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.

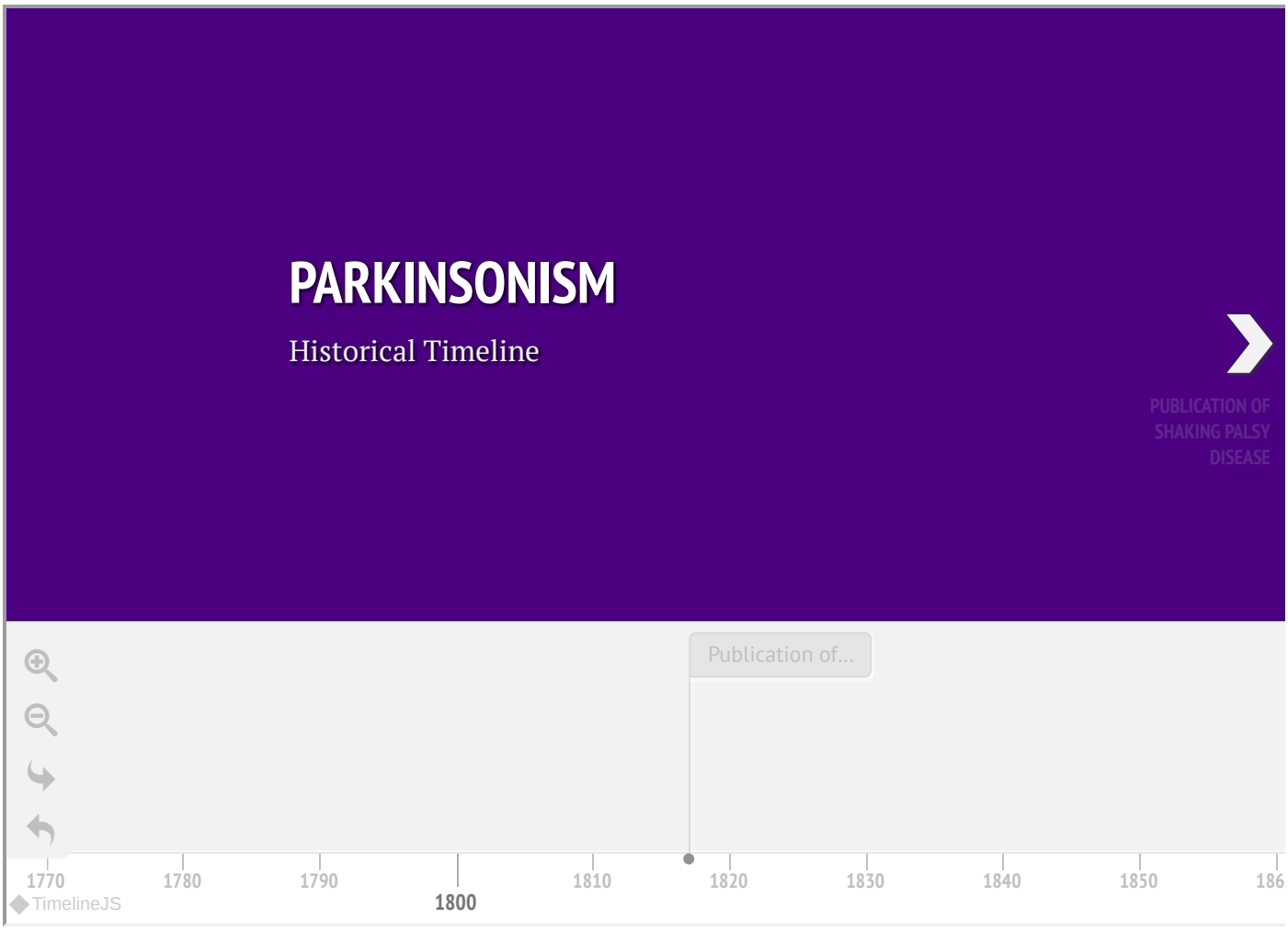
Introduction

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.

Gateways to Disease Onset

As proposed by Braak (see introduction), for many individuals, some of the earliest signs of Parkinson's disease (PD) and Multiple System Atrophy (MSA) may begin outside the brain—in parts of the body like the peripheral nervous system within the gut lining or the tissue inside the nose. Braak suggested that harmful changes might start in these peripheral areas and then gradually spread into the brain. Of the two, the gut has attracted the most sustained attention. When the gut's protective lining becomes "leaky," pathobiont bacteria may invade nearby nerve and immune cells, triggering inflammation. In this environment, the alpha-synuclein (α-synuclein) protein within the nerve can become damaged or misfolded. In PD, these misfolded proteins may travel along the vagus nerve in a prion-like manner, helping to spread the disease. In MSA, however, the misfolded protein may instead be packaged into extracellular vesicles and transferred from nerve cells to nearby glial cells, where the pathway continues toward the formation of aggregates known as glial cytoplasmic inclusions (GCIs).

