential amino acid that is absorbed from the diet in the small intestine and is the precursor of serotonin, melatonin, and the metabolites of the Kynurenine pathway, and the metabolism of all these compounds is significantly affected by gut microbiota. The enzymes indoleamine 2,3-dioxygenase (IDO1, IDO2) and tryptophan 2,3-dioxygenase (TDO) convert about 95% of dietary tryptophan into kynurenine, which can then be converted to neuroprotective kynurenic acid (via kynurenine aminotransferases) or into neurotoxic quinolinic acid (via 3-hydroxykynurenine and kynurenine 3-monooxygenase).

In PD, gut microbiome dysbiosis switches tryptophan catabolism towards the quinolinic acid branch of the Kynurenine pathway, leading to a decrease in the serum and CSF Kynulinic acid/Quinolinic Acid ratio in PD patients, and Quinolinic acid has been shown to activate NMDA receptors and to induce excitotoxic dopaminergic neurodegeneration. The cohort further supported this with direct genomic evidence of reduced microbial pathway capacity to produce indole-3-propionic acid (IPA), a neuroprotective tryptophan catabolite produced specifically by gut microbes, suggesting microbial pathway dysbiosis in PD is causing a perturbation of neuroprotective tryptophan metabolism [59].

### The relationship between genetic factors and gut microbiome in PD

Mutations and copy number variations of the SNCA (α-synuclein), LRRK2 (leucine-rich repeat kinase 2), GBA1 (glucocerebrosidase), PINK1, and Parkin genes explain about 10-15% of PD cases, with LRRK2 G2019S being the most prevalent pathogenic variant in European populations [60].

This has generated a promising avenue of research on the interactions between genes and the microbiome in PD. LRRK2 is highly expressed in intestinal macrophages and Paneth cells, and plays a role in the regulation of innate immune signalling and autophagy pathways related to microbial surveillance [61].

LRRK2 G2019S mutation carriers with and without PD exhibit similar gut microbiome alterations (both Prevotellaceae depletion and Lachnospiraceae reduction) than non-carriers, indicating that LRRK2-induced alterations in gut immune function may affect the dysbiosis underlying the onset and/or progression of PD pathogenesis, independent of motor symptom onset [62].

On a molecular level, the ability of LRRK2 mutations to modulate inflammatory responses to microbial LPS by controlling MAPK signalling pathways offers a plausible molecular link between genetic susceptibility and gut-microbiome-mediated neuroinflammation. The most frequent genetic risk factor for PD is a mutation in the GBA1 gene which codes for

glucocerebrosidase, the enzyme that is lacking in Gaucher's disease, with a frequency of 5–15% of PD patients, depending on the ancestry . Glucocerebrosidase activity in the gut is involved in glycosphingolipid metabolism in colonocytes and immune cells, and variant carriers have a different composition of their faecal microbiome to non-carriers it is not known whether this is the direct result of the mutation on gut epithelial biology or the consequence of a primary PD effect, as the gut microbiome is shaped by many factors. GBA1 status is an important confounder that needs to be stratified in large-scale GBA-PD metagenomic studies [63].

**Targeting Microbiome Therapy with Mechanistic Basis.**

The methods used to treat gut microbiota are based on several biological processes: first, the fermentation of dietary fiber in the small intestine produces SCFAs like acetate, propionate, and butyrate, which regulate neuroinflammation and vagus nerve function; second, gut-derived bacteria produce neurotransmitters like serotonin, gamma-aminobutyric acid (GABA), and dopamine precursors, which modify neural signaling [65].

**Role of SCFAs and Psychobiotics in Brain Function**

SCF Also, by boosting the signaling pathways linked to synaptic flexibility and neuronal survival, they enhance neurogenesis and the integrity of the blood-brain barrier. The prospective therapy impact of microbiome manipulation is the increased expression of neurotrophic factor derived (BDNF), which is triggered by microbiome interventions, according to experimental findings. Psychobiotics are a family of agents that activate the gut-brain axis and promote mental health. They can be probiotics, postbiotics, prebiotics, or synbiotics. Psychobiotics have a psychotropic effect on stress, anxiety, and depression. The neuroendocrine system, immunoregulatory mechanisms, and vagus nerves all stimulate interactions between gut and brain microbes [66].

Psychobiotics can affect the brain's neurotransmitters and proteins, the HPA axis, or of inflammatory chemicals directly associated with depression, or the intellectual and emotional pathways.

**Microbial Therapeutics and Future Prospective in Neurodegenerative Diseases**

Human flora such as Lactobacillus GG and Bifidobacterium infantis 35, 624 stimulate interleukin-10, which in turn decreases pro-inflammatory cytokines and either directly or indirectly maintains the integrity of the blood-brain barrier. Acetylcholine is synthesized by strains of Lactobacillus Lactobacillus odontolyticus and Lactiplantibacillus plantarum. In humans, spore-forming gut microorganisms also cause the enterochromaffin cells to produce more serotonin. When utilized to treat a variety of mental illnesses, such as Parkinson's disease, Alzheimer's disease, Tourette syndrome, autism, and insomnia, psychobiotics—and FMT in particular—have shown positive outcomes. FMT has been shown to be successful in treating symptoms of depression and anxiety.

