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Emerging targets for the diagnosis of Parkinson's disease: examination of systemic biomarkers.

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Abstract

Parkinson's disease (PD) is a highly prevalent and irreversible neurodegenerative disorder that is typically diagnosed in an advanced stage. Currently, there are no approved biomarkers that reliably identify PD patients before they have undergone extensive neuronal damage, eliminating the opportunity for future disease-modifying therapies to intervene in disease progression. This unmet need for diagnostic and therapeutic biomarkers has fueled PD research for decades, but these efforts have not yet yielded actionable results. Recently, studies exploring mechanisms underlying PD progression have offered insights into multisystemic contributions to pathology, challenging the classic perspective of PD as a disease isolated to the brain. This shift in understanding has opened the door to potential new biomarkers from multiple sites in the body. This review focuses on emerging candidates for PD biomarkers in the context of current diagnostic approaches and multiple organ systems that contribute to disease.

Keywords: Parkinson's disease, biomarkers, neurodegeneration, Lewy bodies, substantia nigra, neuroimaging, oxidative stress, mitophagy, reactive gliosis, dopamine, constipation, vagotomy, microbiome, fecal transplant, gut-brain axis autoimmune, neuroinflammation

Introduction

Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder in the United States, affecting 1-2% of the total population over 60 years of age [1]. PD patients typically suffer from debilitating motor and nonmotor symptoms, including bradykinesia, resting tremor, postural instability, difficulty speaking, depression, anxiety, dementia, and ultimately death [1, 2]. More recently, gastrointestinal (GI) symptoms such as constipation, xerostomia (dry mouth), dysphagia, and heartburn have gained recognition as additional hallmarks of PD [2].

On the molecular level, PD is characterized by the presence of Lewy bodies, comprised of aggregates of pathological alpha-synuclein (a-syn) protein, and the selective destruction of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc), a midbrain structure with a crucial role in regulating movement [1]. Because of substantial redundancy of DA circuits within the SNpc, patients may lose up to 70-90% of their DA neurons before clinical symptoms manifest [3]. Unfortunately, since there are no treatments that regenerate or prevent further loss of these neurons, PD patients are left without therapeutic options that halt or slow the progression of their disease. Current treatments, including L-DOPA, monoamine oxidase inhibitors, and catechol-O-methyltransferase inhibitors, among others, partially replenish depleted DA stores to mitigate symptoms and improve quality of life [4].

More than 20 gene mutations have been linked to a risk of PD including *SCNA1, LRRK2, PINK1,* and *Parkin*, among others [5]. However, screening for specific gene mutations associated with PD is not a sufficient predictor of disease. Familial PD is not always monogenic, and age of onset and penetrance of disease varies widely even among individuals with mutations in high risk genes [5]. Further, 85-90% of PD cases are idiopathic or caused by undiscovered mutations [1] and, consequently, undetectable using screening tools currently available. Therefore, developing biomarkers for PD would be a seminal breakthrough to identify individuals at high risk for developing PD prior to symptom onset and clinical diagnosis. To date, most research into PD biomarkers has focused on the changes in the central nervous system (CNS). However, novel insights into peripheral mechanisms contributing to PD have pushed systemic biomarkers to the forefront. Beyond providing new perspectives on PD pathology, peripheral biomarkers can be obtained from relatively accessible sources, making patient identification, disease monitoring, and tracking response to therapy more feasible clinical pursuits. This review will focus on current biomarkers for PD, and emerging systemic biomarker candidates to identify idiopathic PD and track disease progression.