While Braak's theory was a breakthrough, later research has shown that it's not the whole story. Not all cases of PD or MSA appear to begin in the peripheral nervous system. An estimated 51% of patients may experience the first disease-related changes in the central nervous system—specifically, in the brain [citation]. As such, current models are often described as "brain-first" or "body-first," reflecting the observation that PD and MSA are not one-size-fits-all diseases. Instead, they may follow different paths in different people, depending on genetics, prior medical conditions or systemic stressors, immune responses, environmental exposures, and even the makeup of the gut microbiome. Understanding where the disease begins—whether in the brain or the body—is key to catching it earlier and developing better treatments.

Gut Microbiome and the Intestinal Lining

The bacterial components exposed by dysbiosis—such as lipopolysaccharide (LPS) from gram-negative bacteria and peptidoglycans (PGNs) from gram-positive bacteria—are not ignored by the body. Instead, they are detected by specialized immune receptors that initiate inflammatory responses. In Parkinson's disease (PD), the overgrowth of gram-negative Proteobacteria increases exposure to LPS, which is recognized by Toll-Like Receptor 4 (TLR4) on the surface of immune and glial cells. Activation of TLR4 triggers a pro-inflammatory signaling cascade that can compromise the gut barrier and contribute to neuroinflammation.

In contrast, if MSA is associated with a gram-positive skew, the dominant immune trigger may be peptidoglycans—specifically a component called muramyl dipeptide (MDP). Rather than being detected on the cell surface, MDP is sensed inside the cell by an immune receptor called Nucleotide-binding Oligomerization Domain-containing protein 2 (NOD2), which is expressed in innate immune cells such as macrophages and dendritic cells. Like TLR4, NOD2 activation can lead to inflammation, but through a distinct intracellular pathway. Despite using different receptors, both LPS and PGN-driven signaling ultimately converge on chronic inflammation—a shared feature in the progression of both PD and MSA.

The immune system doesn't react the same way to all bacteria—it depends on what kind of bacterial material it encounters and how that material reaches immune cells. In Parkinson's disease (PD), the rise in gram-negative bacteria increases exposure to a molecule called lipopolysaccharide (LPS), which is part of the outer surface of these bacteria. LPS is highly inflammatory and is quickly detected by a surface receptor called TLR4, found on many immune and support cells, including those in the brain.

Once LPS enters the bloodstream—especially if the gut barrier is compromised—it can trigger a widespread, systemic immune response, including inflammation far from the gut.

In contrast, gram-positive bacteria—possibly more involved in Multiple System Atrophy (MSA)—release fragments of their thick cell wall, mainly made of peptidoglycan (PGN). These fragments are often carried within extracellular vesicles, small lipid-enclosed structures released by the bacteria. While traveling in these vesicles, PGN is relatively harmless. It becomes inflammatory only after the vesicle is absorbed by endocytosis into an immune cell, allowing PGN to enter the cell's interior. There, it is recognized by a different immune receptor called NOD2, which senses bacterial components inside the cell. Because this process requires internalization, the resulting immune response tends to be more localized, occurring in the tissues near where the bacterial fragments were released—such as the gut lining or surrounding nerve tissue.

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

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.

Alpha-Synuclein: A Key Protein in Parkinsonism Pathology

Alpha-synuclein (a-syn) is a small, intrinsically disordered protein involved in membrane stabilization, vesicle trafficking, and organelle communication. Under physiological conditions, it plays a central role in supporting mitochondria-associated membranes (MAMs)—transient contact sites between the endoplasmic reticulum (ER) and mitochondria that mediate lipid transfer, calcium signaling, and organelle coordination. Alpha-synuclein also helps regulate synaptic vesicle dynamics, particularly at presynaptic terminals.

The protein is predominantly expressed in neurons, especially those with high metabolic demands such as dopaminergic neurons in the substantia nigra. Under energetic stress, a-syn expression increases, reflecting its role in sustaining membrane interactions under conditions of elevated activity.