Human microbes like Lactobacillus GG and Bifidobacterium infantis 35, 624 augment the interleukin-10 and consequently by trimming down pro-inflammatory cytokines either

directly or indirectly help preserve the integrity of the blood-brain obstacle. Lactobacillus Lactobacillus odontolyticus and Lactiplantibacillus plantarum are strains that produce acetylcholine . There is also a similar increase of serotonin production by the enterochromaffin cells by spore-forming gut microbes in humans. Psychobiotics, and primarily, FMT have recorded positive effects when used in management of numerous mental conditions including but not limited to Parkinson disease , Alzheimer disease , Tourette syndrome, autism , and insomnia . FMT was established to be effective in alleviating the state of depression and anxiety . Therefore, psychobiotics have come as a remedy to all sorts of neurodegenerative disorders. It may be a valuable and promising approach towards healthy well-being. Human studies have not yet been done despite promising results. Additional studies in the field of psychobiotics should be carried out to use it as an alternative treatment in neurodevelopmental and neurodegenerative diseases. [67]

## 1. PROBIOTICS

The ability of probiotics, which are live microorganisms to improve health benefits to the host, has been a focus of research in relation to the microbiome-gut-brain axis . Psychobiotics, originally defined as probiotics with health benefits to the host achieved through gut-brain interactions, has mood-enhancing effects, reductions in anxiety and depression scores, and improvements in stress reactions. This definition has been broadened to encompass other ways to target the microbiome that could potentially improve brain process. Certain strains of bacteria have also been shown to reduce inflammation in preclinical models, including an inflammation model caused by a high-fat diet, where Lactobacillus and Enterococcus strains were able to reduce IL-6, TNF-a and IL-1b, but not anxiety [68].

### Strain-Specific Effects and Neurobehavioral Outcomes

Strain specificity is of great importance; in an example, B. longum was able to reduce depression in individuals with irritable bowel syndrome (IBS), but not anxiety in another study . plantarum also reduced stress Certain probiotics (e.g. Bifidobacteria and Lactobacilli) have demonstrated potential to improve behavioral and neurochemical impairments in preclinical models of ASD, with Bifidobacteria and Lactobacilli enriching behavioral and neurochemical impairments ; but no significant changes in TNF-a, IL-6, and IL-1b or cortisol were found in any group of probiotics tested in a single study [69].

### Psychobiotics and Immune Modulation

Schizophrenia has been linked with peripheral immune abnormalities, although probiotics have not demonstrated much psychiatric effect. The reduction in von Willebrand factor and the modulation of monocyte chemoattractant protein-1, brain-derived neurotrophic (BDNF), chemokine ligand 5, and macrophage inflammatory protein-1β . Pathway analysis indicated that the inhibition of the changes in L. rhamnosus and Bifidobacterium animalis supplementation is associated with the decrease of IL-17 cytokines in the regulation of immune and intestinal epithelial cells, as well as involved changes in mon Positive outcomes on anxiety and depression in schizophrenia and elevation of TNF-related activation-induced

cytokine and IL-22 in respondents are also observed in open-label studies involving Bifidobacterium breve, but lacking placebo arm [70].

**PREBIOTICS**

Prebiotics are an alternate strategy for influencing the microbiome-gut-brain axis. These are indigestible substances that have been hand-picked to influence host microbes in a way that favours good microbes. Fruits, vegetables, entire grains, and even human milk contain prebiotics. Many other foods also include these beneficial bacteria. The proliferation of beneficial microorganisms like Bifidobacterium is one way in which prebiotics may enhance gut health and, by extension, brain health [71].

For example, a review article indicated that healthy individuals can reduce the cortisol awakening response after three weeks of high-dose GOS supplementation, and prebiotics like FOS and GOS have changed the makeup of gut microbes and reduced stress reactions [72]. This suggests that prebiotics may play a role in regulating gut and mental health. Furthermore, prebiotics have the ability to influence in utero VPA-induced alterations in an autism spectrum disorder (ASD) mouse model by lowering levels of specific microbes, increasing intestinal permeability, reestablishing immunological homeostasis, decreasing neuroinflammation (namely, CD68 expression), and improving social behavior and cognition in children exposed to VPA during gestation [73].

Prebiotics also changed levels of corticosterone and BDNF expression in the hippocampus, two important neurobiological pathways associated with neuropsychiatric disorders. To fully understand the therapeutic potential of prebiotics, however, further research in humans is required to determine how these microbes affect mental health.