CNS-based diagnostic tools for PD

PD is characterized by major neurological changes and subsequent motor dysfunction. As PD progresses, patients selectively lose nearly all their DA neurons in the SNpc [1, 5]. Reactive glia surrounding this region of cell death swell [6] and secrete factors that promote inflammation, neuronal death, and disruption of the blood-brain barrier (BBB) [7], a complex and tightly regulated vascular unit that insulates the CNS from the peripheral circulation. Pathological a-syn, the histological signature of PD, spreads from neuron to neuron throughout the midbrain and other regions in the brain, inducing neural damage and accelerating the vicious cycle of disease [8]. Given the substantial neurological damage and resulting clinical motor defects, the search for PD biomarkers has primarily focused on changes in the brain, and recent advances in research and clinical techniques have made CNS-based biomarkers some of the most reliable and promising prognostic and predictive indicators of disease. In that context, neuroimaging techniques currently are employed to identify patients at risk for developing PD, exclude secondary causes of Parkinsonism, and diagnose PD patients. Transcranial ultrasound (TCU) can reveal a hyperechoic SNpc in PD which, according to MDS criteria, characterizes patients with active or prodromal PD [9-11]. Also, several studies have demonstrated the capacity of SNpc hyperechogenecity to predict conversion to PD in prodromal patients and in patients previously diagnosed with essential tremor [12, 13]. Further, neuroimaging can be employed to distinguish PD from other conditions that cause Parkinsonism.

Magnetic resonance imaging (MRI) and nuclear medicine imaging techniques positron emission tomography (PET) and single-photon emission computerized tomography (SPECT) exclude secondary causes of Parkinsonism such as supranuclear palsy, multiple system atrophy, and toxin-induced Parkinsonism [6, 14]. Nuclear medicine imaging offers further insights into DA signaling by radiolabeling metabolites of DA or DA neurons. SPECT imaging of DA transporter (DAT) activity, a marker of DA neurons, is FDA-approved to distinguish PD from other causes of tremor, with a 95% sensitivity and 93% specificity [15]. In late 2019, PET visualization of L-3,4-dihydroxy-6-[18F]fluorophenylalanine (^{18}F-DOPA)-PET, which measures L-DOPA decarboxylase activity [16], was FDA-approved to diagnose PD and monitor disease progression (fda.gov 2019). Additionally, nuclear medicine has potential to identify prodromal PD patients. One of the largest cohort studies analyzing the diagnostic utility of DAT SPECT to identify individuals with a high risk for developing PD revealed that decreases in DAT signal preceded PD development and clinical diagnosis [17]. Recently, this finding was replicated in a study examining the diagnostic utility of ^{18}F-DOPA-PET to predict PD onset, demonstrating that individuals at high risk had decreased levels of ^{18}F-DOPA in the putamen [18].

PET imaging offers further insight into DA metabolism beyond L-DOPA decarboxylase activity. In the putamen, PET reveals a 70-80% decrease of vesicular monoamine transporter 2 (VMAT2) [19, 20] a vesicular protein found in DA neurons. PET also traces DA circuitry by labeling D1 and D2 receptors, which promote and inhibit movement respectively [21]. While D1 binding yields inconsistent results between PD patients and healthy controls [21], PET reveals increased levels of ^{11}C-raclopride, a D2-binding radioligand, in the PD striatum [22]. Furthermore, as PD patients take DA medication, ^{11}C-raclopride levels decrease [23], emphasizing the capacity of using PET imaging to monitor response to therapy. Finally, PET can assess neuroinflammation, a common characteristic of neurodegenerative diseases including PD [24]. As microglia become reactive and inflammatory cytokines are released in the brain, levels of translocator protein (TSPO) increase. Various radioligands that bind TSPO, such as [^{18}F]-radiolabelled phenoxyanilide, ([^{18}F]-FEPPA) have been developed and are currently utilized in PET scans to monitor levels of neuroinflammation [25]. Unfortunately, striatal [^{18}F]-FEPPA levels do not distinguish PD patients from healthy controls [26]. However, as neuroinflammation is both a hallmark and driver of PD pathology [24], monitoring inflammatory processes via PET scan may offer insight into disease progression and response to therapy.

