Parkinsonism

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Abstract

NOTE - this document is in draft currently. If I have the discipline, I will be making additions and edits for the next few months

Parkinsonism is a category of neurological diseases characterized primarily by motor symptoms such as bradykinesia, rigidity, and resting tremor. Specific diseases include Parkinson's Disease (PD), Lewy Body Dementia (LBD), Multiple System Atrophy (MSA), and Progressive Supranuclear Palsy (PSP). While these disorders share overlapping clinical features, their underlying pathologies are complex and heterogeneous. Common pathological features among many forms of idiopathic parkinsonism include misfolded proteins leading to toxic intracellular aggregates, dysfunctional mitochondria resulting in impaired energy production and oxidative stress, chronic central nervous system (CNS) inflammation, and impaired cellular machinery that normally maintains protein homeostasis. The diseases diverge based on the specific cell types affected, the protein that aggregates (e.g., alpha-synuclein in PD, LBD, and MSA; tau in PSP), and the regions of the brain impacted. These pathological changes are reflected in the cargo of exosomes—small extracellular vesicles released by affected cells—which may contain misfolded proteins, markers of mitochondrial dysfunction, inflammatory mediators, and molecular indicators of impaired cellular maintenance. Because exosomes can cross the blood-brain barrier and enter peripheral fluids, they represent a promising avenue for non-invasive biomarker discovery, potentially enabling earlier diagnosis and improved monitoring of disease progression in parkinsonian disorders.