**FMT (Fecal microbiota transplantation)**

FMT is conducted by the application of the therapeutic population of the microbes. FMT is an attractive technique to substitute the pathogenic microbes with the healthy microbes. It entails relocation of healthy microorganisms to the recipient of healthy donors using different delivery methods. The donor should be screened on stringent guidelines . To ensure that the donor infects the recipient, it is required that appropriate stool and blood tests be conducted within 4 weeks prior to transplantation. To the induce immunotolerance by the recipient to the microbes of the donor, it is preferable that a close relative of recipient is used as the donor and not an unrelated donor in the event of a genetic disease like inflammatory bowel disease. [74].

**Clinical Significance and Therapeutic Benefits of FMT**

It was discovered that fecal filtrate with bacterial debris, metabolites, DNA etc. was simply sufficient to treat recurrent Clostridioides difficile infection (CDI). Recurrent CDI was treated with pure culture of intestinal bacteria of one healthy donor only. FMT has been effectively employed in the treatment of antibiotic caused dysbiosis in C. difficile infection. FMT has also been effectively applied in treating recurrent C. difficile infections with a breathtaking percentage of recovery (with the percentage of success significantly lower than those of

CDI). The fecal microbes of the lean mice had to be transferred to the obese mice to restore the butyrate producing bacteria and enhance the production of insulin. Post FMT, the prevalence of antibiotic-resistant pathogens linked to infections of the urinary-tract were radically decreased. Some diseases like alcoholic hepatitis and cirrhosis are linked with the lack of the mucosa-associated invariant T-cells. FTM has been found to restore these T-cells in patients . Likewise, the loss of Bacteroidetes caused by alcohol was recreated following FMT . Successful efficacy Experts related to FMT have carried out a number of clinical trials in the case of liver disorders, evolution of fibrosis, hepatic encephalopathy and alcoholic hepatitis. FMT has been effectively researched in some neurological disorders like autism, sclerosis and Parkinson disease. The fecal microbes transfer of healthy to cancer patients enhanced the response to the immune checkpoint inhibitors [75].

**Limitations, Risks, and Future Directions of FMT**

Determined by the host genes linked to the immune system, the efficacy of the FMT process depends on the heritability of the transferred microorganisms. Research has shown that FMT can effectively treat ulcerative colitis and increase the likelihood of remission. Similarly, FMT treatment was ineffective in cases of chronic Crohn's disease. When β-lactam-resistant Escherichia coli is transferred, immunocompromised persons may suffer from the negative consequences of fecal transmission. In response to the adverse effects of FMT and the health risks associated with foul stools, a new approach to FMT was developed: artificial stool. This method involves fermenting and formulating feces commensals in a controlled environment that mimics the human gut, and then packaging them in a living form. It is also possible to restore the native gut microorganisms by replacing the patient's stool in cases of severe infections [76].

## Table 2 : Functional Roles of Key Gut bacteria in Parkinson Disease Pathogenesis

| Taxon | GBA relevance |
|---|---|
| Faecalibacterium prausnitzii | Butyrate production → anti-inflammatory, gut barrier integrity |
| Akkermansia muciniphila | Mucin degradation, gut permeability regulation |
| *Lactobacillus* spp. | GABA production, vagal signalling, tryptophan metabolism |
| *Bifidobacterium* spp. | GABA, acetate, SCFA; immune modulation |
| *Prevotella copri* | Propionate production; possible neuroinflammation link |
| *Ruminococcus* spp. | Butyrate, cross-feeds other SCFA producers |
| *Enterobacteriaceae* | LPS production → systemic inflammation → neuroinflammation |

# METHOD AND METHADOLOGIES

### The methodology for Shotgun Metagenomics of the Gut Microbiome

The gut microbiome has been described using whole-genome shotgun (WGS) metagenomics, which is the most comprehensive method currently available, and is becoming the gold standard method for mechanistic studies of the gut-brain axis (GBA). In contrast to 16S rRNA amplicon sequencing which can only be used to provide taxonomic profiles at genus level, and lacks direct functional information, shotgun metagenomics randomly fragments and sequences all DNA in a biological sample, including both bacterial, archaeal, viral, fungal and residual host genomes .This method provides a genome-wide, unbiased snapshot of the whole microbial community, allowing species- and strain-level classification, construction of functional gene catalogues, quantification of metabolic pathways and recovery of metagenome-assembled genome (MAG) and profiling of antibiotic resistance genes in a single experimental workflow. For GBA research modelling, where the mechanistic questions focus on the identities of organisms producing neuroactive metabolites (e.g., γ-aminobutyric acid (GABA), short-chain fatty acids (SCFAs) and tryptophan catabolites), and by what biosynthetic route, the functional resolution enabled by the shotgun metagenomics approach is scientifically essential [64].

## 3.1 Sample Collection and Preservation

### Step 1: Faecal Sample Collection

The faecal samples should be collected using validated nucleic acid stabilisation systems, e.g. OMNIgene•GUT OM-200 tube (DNA Genotek). Otherwise immediately freeze in liquid nitrogen [77].