Analyzing cerebrospinal fluid (CSF) is another promising area in CNS-based PD biomarkers research. Among the most widely studied molecular targets in the CSF is a-syn. Aggregated a-syn comprises Lewy bodies, the histological signature of PD found in patient brains, intestines, and other peripheral organs [27, 28]. The role of a-syn in cellular function remains undefined, though it is hypothesized to contribute, in part, to synaptic transmission [29]. A-syn is secreted into bodily fluids and extracellular compartments, making it accessible in blood, saliva, and CSF [30]. Normally present in an unphosphorylated, monomerized form, a-syn becomes pathological in PD when it is phosphorylated and oligomerizes [31]. Higher levels of pathological a-syn are found consistently in the CSF and erythrocytes in PD patients compared to healthy individuals [31-33]. Prominence in pathophysiology, accessibility in bodily fluid, and molecular differences between healthy controls and patients have made a-syn one of the most widely-studied molecules as a potential biomarker for PD.

Studying the aggregation capacity of pathological a-syn is a promising emerging area of research in PD biomarkers [8, 34]. Like the mechanism seen in prion diseases, pathological a-syn induces aggregation and Lewy body formation upon contact with neighboring a-syn, transforming previously healthy protein into neurotoxic clusters [34, 35]. As Lewy bodies are expelled from neurons by membrane-bound exosomes or exocytosis of unbound protein [36], they enter neighboring neurons and induce further a-syn aggregation, mitochondrial degradation, microtubule regressions, and DNA damage [37]. As this mechanism of clustering and spreading is unique to pathological a-syn, aggregation studies can distinguish between healthy and pathological a-syn. Indeed, a-syn extracted from exosomes in PD CSF, but not from control CSF, triggers oligomerization of healthy a-syn in cultured cells [38].

The aberrant effects that PD a-syn has on healthy protein underlie two CSF-based assays that quantify pathological properties of a-syn. Real-time quaking-induced conversion (RT-QuiC) measures the rate at which a-syn isolated from patients induces aggregation of neighboring a-syn. RT-QuiC can distinguish CSF from PD and other neurological disorders with a sensitivity of 92-95% and a specificity of 100%. Indeed, RT-QuiC correctly identified a small cohort of asymptomatic individuals at high risk for developing PD with 100% accuracy, demonstrating its potential to predict clinical diagnosis [39]. Thus, RT-QuiC may become a diagnostic assay that identifies individuals with pre-symptomatic PD. In addition, protein misfolding cyclic amplification (PCMA) quantifies pathological protein levels. In a blinded study, PCMA identified CSF from patients with synucleinopathies with a sensitivity of 88.5% and a specificity of 96.9% [40]. PCMA distinguished mild PD cases from healthy controls, and as motor function declined, the time to amplification decreased [40]. These results suggest that these assays may be useful to diagnostic and detect early disease, and also prognostic and track disease progression.

Beyond examining CSF levels of a-syn, DA and its metabolites are widely studied molecular targets in the search for a CSF-based PD biomarker. L-dihydroxyphenylacetic (L-DOPA), one of the most common medications prescribed to PD patients, is the precursor to DA, which is metabolized into 3,4-dihydroxyphenylacetic (DOPAC) and homovanillic acid (HVA). PD CSF has lower levels of both L-DOPA and DOPAC [41], and this reduction may occur before motor symptoms manifest. A small prospective cohort study revealed that pre-symptomatic PD patients have significantly decreased CSF levels of L-DOPA and DOPAC up to three years before diagnosis [18], suggesting the predictive capabilities of these analytes. The levels of DA metabolite HVA in the CSF of PD patients is highly variable [42]. However, the CSF ratio of xanthine, a purine associated with antioxidant function, to HVA consistently distinguishes PD patients from healthy controls, and continues to increase as pathology progresses [43]. Additionally, levels of HVA correlate with degree of motor impairment in PD [44], making it a possible predictive biomarker to track the efficacy of disease-modifying therapies.