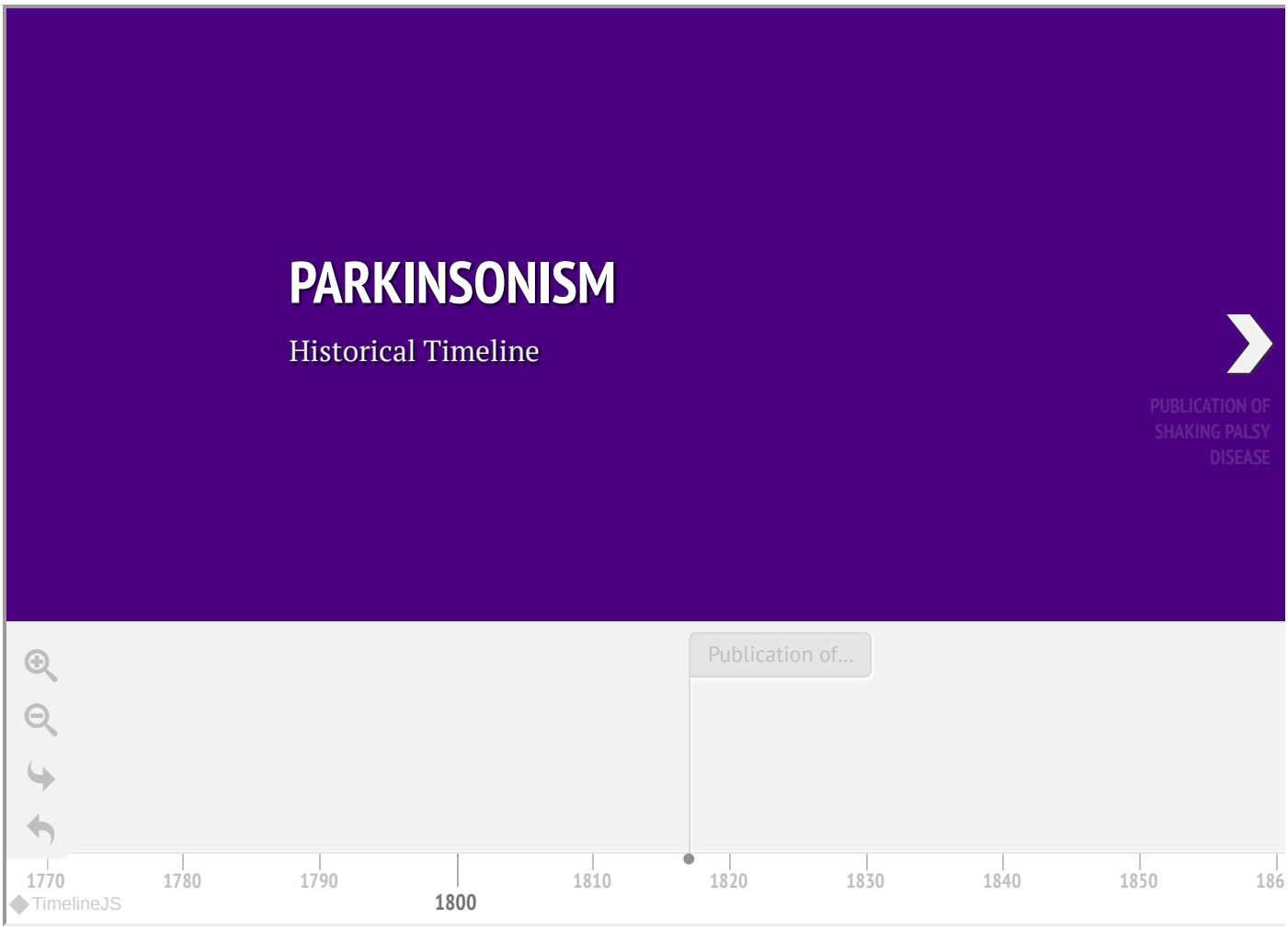
Introduction

The history of Parkinsonism began in 1817, when James Parkinson published An Essay on the Shaking Palsy, describing the characteristic motor symptoms of the condition that would later bear his name. Several decades later, Jean-Martin Charcot refined Parkinson's observations, distinguishing the disease from other movement disorders and formally naming it Maladie de Parkinson. Charcot also noted degeneration of the substantia nigra, an insight later validated as a central pathological feature. Around 1900, Dejerine and Thomas described a distinct degenerative disorder now recognized as Multiple System Atrophy (MSA). Although its pathology differs from Parkinson's Disease, MSA shares overlapping motor symptoms and is often confused with PD in clinical settings—a confusion made more understandable by the later discovery that both diseases involve toxic aggregates of misfolded alpha-synuclein. In 1912, Friedrich Lewy identified intraneuronal inclusions—later called Lewy bodies—marking the first visible pathological hallmark of PD, though their significance would remain unclear for decades.

The mid-20th century brought a major breakthrough when Arvid Carlsson demonstrated the essential role of the neurotransmitter dopamine in motor control and its abundance in the basal ganglia, laying the groundwork for understanding its depletion in Parkinson's Disease (PD). This discovery led to dopamine-replacement therapy, culminating in the widespread use—and eventual FDA approval in 1975—of carbidopa/levodopa, which remains the cornerstone of PD treatment and is also the mainstay therapy for Multiple System Atrophy (MSA), despite its typically limited and short-lived benefits. In the 1980s, an unexpected insight came when individuals exposed to heroin laced with the synthetic drug MPTP developed rapid-onset parkinsonism. The compound's inhibition of mitochondrial Complex I exposed the particular vulnerability of dopaminergic neurons to environmental toxins, marking a pivotal shift away from symptomatic treatments toward mechanistic studies focused on the cellular underpinnings of PD. Subsequent investigations uncovered key roles for mitochondrial dysfunction, oxidative stress, and impaired protein clearance. In the 1990s, mutations in the SNCA gene, which encodes the alpha-synuclein protein, confirmed it as a causative factor in familial PD, and

misfolded alpha-synuclein aggregates were identified as the major component of Lewy bodies, cementing the protein's central role in both inherited and sporadic forms of the disease.

Building on this mechanistic shift, Heiko Braak proposed in 2002 and 2003 that Parkinson's Disease (PD) may originate outside the brain, with misfolded alpha-synuclein initially appearing in the enteric nervous system or olfactory bulb and then spreading in a prion-like fashion to the central nervous system (CNS). Although originally developed to describe PD, similar peripheral origins have been explored for Multiple System Atrophy (MSA), particularly given its early autonomic symptoms—such as constipation, urinary dysfunction, and orthostatic hypotension—and the detection of alpha-synuclein pathology in the peripheral nervous system. More recently, Filip Scheperjans and others have expanded this line of inquiry by implicating the gut microbiota in triggering the chronic inflammation and immune dysregulation that may drive early alpha-synuclein misfolding. These findings have reframed Parkinson's research, and increasingly MSA research as well, shifting focus from late-stage neurodegeneration to the search for prodromal biomarkers and early therapeutic intervention.



All of these scientific developments—from Lewy's discovery to Braak's hypothesis and the rise of microbiome research—have expanded our understanding of Parkinsonism. Yet despite this progress, the clinical diagnosis of Parkinson's Disease (PD) and Multiple System Atrophy (MSA) remains largely reliant on neurologists' evaluation of motor symptoms—such as tremor, rigidity, balance, and coordination—which only manifest well after disease onset. Moreover, these symptoms overlap considerably between PD and MSA, making accurate differentiation difficult in early stages. While several promising biomarkers are under investigation, none are currently used as a front-line diagnostic tool. The closest example, a synuclein-positive skin biopsy, is typically reserved for

confirmatory use after clinical suspicion has already been established. This gap underscores the need for sensitive, disease-specific biomarkers capable of identifying Parkinsonism early—ideally in its prodromal phase—when therapeutic intervention may be most effective.

Peripheral Gateways to Pathogenesis

Peripheral structures like the intestinal lining and olfactory epithelium—first proposed as sites of disease initiation in Braak's staging framework—have emerged as potential gateways for the development of PD and MSA, offering early access points through which environmental triggers may influence disease onset and progression. Of these, the intestinal lining has received the most sustained attention, particularly as a site where breaches in the epithelial barrier may allow microbial products or toxins to interact directly with neurons, glia, and immune cells. Misfolded α-synuclein originating in this environment may then propagate to the central nervous system via the vagus nerve. While Braak's hypothesis initially emphasized anatomical spread, subsequent research has expanded its scope, implicating the gut microbiota and intestinal immune signaling in both initiating and sustaining the chronic inflammation that drives early protein misfolding. These findings have reframed the intestinal mucosa as a dynamic interface—one where microbial, immune, and neuronal signals converge to shape vulnerability or resilience.