### Step 2: Sample Storage Samples

Sample Storage Samples must be stored at −80 °C for long term storage within 15 minutes of voiding. At room temperature, the composition of the gut microbial community changes significantly over a period of 2–4 hours.

After one freeze-thaw (FT), microbial composition is significantly changed [78].

### Step 3 : Use of OMNIgene•GUT System

DNA was stable over 60 days at ambient temperature with the OMNIgene•GUT system and with no measurable distortion of the DNA's composition. This system provides logistical benefits for large multi-site cohort studies while maintaining study analytic integrity [79].

### Step 4: Coordinated Multi-Sample Collection

When matched collection at the same timepoint should contain:

1. Peripheral blood serum

2. Urine

3. Saliva

**Step 5: Blood Serum Analysis Serum**

It is taken from the peripheral blood for:

- Systemic metabolomics
- Inflammatory cytokine
- profiling: TNF-α
- IL-6
- IL-1β

CRP by multiplex ELISA

Gut permeability markers:

Zonulin , fatty acid binding protein-2. LPS-binding protein

**Step 6 : Urine and Saliva Collection:**

At the same time the patient's urine and saliva samples are collected. Untargeted metabolomics is done using urine. Oral microbiome characterisation: collection of saliva. These analyses allow for multi-omic analysis of gut-brain communication pathways to be integrated [80].

**Step 7: Metadata Recording**

The following are important points that comprehensive metadata should include: Exposure to antibiotics in the six months before testing.

All medications including prescriptions and over-the-counter, particularly:

- Proton pump inhibitors , Metformin , SSRIs , Antipsychotics , Laxatives

Dietary patterns judged by:

- Validated 24-hour recall
    i. Food frequency questionnaire
    ii. Bowel habit regularity – quantified using Bristol Stool Scale.
    iii. Body mass index.
    iv. Smoking and drinking.

Psychiatric assessment scores that comprised of:

3. The patient Health Questionnaire-9 (PHQ-9)
4. Generalised Anxiety Disorder-7 (GAD-7)

## 3.3 DNA Extraction

**Step 1: The first step is the selection of the method of DNA extraction.**

The first step is choosing the method for extracting the DNA. The DNA extraction is recommended to be performed using the Qiagen PowerSoil Pro Kit. Shotgun metagenomics is dependent on high quality and high quantity of DNA since each step of the library preparation and quality of the DNA sequencing and subsequent analysis by assembly is sensitive to DNA fragmentation, purity and quantity [81].

**Step 2: Enzymatic Pre-Lysis**

An enzymatic pre-lysis is done with: Lysozyme (10 mg/mL) Mutanolysin (250 U/mL) Samples are cultured for 1 hour at 37 °C.

This partially digests the gram-positive bacterial cell walls before disrupting them mechanically.

**Step 3: Mechanical Disruption Bead beating**

It is done at a speed of 6.0m/s for 40s. A combination of: Garnet beads 0.1 mm glass beads This is using an MP Biomedicals FastPrep-24 5G instrument.

**Step 4: A gram-positive bacterial lysis occurs**

The enzymatic-mechanical method is more effective than bead-beating alone in reaching a much higher degree of lysis of Firmicutes. This will minimize the systematic under-representation of gut-brain axis gram-positive taxa.

**Step 5 : Assessment of DNA purity**

Spectrophotometrically, the purity of DNA is determined.

Target values: $OD_{260/280}$ ratio: 1.8–2.0 , $OD_{260/230}$ ratio: >1.7 Deviations indicate residual: Protein , Polysaccharide and Humic acid contamination

**Step 6: DNA Quantification**

To measure the quantity of DNA, the following is used:

Qubit 4 Fluorometer

dsDNA Broad Range Assay Kit (Thermo Fisher Scientific)

Target yield: 500 nanograms to 5 micrograms

**Step 7: DNA Integrity Analysis**

The integrity of DNA is checked by: Agilent TapeStation Genomic DNA ScreenTape analysis. Acceptable quality: DNA Integrity Number (DIN) ≥7 Strong band at frequencies of more than 20 Kb.

**Step 8 Quality Control Procedures**

There are two procedural controls in each extraction batch:

Extraction Blanks uses only processing chemicals and not biological material.

Used for background contamination for kit associated ("kitome").

18 ZymoBIOMICS Microbial Community Standard D6300 Mock community containing:

Eight bacterial species and Two fungal species

Used to monitor: Extraction efficiency, Lysis completeness and Inter-batch reproducibility.

**Step 9 : The host DNA is depleted**

For GBA samples containing high amount of human DNA: MolYsis Complete5 kit Saponin-based differential lysis Applied before being mined. Microbial reads are 3- to 10-fold higher with host depletion and sequencing cost is a fraction of what it is without host depletion.