Further studies have taken a broader approach to extracting biomarkers from PD patient CSF, assembling varied panels of proteins to study. However, these efforts have yielded mixed results. Out of 15 CNS proteins, Dos Santos et al. discovered that levels of a-syn, beta-amyloid (Aβ42), tau (p-Tau and t-Tau), protein deglycase (DJ-1), and S100β were significantly dysregulated in PD patients [45]. However, when replicated in a larger cohort, decreased Aβ42 levels was the only consistent finding [45]. Interestingly, decreased CSF Aβ42 levels in PD patients has been associated with gait impairment, cognitive decline, and early onset dementia [46-48]. However, low CSF Aβ42 levels are general indicators of cortical atrophy, which also occurs in Alzheimer's disease, mild cognitive impairment, and normal aging [49, 50]. Therefore, Aβ42 CSF levels may be useful for monitoring PD patients who are already diagnosed, but are not specific to PD.

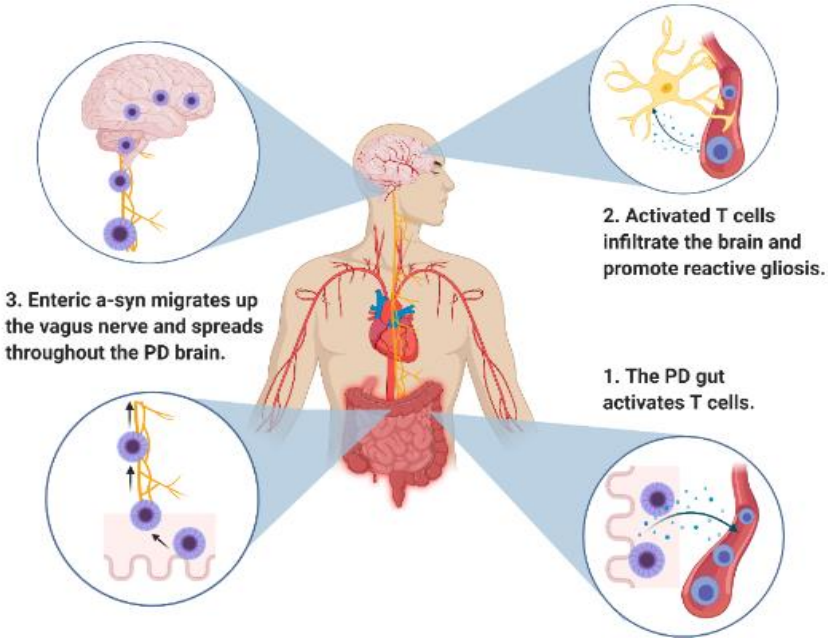

Figure A. The gut, immune system, and brain interact to promote PD.

1) The PD intestinal barrier is more permeable than in healthy individuals, allowing components of the gut to leak into the peripheral bloodstream. These antigens, normally insulated from peripheral circulation, activate immune cells, leading to chronic low-grade inflammation. This also may represent a point of exposure of autoreactive T cells to enteric a-syn, which is present in the PD gut and brain.

2) Activated immune cells, especially T cells, infiltrate the PD brain. Once in the CNS, inflammatory cytokines from T cells induce reactive gliosis in microglia and astrocytes. T cells and gliosis are present in higher levels in the PD brain, and have been demonstrated to promote neurodegeneration in mouse models of PD.

3) The gut has emerged as a potential site of PD onset. Indeed, the vagus nerve may be a route through which Lewy bodies, formed from enteric a-syn, migrate from the gut to the brain in PD pathogenesis. Once in the midbrain, Lewy bodies promote neurodegeneration. Most PD patients experience GI dysfunction prior to motor deficits, and vagotomized patients have half the rate of PD as intact individuals.

Molecular insights in pathophysiology offer novel peripheral biomarkers

Despite the benefits of studying changes in the CNS as PD progresses, focusing exclusively on the CNS has significant drawbacks. CFS sampling is invasive, which may limit the ability to monitor disease progression and response to therapy in PD patients. Furthermore, emerging research has revealed that peripheral changes in the gut and immune system may be major mediators or initiators of disease (Figure A). Thus, exclusively using a CNS-based biomarker may fail to identify patients in the earliest stages of PD development. Molecular signatures that offer windows into multiple regions affected by the disease could help clinicians identify at risk individuals (diagnostic biomarkers), better understand the origins of PD (mechanistic biomarkers), track progression of pathology (prognostic biomarkers), and assess responses to medications (predictive biomarkers). Here, we examine peripheral systems driving or affected by PD pathology and identify the most promising biomarkers outside of the CNS.