Microbiome and the Intestinal Lining

In the context of dysbiosis, different microbial populations may drive distinct but convergent inflammatory pathways in Parkinsonism. In PD, dysbiosis is typically characterized within the gut by an overrepresentation of gram-negative bacteria, whose outer membranes contain lipopolysaccharide (LPS)—a potent immune stimulator recognized by Toll-Like Receptor 4 (TLR4) on glial and immune cells. In contrast, MSA is more often associated with gut gram-positive dysbiosis, in which peptidoglycans (PGNs)—major components of the gram-positive cell wall—serve as the primary immunogenic signal. These peptidoglycans, particularly through fragments such as muramyl dipeptide (MDP), are detected by the intracellular pattern recognition receptor Nucleotide-binding Oligomerization Domain-containing protein 2 (NOD2), which is expressed on innate immune cells such as macrophages and dendritic cells. Although LPS and PGNs activate distinct receptors (TLR4 vs. NOD2), both initiate downstream pro-inflammatory signaling cascades, converging mechanistically through chronic inflammation—a common hallmark of most neurodegenerative disease progression, including PD and MSA.

ADD TABLE OF DYSBIOSIS FOR BOTH PD AND MSA

This distinction underscores a fundamental difference in how dysbiosis may contribute to disease pathogenesis. While gram-negative bacterial products like LPS typically require a leaky gut to access host immune receptors at the cell surface, gram-positive bacteria may bypass this barrier by releasing extracellular vesicles (EVs). These EVs can be endocytosed by immune cells, delivering immunogenic peptidoglycan fragments such as muramyl dipeptide (MDP) directly into the cytosol, where they activate NOD2 and initiate inflammatory signaling.

The intestinal lining forms a complex, multilayered barrier that regulates the interface between the gut microbiota and host tissues. At its outermost surface—and directly adjacent to the gut microbiome—lies a single layer of epithelial cells joined by tight junction proteins, which together constitute the first physical and immunological barrier to microbial intrusion. In a healthy gut, this epithelial layer prevents bacterial products like LPS from entering deeper tissue compartments; however, MDP may bypass this barrier when delivered intracellularly via bacterial extracellular vesicles (EVs). However, when this barrier is compromised—a condition often referred to as "leaky gut"—microbial fragments from both gram-negative and gram-positive bacteria can cross the epithelium and access the

underlying lamina propria. This connective tissue layer hosts a rich network of immune cells, including macrophages and dendritic cells, as well as enteric glial cells that support local neuronal populations. It is within the lamina propria that much of the innate immune response is initiated, particularly through pattern recognition receptors like TLR4—previously activated by LPS—and NOD2, which detects bacterial peptidoglycans. As such, the integrity of the intestinal epithelium plays a central role in determining whether microbial signals are safely contained or become the spark for a sustained inflammatory response.

Enteric neurons reside within the gut wall in close proximity to immune and glial cells, forming a dense network governing gastrointestinal motility, secretion, and immune surveillance. Unlike dopaminergic neurons in the peripheral or central nervous systems, dopaminergic neurons in the enteric nervous system are unmyelinated, leaving the axonal membrane continuously exposed to the extracellular environment and rendering them more vulnerable to inflammatory damage. Their anatomical position —embedded within the lamina propria and submucosal plexus—places them in direct communication with both the gut epithelium and resident immune cells. This intimate spatial relationship makes enteric neurons among the first neuronal populations exposed to microbial products and other toxins. Although unmyelination is a common feature of enteric neurons, the selective vulnerability of dopaminergic populations likely reflects additional factors—such as their metabolic burden and reliance on α-synuclein—a topic explored further in a later section. This combination of environmental exposure and structural vulnerability positions enteric neurons as early targets in the pathogenesis of synucleinopathies.

Enteric glial cells (EGCs) are multifunctional support cells that help regulate gut neuroimmune interactions. In a healthy gut, these cells help maintain neuronal function, regulate synaptic activity, and support epithelial barrier integrity. However, enteric glia, akin to immune cells, also have innate immune capabilities: they use pattern recognition receptors, including members of the Toll-Like Receptor family, to detect microbial products such as LPS and respond by releasing pro-inflammatory cytokines and reactive oxygen species. Under conditions of chronic exposure to microbial stressors or persistent dysbiosis, these cells can adopt an active state that amplifies local inflammation and contributes to neuronal dysfunction. As such, EGCs sit at the intersection of microbial sensing, immune signaling, and neuronal support—poised to either protect or endanger dopaminergic neurons depending on the context of gut health. Together, these properties enable enteric glial cells to mediate early immune-neuronal interactions that contribute to the onset and progression of PD and MSA.

In addition to classical immune receptors like TLR4 and NOD2, emerging research highlights the role of neurotransmitter receptors—expressed on neurons, glial cells, and immune cells—in shaping the intensity and persistence of inflammation. Among these, the serotonin receptor 5-HT2A is of particular interest. It is expressed widely in the gastrointestinal tract, including on enteric glia and mucosal immune cells, and has been shown in some models to amplify cytokine production in response to microbial stressors. Other modulatory receptors, such as α7 nicotinic acetylcholine and purinergic receptors, may also contribute to this immunological tuning. This convergence of neurotransmission, microbial sensing, and immune regulation creates a self-reinforcing environment in which early signals from dysbiosis may be prolonged through receptor-mediated inflammatory feedback loops. This receptor-mediated feedback may represent a key mechanism by which inflammation persists beyond the initial microbial trigger.