## DNA Fragmentation and Library Preparation

17 Our first step is to break apart the genome in order to prepare a library.The first step is to fragment the DNA and prepare a library. The workflow to prepare libraries transforms high molecular weight genomic DNA into short, uniformly sized, sequencer compatible, adapter and index sequence tagged DNA molecules. Acoustic fragmentation is recommended via the 15 Covaris E220 Focused-ultrasonicator at 350 watts peak power, 5% duty factor, 200 cycles per burst and a treatment time of 60 to 80 seconds for Illumina 2×150 base pair paired-end chemistry products with a median insert size of 200-350 base pairs

Unlike enzymatic alternatives to shearing like Nextera XT tagmentation, which have a known insertion sequence bias and preferentially fragment accessible chromatin regions in the genome, Covaris acoustic shearing generates a consistent and highly reproducible size distribution with no insertion sequence bias across the genome. Size selection after fragmentation by removing DNA fragments < 100 bp (which yield non-informative adapter-dimer reads) and residual genomic DNA > ~800 bp (which would adversely affect bridge amplification clustering efficiency for Illumina sequencing) is achieved by using the AMPure XP paramagnetic bead purification system at the 0.6 vol : vol ratio.

21 The size distribution is checked by Agilent Bioanalyzer DNA 1000 chip or TapeStation D1000 ScreenTape, which shows a tight peak at the targeted insert size with the added 130 base pairs from the ligated adapters. Both end repair and dA-tailing are done with the NEBNext Ultra II FS DNA Library Prep Kit (New England Biolabs) using T4 DNA polymerase for blunt end generation and Klenow fragment exonuclease for 3'-dA overhang addition.

29 Illumina sequencing adapters containing unique dual indexes (UDIs), are ligated with T4 DNA Ligase at 20°C for 15 minutes and then the hairpin loop structure in the adapter is cut by USER enzyme. For multiplexed NovaSeq sequencing using patterned flow cells, the use of unique dual indexes (rather than combinatorial dual indexes) is required, and index hopping rates of 0.1–1% have been reported, which could result in non-trivial levels of cross-

sample contamination in cases where many GBA-relevant taxa are detected at sub-0.1% relative abundance.

Library preparation is done using 8–12 cycles of PCR amplification with KAPA HiFi HotStart ReadyMix (Roche) to minimise GC-content amplification bias and chimera formation; if sufficient input DNA (≥1 µg) is available, PCR-free library preparation (Illumina TruSeq PCR-Free or KAPA HyperPrep PCR-free) is strongly recommended to eliminate amplification bias completely and to improve coverage uniformity across high-GC genomic regions that are over-represented in Actinobacteria, such as the GBA-relevant Bifidobacterium species.

Final library quantification is performed using both Qubit dsDNA High Sensitivity fluorometry and quantitative PCR using an Illumina-compatible library quantification standard (KAPA Library Quantification Kit) and individually quantified libraries are further normalised to a 4 nM working concentration, pooled equimolarly across the sample cohort, denatured to 20 pM, and supplemented with a 1% PhiX control library spike-in to provide increased nucleotide diversity during Illumina base-calling.

## Sequencing Platform and Depth

The platform is the surface on which the ball is placed. Platform is the area the ball rests on. Current platforms for large-scale gut metagenomic studies are the Illumina NovaSeq 6000 or NovaSeq X Plus, which uses patterned flow cells and either 2×150 or 2×250 base pair paired end sequencing, and provide between 300 gigabases and 6 terabases per run at around £150 – 400 per sample, depending on sequencing depth and batch size.

To ensure reliable metabolic pathway reconstruction using HUMAnN3, a minimum sequencing depth of 10 gigabases per sample (around 50 million paired-end read pairs) is required; less than about 5 million reads mapped to coverage of metabolic pathways of low abundance microbial contributors to the GBA-relevant metabolic routes is not reliable.

Sequencing depths of 20–30 gigabases per sample are sufficient to get the contig depth coverage (>10× median coverage) needed for reliable assembly and binning for studies focused on MAG recovery and novel species characterisation. Shallow shotgun metagenomics, meaning sequencing at a depth of 1–2 gigabases per sample, has been shown to be a cost-effective method for species-level taxonomic profiling and coarse functional classification, and has proven to be effective in several large GBA cohort studies.

Long-read sequencing platforms, like those of Oxford Nanopore Technologies (PromethION) with ultra-long reads (50-100 gigabases per flow cell) and accuracy over 99.9%, and PacBio (Revio), 15-20 kilobase HiFi reads, are also shown to provide complementary data to support strain-level resolution, near-complete MAG assembly, and detection of structural variants.

For new species characterization from GBA cohorts, hybrid assembly approaches that leverage the strengths of both long and short read data are the current best practices for metagenomic studies.