Non-invasive sampling: blood draws and skin scrapings

While PD blood-based biomarkers is still an emerging field of research, multiple investigators have demonstrated the promising potential of simple blood draws to help identify PD patients and monitor disease progression. As in the CSF, PD patients have significantly lower levels of L-DOPA and DOPAC circulating in the blood, and L-DOPA levels inversely correlate with PD patients' clinical scores of motor impairment [51]. PD erythrocytes also express higher levels of pathogical a-syn when compared to healthy controls [33]. Furthermore, plasma containing a-syn from PD patients, but not from healthy individuals, induces cell death and release of Lewy bodies from primary rat neuron cultures [52], demonstrating the presence of pathological a-syn in circulation.

Novel evidence suggests that PD may be, in part, an autoimmune disorder, deepening the potential insight that blood-based biomarkers may offer. PD patients share 17 genetic loci common to established autoimmune diseases, like Crohn's disease, multiple sclerosis, and rheumatoid arthritis [53]. CD4 and CD8 T cells are found in postmortem SNpc's of PD patients [54, 55] and MPTP mice [55] at higher levels than that of typical immune cell extravasation into the brain after death. Further, mice treated with MPTP but lacking CD4 T cells do not develop a PD-like pathology, while adoptive transfer of CD4 T cells back into these mice restores MPTP-induced neurodegeneration [55].

While the precise mechanisms through which the immune system mediates pathology in PD remain elusive, immunological signatures unique to the disease may identify blood-based PD biomarkers. In that context, sera from early-stage PD patients can be distinguished from sera from healthy individuals by differential expression

of MARK1, IL20, and CCL19 [56]. These genes function in neuronal integrity (MARK1) and systemic inflammation (IL20, CCL19), and may reflect the response to DA neuronal debris escaping through a leaky BBB and entering the peripheral circulation. PD patients also have fewer naive T cells and a higher proportion of activated T cells in circulation [57]. T cells from both PD patients and mice treated with MPTP express lower levels of DA receptor D3R [51, 58], although the specific role that this receptor plays in PD progression remains controversial [59, 60]. Further, peripheral T cells from PD patients, but not from healthy controls, recognize a-syn and produce the pro-inflammatory cytokines IL-5 and IFNγ in its presence [61], and pathological a-syn induces an inflammatory phenotype in immune cells [62]. Together, these observations may provide an opportunity to identify pre-symptomatic PD patients, offer insight into an autoimmune mechanism underlying pathology, and reveal a potential therapeutic target. As T cells gain increasing recognition as drivers of PD, the role of specific subsets in the pathophysiology of disease is being explored. PD patents have a higher percentage of Th17 T cells in the peripheral circulation, which induce midbrain neuronal cell death in an IL-17-dependent manner [54]. Further, IL-17A exacerbates MPTP-induced neurodegeneration and neuroinflammation in mice by driving microglial activation [63]. Similarly, RANTES, a chemokine that promotes Th17 T cell migration, has been identified in the SNpc of PD patients [64] and circulates in the blood at higher levels in PD patients as compared to healthy individuals [65].