However, this conformationally flexible protein can misfold into rigid, β-sheet-rich structures that promote oligomerization, fibril formation, and ultimately the accumulation of insoluble aggregates. These aggregates present as Lewy Bodies (LBs) in Parkinson's Disease (PD) and Glial Cytoplasmic Inclusions (GCIs) in Multiple System Atrophy (MSA). Although both disorders involve pathological a-syn accumulation, they differ in cell type involvement, aggregate structure, and likely pathogenic mechanisms.

A key mechanistic question in MSA is how a neuron-enriched protein like a-syn forms aggregates in oligodendrocytes, which do not normally express it at high levels. Current evidence suggests that a-syn is transferred from neurons to glial cells via exosomes, endocytosis, or other forms of vesicle-mediated uptake. Once inside the oligodendrocyte, the protein misfolds and accumulates as GCIs, impairing glial functions including myelination and metabolic support of axons.

Protein misfolding is not inherently pathological—cells routinely produce misfolded proteins, and have evolved sophisticated mechanisms to manage them. These include molecular chaperones (such as heat shock proteins and chaperonins) that assist in proper folding, and degradation pathways like the ubiquitin-proteasome system and the autophagy-lysosome pathway, which eliminate irreversibly misfolded proteins. Under normal conditions, misfolded a-syn is rapidly cleared by these systems.

However, in neurodegenerative diseases like PD and MSA, this proteostasis network becomes compromised—either through chronic cellular stress, genetic predisposition, or an overproduction of misfolded a-syn. These conditions allow soluble oligomers to accumulate, evade quality control, and begin seeding further aggregation. These oligomeric intermediates, rather than the final aggregates,

are now believed to be the most toxic forms of a-syn, capable of disrupting membranes, damaging mitochondria, and triggering inflammatory cascades.

Importantly, the toxic mechanisms and cellular responses differ between neurons and oligodendrocytes due to their divergent metabolic strategies. In PD, a-syn oligomers directly impair the electron transport chain (ETC)—particularly Complex I—leading to mitochondrial depolarization and activation of the PINK1/Parkin mitophagy pathway. In contrast, MSA shows little to no upregulation of PINK1/Parkin, suggesting that mitochondrial dysfunction occurs upstream of the ETC, possibly due to disrupted MAM integrity or impaired lipid and calcium flux. Oligodendrocytes, which rely more on glycolytic intermediates for lipid production than on mitochondrial ATP generation, may be especially vulnerable to these early-stage disruptions.

Interestingly, this differential stress response highlights the potential of PINK1 and Parkin as disease-specific biomarkers—elevated in PD but not MSA. While such markers may eventually support targeted diagnostics or therapeutics, a broader and more dynamic molecular signature may lie in transfer RNA fragments (tRFs). These fragments, discussed in the next section, offer a promising window into mitochondrial stress states and disease subtype, potentially enabling personalized therapeutic strategies.

Diagnostic Insights from a-syn Aggregation

Although a-syn aggregates are central to pathology, their diagnostic utility lies in when and where they appear. Importantly, misfolded a-syn is not restricted to the brain—it also accumulates in the peripheral nervous system, including unmyelinated fibers in the skin. This has enabled development of the Syn-One Test, a minimally invasive skin biopsy used to detect phosphorylated a-syn aggregates. While currently deployed to confirm clinical diagnoses, studies suggest that aggregate location and ultrastructure differ between PD and MSA, raising the possibility of earlier and more refined diagnostic use.

At an earlier disease stage, seeding amplification assays such as Protein Misfolding Cyclic Amplification (PMCA) and Real-Time Quaking-Induced Conversion (RT-QuIC) have demonstrated the ability to detect misfolded a-syn by amplifying minute amounts from cerebrospinal fluid (CSF). These assays show strong sensitivity for preclinical diagnosis and offer mechanistic insights into aggregate propagation. However, they require lumbar puncture for CSF collection—an invasive procedure unsuitable for point-of-care (POC) use—despite the growing recognition that early, routine testing will be essential for meaningful intervention.

Emerging Tools for Early, Routine Detection of Synucleinopathies

While several groups are actively pursuing alpha-synuclein (a-syn)–based diagnostic strategies, two recent approaches deserve particular attention for their potential to transform detection from a specialist task into a routine, point-of-care procedure: the Soluble Oligomer Binding Assay (SOBA) and the CandyCollect saliva-based collection device.

SOBA is a blood-based assay designed to detect α-sheet-rich oligomeric forms of a-syn, believed to represent the most pathogenic and earliest detectable conformation in synucleinopathies. By capturing these species before fibrils or large aggregates form, SOBA aims to function as a true prodromal diagnostic tool—one capable of identifying disease before clinical symptoms emerge. Crucially, SOBA is compatible with POC diagnostic workflows, overcoming the accessibility and invasiveness limitations associated with CSF- or biopsy-based testing. Early studies suggest it may not only support early diagnosis, but also help distinguish between PD, MSA, and other synucleinopathies based on oligomer profile and conformational signature.