## Bioinformatic Processing: Quality Control and Host Decontamination

Prior to any analysis of the sequences, a standardised quality control and host decontamination pipeline is applied to the raw sequencing reads. Quality assessment is carried out using FastQC and multi-sample reports using MultiQC and requiring a minimum of 80% of bases at Phred quality score Q30 or higher, acceptable per-base quality scores across the length of the reads, consistent distribution of GC content with respect to the expected microbial community, and low levels of adapter contamination, less than 1% of reads. Adapter trimming and quality filtering are performed using fastp with the following parameters: per-base quality score minimum, Q20; minimum length of the read after adapter trimming, 50 bp; automatic detection and removal of paired-end adapters. All human faecal metagenomic samples must undergo host read decontamination prior to taxonomic and/or functional analysis. The reads are quality filtered and aligned against the human reference genome GRCh38 with Bowtie2 with sensitive-local alignment parameters, all concordantly and discordantly mapping read pairs are removed and the unmapped read pairs are retained as the microbial fraction for downstream processing. The high proportion of reads that map to the human genome typically ranges from 10 to 50%, and can be significantly higher for faecal samples that contain mucosal epithelial cells, blood or inflammatory exudate, as might be found in GBA studies of patients with intestinal barrier dysfunction, inflammatory bowel disease, or colonic biopsies . Post-decontamination samples with fewer than 5 million microbial read pairs are marked and either not used for pathway-level analysis or only used for shallow-shotgun taxonomic analysis because the number of reads at this level is not sufficiently deep to support power-ful pathway reconstruction .

## Taxonomic Profiling

Host-depleted metagenomic reads are taxonomically profiled at the species level and subspecies level using MetaPhlAn4 which uses around 5.1 million clade-specific marker genes from 99,000 reference genomes and high-quality metagenome-assembled genomes to obtain relative abundance profiles with a very low false-positive rate through taxonomic profiling. To complement and cross validate, a complementary and less sensitive approach to Kraken2 is applied with the Bracken abundance estimation method using the reference database PlusPF, which includes all RefSeq bacterial, archaeal, viral, fungal, and protozoan sequences from NCBI. Taxonomy assignments from both tools are cross checked and merged to achieve the highest possible level of sensitivity and specificity in the final abundance table.

## Functional Profiling

Functional pathway analysis is carried out with HUMAnN3, a three steps computationally efficient functional pathway annotation approach. In the first tier, species profiles are identified by MetaPhlAn4 and used to select species-specific sequence databases from the ChocoPhlAn v296 database that are then aligned to reads using Bowtie2 to calculate per-

gene-family read counts. In the second tier, then the reads that are not detected by the first tier are searched for translated sequences against the UniRef90 protein sequence database using DIAMOND to retrieve functional signal from genes in poorly characterised or undetected organisms. Normalisation is based on read counts per gene (RPK), and then gene families are aggregated into the abundance of metabolic pathway using MinPath for both MetaCyc and KEGG pathway databases (copies per million sequenced reads). The functional outputs of particular interest for GBA research are the pathways for GABA biosynthesis (MetaCyc PWY-5022), the pathways for biosynthesis of tryptophan and catabolism of kynurenine (MetaCyc TRPSYN-PWY, KYNURENINE-PWY), pathways for synthesis of SCFA including butyrate (BUTANESYN-PWY), propionate (P108-PWY), and conversion to acetate, enzyme families for DOPA decarboxylase enzyme functions relevant to microbial biosynthesis of dopamine precursors , and the capacity to synthesize LPS as a proxy for systemic inflammatory potential.

## Strain-Level Analysis

Strain-level resolution below the species level is enabled by SNP based-profiling of per-sample consensus sequences that can be reconstructed at the clade-specific positions of marker genes defined by the MetaPhlAn4 algorithm, distributed in bioBakery3 suite, and by multi-sample phylogenetic analysis of these sequences, to detect strain-level population structure and transmission events. This strain-level resolution is completely unachievable through 16S sequencing, and needed when looking at the functional properties of probiotic versus commensally co-occurring strains, as in the case of Lactobacillus rhamnosus JB-1, which is known to produce GABA and have anxiety-attenuating effects, versus other L. rhamnosus strains without the same properties (Bravo et al., 2011). inStrain (Olm et al., 2021) offers complementary within-sample and between-sample average nucleotide identity and nucleotide diversity calculations, which can be used to monitor strain colonisation patterns, micro-evolutionary changes and transmission chains over the time points of a longitudinal GBA intervention study.

The metagenome assembly and MAG recovery step. The metagenome assembly and MAG recovery step is step 3.10. Host depleted reads are assembled de novo using MEGAHIT for single assembly or metaSPAdes for complex microbial communities for assembly purposes that are computationally efficient and suitable for studies that aim to characterise new uncultured species of microbes with putative GBA-relevant properties. Contigs are assembled and binned into MAGs using an ensemble of three assemblers (MetaBAT2 , CONCOCT and MaxBin2 and the best non-redundant set of bins is selected by DAS_Tool MAG quality is evaluated with CheckM2 and high quality MAGs are those with ≥90% completeness and ≤5% contamination according to MIMAG guidelines. GTDB-Tk is used to classify taxa and eggNOG-mapper to annotate functions based on eggNOG 5.0 database, including screening for gene families relevant to GBA: glutamate decarboxylase (gadA/gadB for GABA synthesis), butyryl-CoA transferase (but), tryptophan synthase (trpAB), and tyrosine decarboxylase (tdc).