Tryptophan metabolism provides an additional regulatory axis linking microbial imbalance, immune activation, and neurotransmitter availability. Under normal conditions, tryptophan serves as a precursor for serotonin synthesis in the gut. However, during systemic inflammation, homeostasis is perturbed, further biasing tryptophan metabolism toward the kynurenine pathway. This shift yields neuroactive metabolites that can modulate glial cell behavior, increase oxidative stress, and potentially promote alpha-synuclein misfolding. The loss of serotonin, combined with the rise of kynurenine-derived compounds, may further dysregulate the enteric environment, compounding neuronal vulnerability and inflammatory persistence. By altering the availability and metabolic fate of

tryptophan, chronic inflammation not only reshapes neurotransmitter signaling but also deepens the pathological loop central to PD and MSA.

ADD GRAPHIC OF TRYPTOPHAN PATHWAY

While dysbiosis promotes inflammation through LPS, PGNs, and microbial vesicles, a healthy gut microbiota performs critical anti-inflammatory functions. One major class of protective metabolites is the short-chain fatty acids (SCFAs), particularly butyrate, propionate, and acetate, which are produced through the fermentation of dietary fiber by gram-positive anaerobes. SCFAs enhance epithelial barrier integrity, support tight junction formation, and promote the development of regulatory T cells (Tregs) that help suppress immune overactivation. Butyrate, in particular, has been shown to inhibit pro-inflammatory cytokine production. In the context of PD and MSA, a loss of SCFA-producing bacteria has been observed, resulting in diminished immune tolerance, impaired barrier maintenance, and a permissive environment for sustained inflammation. This suggests that the shift in microbiota composition not only introduces pro-inflammatory signals but also removes essential counter-regulatory mechanisms.

Although this section focuses on the gut microbiome, it is important to note that other microbial ecosystems within the digestive tract—particularly the oral microbiome—may also contribute to systemic and neurological inflammation. Recent studies have identified associations between oral dysbiosis, periodontal disease, and neurodegenerative conditions such as Parkinson's Disease and Alzheimer's Disease. However, microbial patterns in the oral cavity differ substantially from those in the gut. For example, while PD-associated dysbiosis in the colon often involves an overrepresentation of gram-negative bacteria and loss of SCFA-producing gram-positive species, these trends may not hold true in the oral environment. The oral microbiome is shaped by distinct environmental factors, immune interfaces, and microbial dynamics—including biofilm formation and epithelial invasion—making it a separate, though potentially convergent, source of inflammatory stimuli. For the purposes of this section, our discussion is restricted to the gut microbiome, with the oral biome reserved for future consideration or complementary investigation.