Outside the immune system, blood-based exosomes have emerged as another major area of focus in PD biomarker research. Exosomes are membrane-bound nanosized particles formed from the invagination of late endosomes, and released into bodily fluid [66]. They therefore reflect intracellular changes that occur in their cell of origin during pathology. Exosomes isolated from PD patient plasma harbor several unique characteristics, such as increased a-syn [67, 68], increased DJ-1 [69], and abnormal expression levels of microRNAs associated with neuronal apoptosis [70]. PD plasma exosomes also contain lower levels of mitochondrial complexes ATP5A, NDUFS3, and SDHB, as compared to healthy donors [71]. This finding is particularly significant given the major role that mitochondrial dysfunction plays in PD pathology. Nearly all high-risk gene mutations for PD, including *PINK1*, *parkin*, *DJ-1*, and *LRRK2*, impair mitophagy of damaged mitochondria and target complexes in the electron transport chain [72]. Samples obtained from PD patients at autopsy frequently reveal a deficit in complex I of the respiratory pathway in mitochondria in both the SNpc and peripheral tissues [73], even in patients without gene mutations associated with PD. Mitochondrial dysfunction is so widely recognized as a source of PD that the most common mouse models of environmental PD, like MPTP, 6-OHDA, and rotenone, directly target complexes in the mitochondrial electron transport chain to induce pathology [74]. Therefore, the ability to use noninvasive methods to identify changes in mitochondrial function would represent a significant breakthrough in predicting PD pathology and monitoring disease progression. Recent advances in research have extended beyond measuring mitochondrial proteins in plasma-derived exosomes. In an exciting new study, fibroblasts were isolated from skin scrapings taken from PD patients, healthy controls, and preclinical patients with gene mutations that placed them at high risk for developing PD. The fibroblasts were stressed, which induces mitophagy in healthy cells to clear damaged mitochondria via a homeostatic process of organelle regeneration. While all the control fibroblasts lost their expression of Miro-1, a mitochondrial surface marker, 94% of PD patients retained their Miro-1 levels, indicating an inability to digest damaged mitochondria. Importantly, fibroblasts isolated from high risk individuals who did not yet have PD symptoms retained high levels of Miro-1 [75], revealing the potential for using Miro-1 expression in peripheral tissues as an accessible diagnostic biomarker to identify pre-symptomatic PD patients.

Olfactory mucosa and gut: sites of PD initiation

While the neurological impacts of PD have been most widely studied, there is a growing body of evidence that PD may begin in the gut and nasal mucosa, which offers novel targets for identifying PD patients prior to motor dysfunction [76]. 75-95% of PD patients present with olfactory impairment [76], which usually manifests prior to motor symptoms. In fact, one study found that 67% of individuals presenting with hyposmia combined with a DAT deficit developed PD with motor impairment within four years [77]. Beyond olfactory impairment, about

80% of PD patients report constipation [78], and a wide range of gastrointestinal (GI) symptoms including constipation, delayed gastric emptying, early satiety, xerostomia, and difficulty swallowing often manifest up to 20 years before motor impairment [79]. Further, Lewy bodies have been identified in colon biopsies from both preclinical and symptomatic PD patients in multiple studies [80, 81], although the utility of using a-syn in the GI tract as a specific marker for PD remains controversial [82].

The dual-hit theory of Parkinson's pathogenesis posits two prongs of sporadic disease initiation. The first arm of the theory states that PD pathogenesis begins in the nasal mucosa, enters the olfactory bulb, and migrates to the temporal lobe before spreading to other brain regions [83]. Indeed, phosphorylated a-syn inclusions have been identified in PD olfactory bulbs [84], and De Luca et. al. recently demonstrated that the aggregation assay RT-QuIC can distinguish PD patients from healthy individuals using a-syn isolated from the olfactory mucosa [85]. In conjunction with the olfactory mucosa-to-brain route of PD pathogenesis, the second arm of the dual-hit theory posits the Braak hypothesis, which states that sporadic PD begins when a pathogen enters the nasal cavity, is swallowed, and is trafficked to the gut. There, it initiates the formation of Lewy bodies, which then migrate up the vagus nerve and spread throughout the brain [86], causing neurodegeneration. Recently, this theory was bolstered by a study in which pre-formed synthetic a-syn fibrils (PFF) were injected into the gut of vagotomized and intact mice. In this model of PD, intact mice lose a significant number of DA neurons and develop motor impairment, while vagotomized mice are protected from DA neuron loss and motor dysfunction [34]. Similarly, PD rates in vagotomized patients are half that of individuals with intact vagus nerves [87].

Independently from its vagal connection with the brain, current research suggests that the gut also may initiate PD through interactions with the immune system. While the sequence of events remains controversial, novel studies reveal a relationship between gut inflammation, loss of intestinal barrier integrity, systemic immune cell activation, and propagation of PD pathology.