## Statistical Analysis

The abundance differences of each clinical group compared to the other groups are analyzed using ANCOM-BC2, an explicit model and correction for compositional nature of metagenomic count data and total variation of microbial biomass in different samples, resulting in unbiased estimation of log fold-change with false discovery rate correction by Benjamini-Hochberg procedure. Multivariable analyses involving GBA-relevant confounders such as psychiatric diagnosis, drug exposure, dietary patterns, bowel transit time, and demographic variables use an implementation of the LASSO-regularised variable selection method for fitting GLMs to identify microbial features that are independently associated with GBA phenotypes of intere. Alpha diversity indexes (Shannon entropy, observed species, Faith's Phylogenetic Diversity) are compared between groups either by Kruskal-Wallis test with Dunn post hoc test, or by linear mixed models for longitudinal data; beta diversity (Bray-Curtis dissimilarity and Aitchison distance) is analysed by PERMANOVA with 999 permutations and with homogeneity of dispersions verified by PERMDISP2. Within the mixOmics framework, DIABLO is used to integrate metagenomic pathway data with metabolomics, host transcriptomics and clinical neuroimaging data, using sparse partial least squares discriminant analysis to identify correlated cross-omic signatures that are linked to GBA phenotypic outcomes.

**Table 1 : Integrated multi-omics platforms and representative bioinformatics tools employed for comprehensive analysis of microbial communities.**

| Omics Category | Tool Name | Function | Application | Key Features |
|---|---|---|---|---|
| Biomarker Profiling | Random Forest (ML-based) | Microbiota composition | Biomarker discovery & classification | Combines statistical significance with biological relevance |
| Metagenomics | QIIME 2 | Microbiota functional gene capacity | 16S rRNA & shotgun data analysis | Modular, reproducible workflows |
| Metabolomics | XCMS | Metabolic productivity | LC-MS data processing | Peak detection & alignment |
| Metatranscriptomics | HUMAnN3 | Microbial functional gene Expression | Functional activity profiling | Gene expression analysis |
| Metaproteomics | MaxQuant | Protein Expression | Protein identification & quantification | High-resolution MS data analysis |

## 16S rRNA Gene Sequencing

**Step1:** Collect the sample. Gut, skin, soil — where microbes reside

**Step 2:** Extract DNA Lyse cells to release all microbial DNA The 16S rRNA gene was amplified

**Step 3:** Involved amplifying the 16S rRNA gene. Copy a "barcode" gene that is present in in ALL bacteria — NOT in human cells The 16S gene Has 9 "variable" regions (V1–V9). Like a fingerprint for each species

**Step 4:** Sequence the DNA Read the A, T, G, C letters in the gene.

**Step 5:** Match to a database Compare sequences to other known sequences; find similarities and differences Please provide an identifier for your sequences, such as SILVA or Greengenes database.

**Step 6:** Bioinformatics analysis Cluster into OTUs/ASVs, and count the number of species Objective: Who are the people that inhabit this area? Which bacteria, how many and in what way do they enter the system? what proportion.

**Procedure**

1. **Collect** — swab, stool, soil or water sample that has microbes in it.
2. **Extract DNA** — the cells are broken open chemically, all of the DNA from each microbe is extracted simultaneously.
3. **Amplify (PCR)** – primers bind to the conserved flanking regions, scientists make millions of copies of only the 16S gene from each organism present.
4. **Sequence** – modern machines read the DNA letter by letter (Next-Generation Sequencing can read millions of letters at once).
5. **Match to database** — each sequence is compared to reference databases. If it is 97%+ identical to a recognized bacterium, then it is labelled in that way.
6. **Analyze** – sequences are grouped into OTUs (Operational Taxonomic Units) or ASVs (Amplicon Sequence Variants) which are essentially groups of organisms. They are counted and compared using software.
7. **Results**: Your picture of your microbial community is revealed: What bacteria are present, how abundant and how diverse?

**RESULT**

The thorough review of published literature revealed a consistent and reproducible change in the gut microbiome of Parkinson disease (PD) patients. In both cross-sectional and metagenomic studies, there was a marked decrease in the levels of short-chain fatty acid (SCFA)-producing bacteria, such as Faecalibacterium prausnitzii, Roseburia intestinalis, and members of the Lachnospiraceae and Ruminococcaceae families. Simultaneously, a greater number of Gram-negative bacteria, such as Enterobacteriaceae and Akkermansia muciniphila, was found to be pro-inflammatory, consistently. These microbial changes were highly correlated with functional changes, such as reduction in the production of short-chain fatty acids, increase in production of lipopolysaccharide (LPS), and impairment of intestinal barrier integrity. Increased levels of inflammatory markers like tumor necrosis factor alpha(TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and gut permeability markers (zonulin and fatty acid binding protein-2) were found in PD patients and were associated with the severity and duration of the disease. Dysbiosis of the gut microbiome was found to affect multiple pathways involved in the pathogenesis of PD through mechanistic insights. There is evidence that the vagus nerve plays a role in the propagation of misfolded α-synuclein from the gut to the brain. Further, a decreased production of neuroprotective metabolites such as butyrate and indole-3-propionic acid makes a contribution to neuroinflammation and dopaminergic neuronal loss. High-resolution functional validation was achieved using shotgun metagenomic studies which showed a downregulation of microbial pathways associated with SCFA biosynthesis and upregulation of pathways associated with inflammatory responses. Importantly, dysbiosis was found in prodromal conditions, such as idiopathic REM sleep behaviour disorder, indicating that dysbiosis is present prior to onset of motor symptoms. In general, the findings confirm the correlation between dysbiosis of the