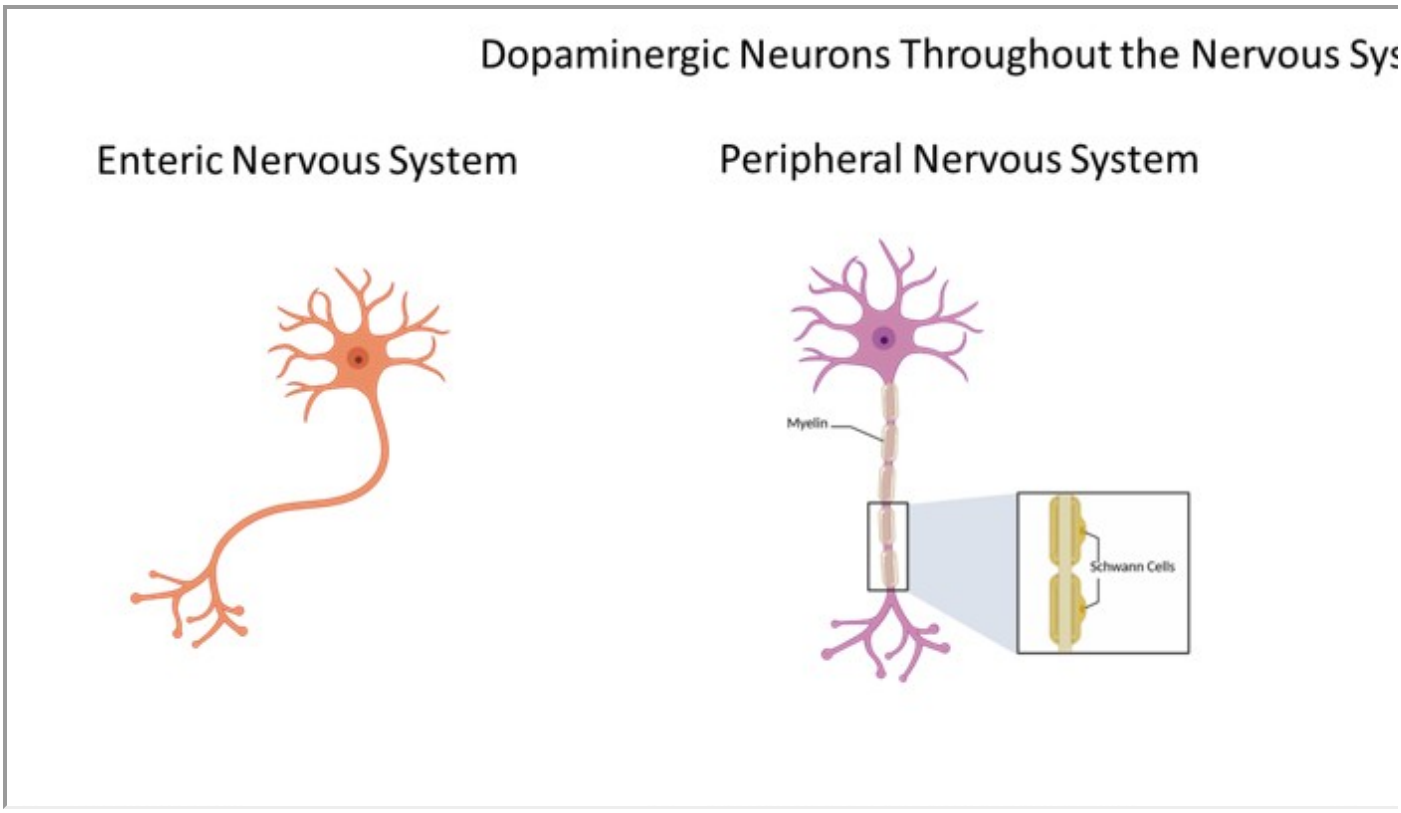
Early identification of chronic gut-derived inflammation—prior to the onset of overt motor symptoms—may present a critical window for therapeutic intervention. Measuring biomarkers associated with PD and MSA during this early prodromal stage opens the door to novel treatment strategies that address the disease before it becomes clinically entrenched. Central to this is the tipping point between neuroplasticity and neurodegeneration: as degeneration begins to outpace the brain's adaptive capacity, therapeutic windows narrow. A two-pronged strategy may help shift this balance. The first arm would target inflammatory potentiators such as the serotonin receptor 5-HT2A, using agonists in the R-DOI family or compounds with psychotropic or psychedelic properties to modulate receptor activity and attenuate cytokine amplification. The second would aim to restore metabolic balance, including interventions that reduce the accumulation of kynurenine-derived neurotoxic metabolites or promote serotonin availability. Enhancing neuroplasticity through lifestyle interventions—such as aerobic exercise, cognitive stimulation, and dietary modification—as well as emerging pharmacological therapies that promote neuronal resilience, may further strengthen the brain's ability to resist degeneration. Together, these approaches may delay or prevent the neurodegenerative cascade that characterizes later-stage PD and MSA, offering a rationale for developing early detection strategies that shift intervention further upstream in the disease course.

ADD TABLE NEURODEGENERATION STRATEGIES AND NEUROPLASTICITY STRATEGIES

Mounting evidence suggests that the pathogenesis of neurodegenerative diseases like Parkinson's Disease (PD) may begin in the Enteric Nervous System (ENS), progress through the autonomic branches of the Peripheral Nervous System (PNS), and ascend via a neural bridge—most notably, the

vagus nerve—into the Central Nervous System (CNS). While this ENS-to-CNS progression is well supported in PD, similar pathways are being explored in Multiple System Atrophy (MSA), especially in cases where early autonomic symptoms and peripheral alpha-synuclein pathology are evident. Understanding the structural anatomy of these three interconnected systems is therefore critical to realizing how synucleinopathies may develop and spread through the body. A key to this understanding lies in comparing dopaminergic neurons as they appear in each of these environments: the Enteric Nervous System (ENS), the Peripheral Nervous System (PNS), and the Central Nervous System (CNS). These comparisons not only illustrate structural distinctions but also highlight how each system is uniquely affected in diseases like PD and MSA.

To visually anchor this comparison, we present a labeled illustration of representative neurons from each system. This drawing forms the centerpiece of this section and includes bullet points beneath each neuron to highlight their key structural and functional characteristics. These visual summaries also reinforce each system's relevance to synucleinopathies like PD and MSA.



Target Biomarkers

In this study, we focus on three classes of biomarkers: exosomes, alpha-synuclein (α-syn), and transfer RNA fragments (tRFs). Each offers a distinct advantage in the early detection and stratification of Parkinsonism, including Parkinson's Disease (PD) and Multiple System Atrophy (MSA).

Exosomes are small extracellular vesicles that encapsulate molecular cargo reflective of their parent cells' physiological and pathological states. While blood-based biomarker detection has shown promise, systemic noise from abundant plasma proteins and fragmented RNAs often masks subtle early signals—especially in neurodegenerative disease. Exosomes, by contrast, offer a means to selectively enrich disease-relevant cargo from the affected cell population, potentially improving signal-to-noise ratios. Although definitive clinical validation is still underway, preliminary studies suggest that targeted isolation of exosomes—particularly those derived from neurons or oligodendrocytes—may enhance early detection sensitivity in synucleinopathies such as PD and MSA. As we plan to isolate and characterize exosomes by parent cell type, we propose leveraging their protein and RNA cargo as a core source of biomarkers for early and differential diagnosis.