CandyCollect, meanwhile, introduces a novel collection format for an alternative biofluid: saliva. This lollipop-inspired, open-microfluidic sampling device was originally developed for infectious disease surveillance but has recently been adapted for capturing extracellular vesicles (EVs) from saliva. In a preclinical study, researchers demonstrated that EVs collected using CandyCollect could be processed for molecular characterization of a-syn species—offering a non-invasive, scalable option for early detection.

Together, these methods offer significant benefits:

- Non-invasive sampling (blood or saliva)
- POC compatibility, requiring no advanced clinical infrastructure
- Repeatability, enabling longitudinal monitoring of disease state or therapy response
- Feasibility for general practitioner use, paving the way for mass screening

While further clinical validation is needed, both SOBA and CandyCollect represent a meaningful departure from invasive, episodic testing. They point toward a future where screening for Parkinson's Disease and related disorders could be as routine as a complete blood count—a shift with profound implications for diagnosis, treatment timing, and patient outcomes.

An intriguing future direction lies in combining the strengths of these two approaches: applying assays like SOBA not to whole blood, but to extracellular vesicle (EV) fractions isolated from saliva or plasma. Because EVs are cell-derived and carry molecular cargo specific to their origin, they may provide a less noisy, more disease-relevant readout than unfractionated biofluids. Integrating EV isolation with conformation-specific assays could enable even earlier and more precise detection, potentially pushing the diagnostic window further upstream into the prodromal phase—before irreversible neurodegeneration begins.

Transfer RNA Fragments

Some text....

The Design of a Point-of-Care Diagnostic Device

Some text

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

`Heading 2`'s are recommended for broad sections such as *Abstract*, *Methods*, *Conclusion*, etc.

`Heading 3`'s and `Heading 4`'s are recommended for sub-sections.

Links

Bare URL link: https://manubot.org

Long link with lots of words and stuff and junk and bleep and blah and stuff and other stuff and more stuff yeah

Link with text

Link with hover text

Link by reference

Citations

Citation by DOI [1].

Citation by PubMed Central ID [2].

Citation by PubMed ID [3].

Citation by Wikidata ID [4].

Citation by ISBN [5].

Citation by URL [6].

Citation by alias [7].

Multiple citations can be put inside the same set of brackets [1,5,7]. Manubot plugins provide easier, more convenient visualization of and navigation between citations [2,3,7,8].

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-
        svyazyvanie-insulyatornyh-belkov-vtransfetsirovannyh-geneticheskih-
        konstruktsiyah"
```

Code block with no syntax highlighting:

```
Exporting HTML manuscript
Exporting DOCX manuscript
Exporting PDF manuscript
```

Figures

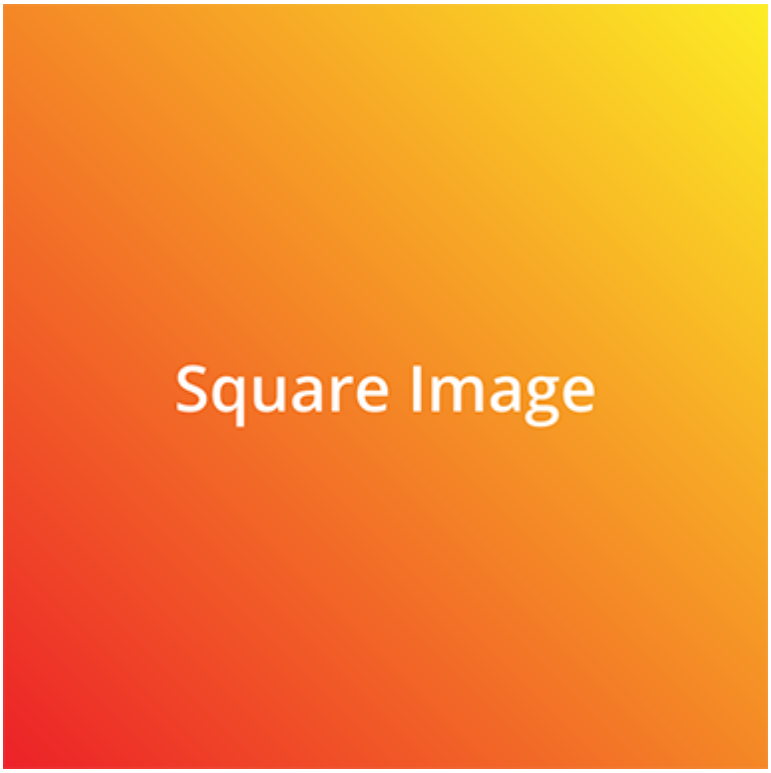

Figure 1: A square image at actual size and with a bottom caption. Loaded from the latest version of image on GitHub.

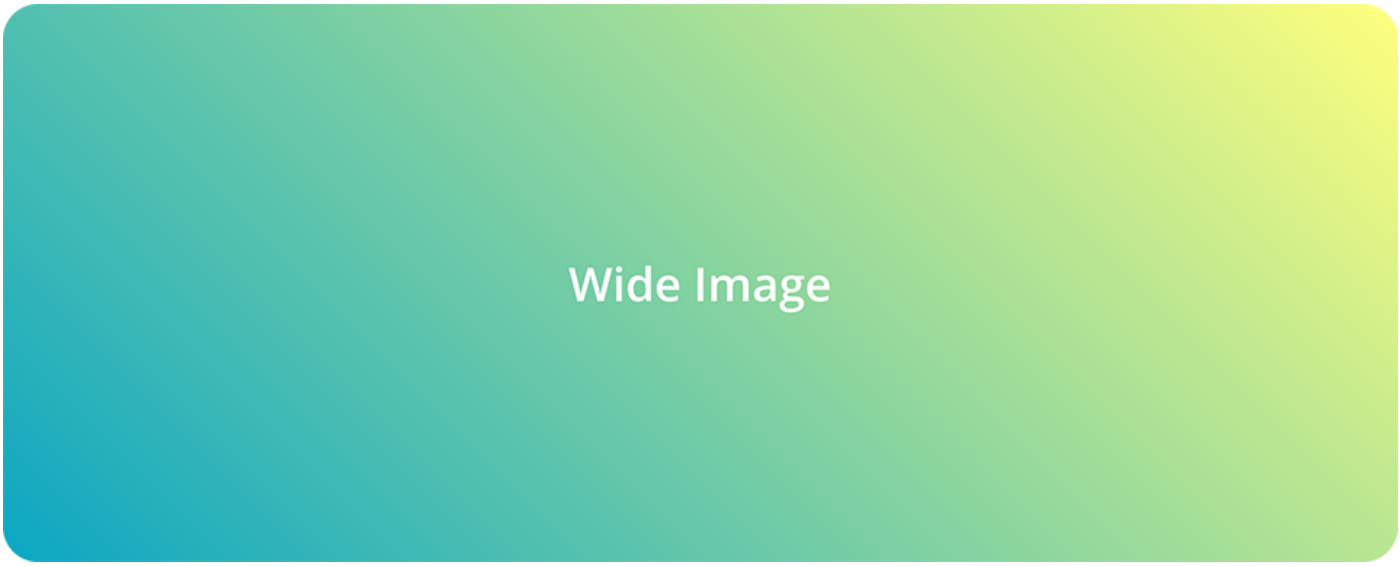

Figure 2: An image too wide to fit within page at full size. Loaded from a specific (hashed) version of the image on GitHub.

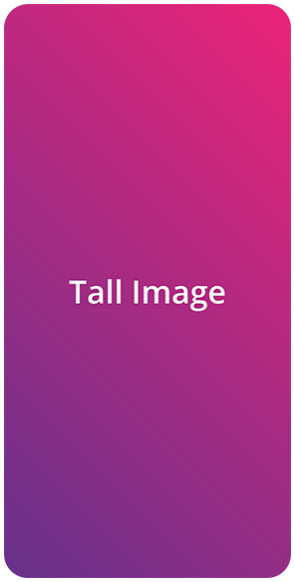

Figure 3: A tall image with a specified height. Loaded from a specific (hashed) version of the image on GitHub.

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:

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

Special

⚠ **WARNING** *The following features are only supported and intended for `.html` and `.pdf` exports. Journals are not likely to support them, and they may not display correctly when converted to other formats such as `.docx`.*

LINK STYLED AS A BUTTON

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

> Manubot Manubot Manubot Manubot Manubot. Manubot Manubot Manubot Manubot. Manubot Manubot Manubot. Manubot Manubot. Manubot.

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):

> Manubot Manubot Manubot Manubot Manubot. Manubot Manubot Manubot Manubot. Manubot Manubot Manubot. Manubot Manubot. Manubot.

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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