microbiota and PD, and its involvement in the disruption of the intestinal barrier, systemic inflammatory response and neurodegenerative .

**Conclusion**

In summary, gut microbiome is an important and complex player in the pathogenesis of PD via the gut-brain axis. The evidence that microbial dysbiosis is involved in the pathogenesis of α-synuclein pathology, neuroinflammation and metabolic disturbances is growing and all of these are implicated in dopaminergic neurodegeneration. Consistent microbial signatures and functional alterations in PD offer a solid basis for the development of novel diagnostic biomarkers, especially in early and/or prodromal stages of PD. The use of metagenomic technologies has also enabled a greater understanding of the function of the microbiota, including a high-resolution view, further supporting the mechanistic understanding of the gut-brain axis. These progressions notwithstanding, there are still some problems to solve such as variations across studies, confounding factors and establishing causality. In the future, further studies are needed to establish a time course and to develop standard methods and integrative multi-omics analysis to understand the association of dysbiosis and disease onset. Other novel therapies for PDs that have recently gained momentum are known as microbiomics therapy, such as psychobiotics and fecal microbiota transplant. They will need strong clinical trials to be translated into clinical practice and more knowledge about host-microbiome interactions. In sum, the study of the microbiome in the gut provides a novel and promising opportunity to understand the physiology, diagnosis and treatment of Parkinson disease from a new perspective, as a multi-organ disorder rather than a purely neurological one.

**DISCUSSION**

In this thesis , discuss the increasing evidence that the gut-brain axis is a key factor in the pathogenesis of Parkinson's disease. PD is regarded as a disorder primarily affecting the substantia nigra, but is now recognized as a systemic disorder with early pathological changes in the gastrointestinal tract. The “body-first” theory is a powerful model that suggests peripheral α-synuclein aggregation is responsible for central neurodegeneration through the vagus nerve. The changes in gut microbiome in PD patients are one of the most consistent results that have been observed in various studies. Decreases in the abundance of SCFA-producing bacteria, like Faecalibacterium prausnitzii and Roseburia intestinalis, indicate loss of anti-inflammatory and neuroprotective metabolites, specifically butyrate. This is significant for intestinal barrier integrity and immune regulation. However, the rise in pro-inflammatory bacteria (Enterobacteriaceae) supports the role of microbial induced inflammation in PD progression. The mechanisms are several that are interconnected. First, the loss of the normal microbial balance can lead to misfolding of α-synuclein in gut neurons by molecular mimicry or by bacterial amyloids. Second, translocated bacterial endotoxins like lipopolysaccharide (LPS) cause systemic inflammation and activation of microglial cells in the brain. Third, microbial metabolism disruption, in particular, SCFAs and tryptophan-derived metabolites, has an impact on neurotransmission, neuroinflammation and neuronal survival. The role of the kynurenine pathway is particularly interesting because an increase in its neurotoxic metabolites such as quinolinic acid could be responsible for excitotoxicity and

loss of dopaminergic neurons. Likewise, serotonin production and signaling changes account for some of the early non-motor symptoms (depression and constipation). New technologies have greatly improved the knowledge of the functionality changes in the microbial community, particularly the shotgun metagenomics approach. These methods allow pathway-level analysis, providing insights into changes in metabolic pathways, including pathways involved in SCFA biosynthesis, LPS production, and neurotransmitter pathways, which are not possible with 16S rRNA sequencing. But methodological variability, confounding factors (such as medication, diet, BMI), and the lack of standardization are significant challenges. Importantly, alteration of the microbiome has been seen in predromal cohorts (e.g. idiopathic REM sleep behaviour disorder) before clinical PD. This provides opportunities for early diagnosis and intervention. But the cause of dysbiosis remains uncertain, as it could be a cause of PD, or a result of disease progression is still being explored. In terms of therapeutic potential, microbiome-targeted approaches, including probiotics, prebiotics, and fecal microbiota transplantation (FMT), hold promise for regulating inflammation and neurochemical processes. However, there is still a lack of clinical proof, and problems like strain specificity, safety and long-term efficacy need to be resolved before the widespread use of this approach.