Alpha-synuclein (α-syn) is a central pathological hallmark of all synucleinopathies. While early oligomeric or post-translationally modified forms of α-syn may serve as disease-specific markers, we view α-syn primarily as a downstream convergence point that reflects prior upstream assaults—whether inflammatory, toxic, or genetic. As such, α-syn is best interpreted within the context of active or late-stage pathology, complementing upstream indicators rather than replacing them.

tRFs offer a window into upstream disease mechanisms. These small RNA fragments, derived from the enzymatic processing of specific tRNAs, display disease-specific expression patterns influenced by mode of onset—idiopathic, environmental, or hereditary. While still an emerging field, we anticipate that tRFs may carry contextual signatures of the disease's point of origin, potentially reflecting whether early immune and metabolic stress began in the gut, nasal mucosa, bloodstream, or elsewhere. Though this mapping remains speculative, we consider it a worthwhile direction for research, with the potential to inform future efforts toward personalized therapeutic interventions targeting early—and potentially disparate—proinflammatory pathways.

Given the complexity and heterogeneity of Parkinsonism pathogenesis, we propose that tRFs be integrated alongside exosome profiling and α-synuclein characterization, forming a composite biomarker strategy that leverages the strengths of each while mitigating individual limitations. This multi-dimensional approach increases diagnostic robustness and enhances the potential to both detect disease earlier and tailor interventions to individualized etiological profiles.

Exosomes

Exosomes are a specific subtype of extracellular vesicles (EVs), distinct from microvesicles and apoptotic bodies, and are defined by their endosomal origin. They form as intraluminal vesicles within multivesicular bodies (MVBs) and are released into the extracellular space via exocytosis. This mode of biogenesis ensures that their cargo—proteins, lipids, and RNAs—reflects the internal physiological and pathological state of the parent cell. In contrast, microvesicles bud directly from the plasma membrane and may carry more surface-shed material, while apoptotic bodies arise from fragmented dying cells and contain mixed cellular contents.[1] Because of their intracellular origin and selective packaging, exosomes offer a particularly rich source of early, disease-relevant molecular signals, especially in disorders like Parkinson's Disease (PD) and Multiple System Atrophy (MSA).

These synucleinopathies are characterized by long prodromal phases during which non-motor symptoms may precede diagnosis by years. Since exosomes can be isolated from peripheral fluids such as blood or saliva, they offer a minimally invasive route to molecular screening that could detect disease activity well before clinical presentation.[2]

Another defining strength of exosomes is their ability to cross the blood-brain barrier (BBB) bidirectionally, allowing molecular signatures of central nervous system (CNS) pathology to be captured from peripheral samples.[3] In neurodegenerative conditions, however, a compromised BBB may also allow exosomes bearing toxic protein aggregates (e.g., oligomeric α-synuclein), metabolic stress markers, and pro-inflammatory molecules to circulate systemically.[4] These vesicles may contribute to disease progression by amplifying inflammation, spreading misfolded proteins, and disrupting neuronal homeostasis—making exosomes both diagnostic tools and potential pathogenic agents.

Current Workflow for Exosome Isolation and Characterization

Despite their diagnostic promise, current methods for isolating and analyzing exosomes are technically complex and confined to research laboratories. The typical workflow includes:

1. Removal of cellular debris: Red and white blood cells are separated via low-speed centrifugation or filtration.
2. Depletion of plasma proteins: High-abundance proteins such as albumin and immunoglobulins are removed to reduce background noise.
3. Size-based separation: Differential ultracentrifugation or gradient density centrifugation isolates vesicles in the 30–150 nm range characteristic of exosomes.
4. Immunoaffinity capture: Exosomes are sorted based on surface protein markers that reflect their parent cell identity: * L1CAM (CD171) and NCAM for neuron-derived exosomes, including dopaminergic neurons[5] * CNPase and MOG for oligodendrocyte-derived exosomes[6]
5. Lysis and molecular analysis: Once isolated, exosomes are lysed to release their internal contents (e.g., proteins, miRNAs, tRFs), which are then analyzed using methods that are adaptable to Point-of-Care settings, including emerging platforms for rapid detection of nucleic acids and disease-specific proteins.

This multi-step workflow demands specialized equipment (e.g., ultracentrifuges, immunoprecipitation platforms) and highly skilled personnel, making it unsuitable for routine clinical application—particularly in Point-of-Care (POC) environments. Additionally, the labor-intensive nature of the process introduces inter-lab variability and limits the scalability of exosome-based diagnostics.

Current Limitations and the Path Forward

While the molecular resolution enabled by exosome analysis is powerful, its clinical utility remains constrained by its technical inaccessibility. Without a simplified, reproducible platform for clinical use, exosome-based early detection remains largely academic and inaccessible to most patients. This presents a critical opportunity for innovation in microfluidics and lab-on-a-chip platforms, which we explore in the following section.

Future Directions and Translational Implications

Despite significant advances in our mechanistic understanding of Parkinson's Disease (PD) and Multiple System Atrophy (MSA), clinical diagnosis remains fundamentally reactive—triggered by overt motor symptoms that arise well after disease onset. Even when PD and MSA are correctly identified, treatment remains largely symptomatic, with little to no impact on long-term progression. Yet early and accurate differentiation still carries meaningful value. For patients and families, it shapes prognosis, expectations, and long-term planning. For clinicians, it informs therapeutic priorities, including the decision to avoid prolonged levodopa trials in likely MSA cases. And for researchers, it allows cleaner stratification in clinical trials and accelerates the development of disease-specific interventions.

Looking ahead, early diagnostic clarity may also enable cross-disease therapeutic repurposing. Although MSA is rare and underrepresented in drug development pipelines, it shares key downstream pathologies with more common disorders. One such overlap is with Multiple Sclerosis (MS), where demyelination—though driven by autoimmune mechanisms—results in similar oligodendrocyte dysfunction and axonal compromise. If emerging MS therapies aimed at remyelination or glial support prove safe and broadly effective, they may offer therapeutic potential for MSA as well, particularly when initiated during its early, preclinical phase.

This principle echoes the trajectory of CAR-T cell therapy, which was first developed and approved for common hematologic malignancies like leukemia and lymphoma before being adapted to treat multiple myeloma. Although the diseases differ in etiology, they share enough downstream immunological architecture to allow therapeutic crossover. The same logic may apply in neurology:

therapies developed for more prevalent demyelinating diseases like MS may hold untapped potential in rare disorders such as MSA—and early diagnosis of MSA may enable timely exploration.

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1. Ordered list item
2. Ordered list item
    a. Sub-item
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Putting each sentence on its own line has numerous benefits with regard to editing and version control.

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two spaces at end of line.

Document organization

Document section headings:

Heading 1

Heading 2

Heading 3

Heading 4

Heading 5

Heading 6

A heading centered on its own printed page

Horizontal rule:

`Heading 1`'s are recommended to be reserved for the title of the manuscript.

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Citation tags (i.e. aliases) can be defined in their own paragraphs using Markdown's reference link syntax:

Referencing figures, tables, equations

Figure 1

Figure 2

Figure 3

Figure 4

Table 1

Equation 1

Equation 2

Quotes and code

> Quoted text

> Quoted block of text
>
> Two roads diverged in a wood, and I—
> I took the one less traveled by,
> And that has made all the difference.

Code `in the middle` of normal text, aka `inline code`.

Code block with Python syntax highlighting:

```
from manubot.cite.doi import expand_short_doi

def test_expand_short_doi():
    doi = expand_short_doi("10/c3bp")
    # a string too long to fit within page:
    assert doi == "10.25313/2524-2695-2018-3-vliyanie-enhansera-copia-i-
        insulyatora-gypsy-na-sintez-ernk-modifikatsii-hromatina-i-
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Figures

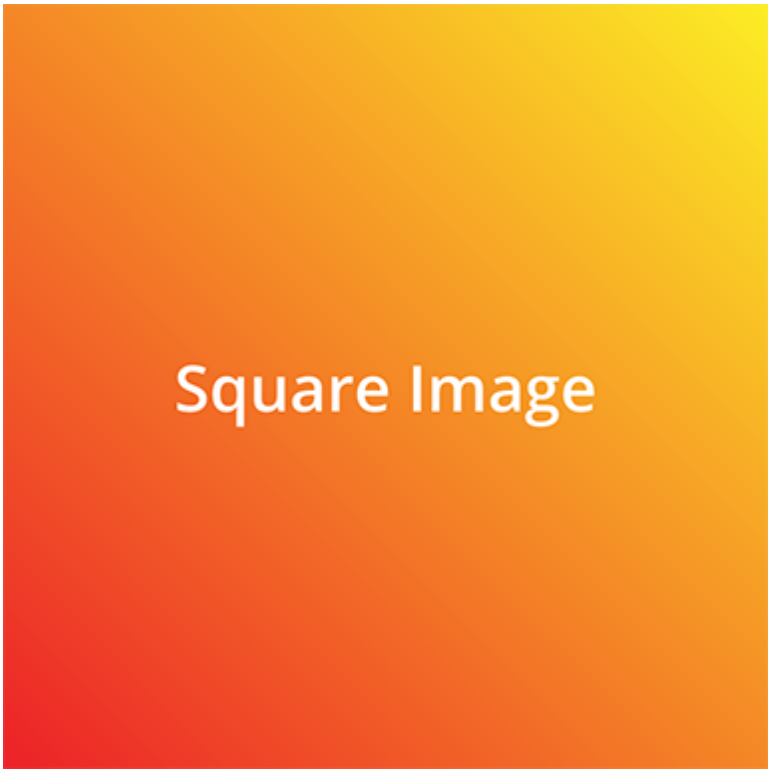

Figure 1: A square image at actual size and with a bottom caption. Loaded from the latest version of image on GitHub.

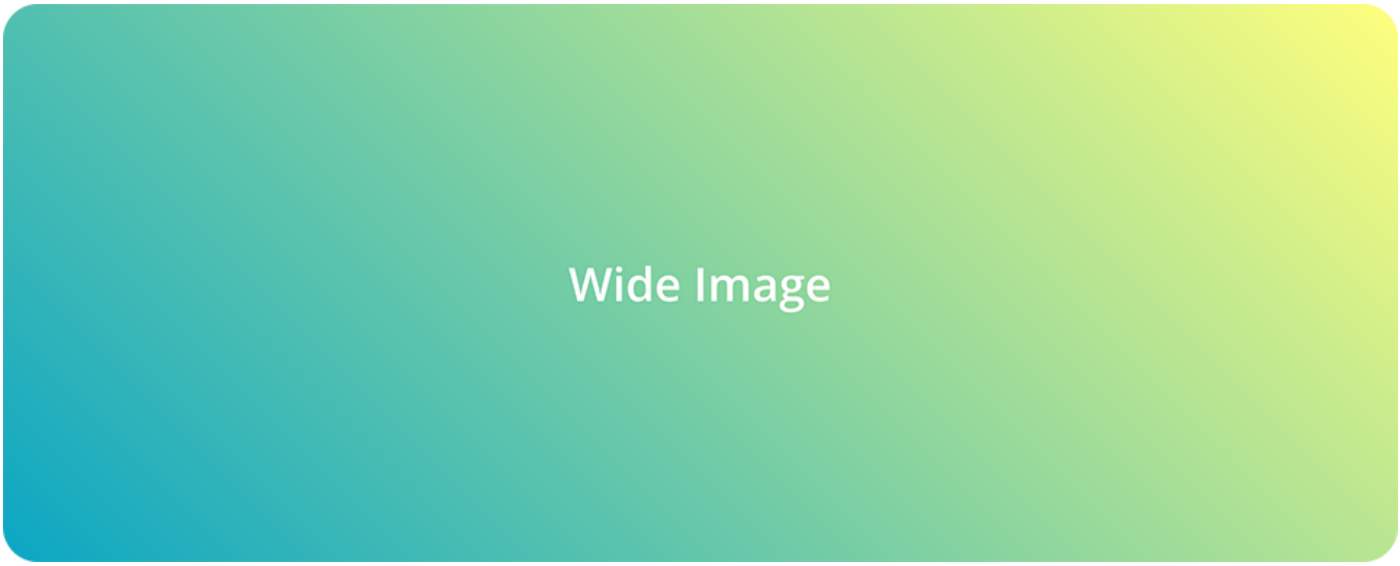

Figure 2: An image too wide to fit within page at full size. Loaded from a specific (hashed) version of the image on GitHub.

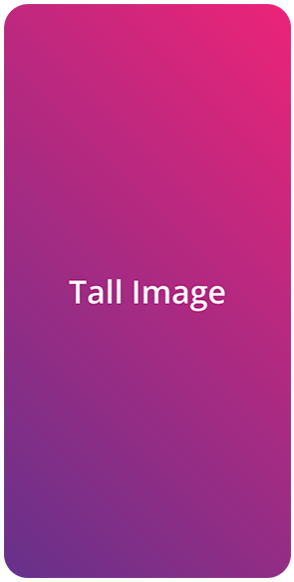

Figure 3: A tall image with a specified height. Loaded from a specific (hashed) version of the image on GitHub.

Figure 4: A vector `.svg` image loaded from GitHub. The parameter `sanitize=true` is necessary to properly load SVGs hosted via GitHub URLs. White background specified to serve as a backdrop for transparent sections of the image. Note that if you want to export to Word (`.docx`), you need to download the image and reference it locally (e.g. `content/images/vector.svg`) instead of using a URL.

Tables

Table 1: A table with a top caption and specified relative column widths.

Bowling Scores	**Jane**	**John**	**Alice**	**Bob**
Game 1	150	187	210	105
Game 2	98	202	197	102
Game 3	123	180	238	134

Table 2: A table too wide to fit within page.

	Digits 1-33	**Digits 34-66**	**Digits 67-99**	**Ref.**
pi	3.14159265358979323 846264338327950	28841971693993751 0582097494459230	78164062862089986 2803482534211706	`piday.org`
e	2.71828182845904523 536028747135266	24977572470936999 5957496696762772	40766303535475945 7138217852516642	`nasa.gov`

Table 3: A table with merged cells using the `attributes` plugin.

	Colors	
Size	**Text Color**	**Background Color**
big	blue	orange
small	black	white

Equations

A LaTeX equation:

$$\int_0^\infty e^{-x^2} dx = \frac{\sqrt{\pi}}{2} \tag{1}$$

An equation too long to fit within page:

$$\begin{aligned} x = a + b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t \\ + u + v + w + x + y + z + 1 + 2 + 3 + 4 + 5 + 6 + 7 + 8 + 9 \end{aligned} \tag{2}$$

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Adding arbitrary HTML attributes to an element using Pandoc's attribute syntax:

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Adding arbitrary HTML attributes to an element with the Manubot `attributes` plugin (more flexible than Pandoc's method in terms of which elements you can add attributes to):

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Available background colors for text, images, code, banners, etc:

`white` `lightgrey` `grey` `darkgrey` `black` `lightred` `lightyellow` `lightgreen` `lightblue` `lightpurple` `red` `orange` `yellow` `green` `blue` `purple`

Using the Font Awesome icon set:

✔ ? ★ 🔔 ⊗ ⋯

Light Grey Banner
useful for *general information* - manubot.org

Blue Banner
useful for *important information* - manubot.org

Light Red Banner
useful for *warnings* - manubot.org

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