Proinflammatory markers including CXCL8, IL1α, and CRP are upregulated in the stool of PD patients [88]. Also, mRNA levels of proinflammatory cytokines, like IL-1β, TNFα, and IFNγ [89] are increased in colons of PD patients, and these levels mirror cytokine patterns in inflammatory bowel disease (IBD). In fact, a recent retrospective cohort study determined that IBD patients are 22% more likely to develop PD [90], possibly reflecting a role for the inflamed gut in PD progression. Levels of cytokine expression were independent of Lewy body density and inversely correlated with disease progression [89], suggesting that an inflamed gut may be an early or inciting event of PD. Further, pro-inflammatory cytokines, including but not limited to, TNFα, IL-1β, and IFNγ, downregulate tight junctions that seal the intestinal barrier, like claudins and occludins [91-93]. Indeed, PD guts are more permeable than those in age-matched controls, even in early disease [94, 95], and increased gut permeability has been correlated with higher levels of enteric a-syn [95].

Early loss of barrier integrity may contribute to PD, since peripherally circulating immune cells more easily infiltrate a permeable gut, where they release inflammatory cytokines that exacerbate a-syn misfolding and aggregation while further disrupting the intestinal barrier. When mice receive intraperitoneal (IP) injections of lipopolysaccharide (LPS), a bacterial toxin that induces systemic inflammation, their colons become more permeable and develop phosphorylated a-syn inclusions [96]. A more recent study revealed that inducing a-syn overexpressing mice with chronic, low-grade experimental colitis, was associated with DA neuron loss in the SNpc and motor impairment [97], suggesting a relationship between intestinal inflammation and the development of PD.

The microbiome

The microbiome has emerged as a key feature discriminating PD patients from healthy controls and may represent an important driver of disease [98]. While the precise demographics of bacterial populations have varied between cohorts, PD patients consistently have microbiome signatures distinct from healthy controls within the same country and consuming comparable diets [99, 100]. Further, PD patients have lower levels of the three most abundant short chain fatty acids (SCFAs), including butyrate, propionate, and acetate, which are

metabolic products of intestinal microbes [101]. While the clinical impact of this observation is not yet fully understood, analyzing microbial populations and their metabolites may yield promising results for developing PD biomarkers, especially when applied to patients presenting with hallmark GI symptoms.

Beyond their potential as PD biomarkers, gut bacteria may serve as therapeutic targets for future disease-modifying treatments. In a mouse model of PD that over-expresses a-syn (ASO), a normal microbiome is associated with high levels of pro-inflammatory cytokines in the gut, development of intestinal a-syn aggregates, and the onset of constipation. These effects are dampened by antibiotic administration, or in germ-free mice with depleted microbiomes [102]. Moreover, targeting the microbiome may ameliorate PD-like symptoms outside of the GI tract. Indeed, ASO mice with normal microbiomes develop more reactive microglia, higher levels of inflammatory cytokines in the brain, and more severe motor deficits. Like in the gut, these PD-like symptoms are mitigated by both antibiotics and germ-free environments. Further, when germ-free ASO mice receive microbiota purified from human PD colons, they develop striking motor deficits compared to microbiota from healthy controls [102]. The potential of the intestinal microbiota as a therapeutic target in PD can be appreciated by considering that the efficacy of fecal transplants as a treatment currently is being explored in clinical trials (ClinicalTrials.gov identifier NCT03808389).

A gut-brain axis in PD pathophysiology

The impact of changes in the gut on neurodegeneration in PD models underscores the need to better define the contribution of the gut-brain axis to disease pathophysiology. To this end, several groups are examining the role of endocrine loops that signal from the GI tract to the brain in PD. Ghrelin is a hormone released from the stomach and small intestine that binds to receptors in the hypothalamus to promote satiety. Perhaps surprisingly, ghrelin also has been implicated in the pathophysiology of PD. Indeed, PD patients have lower circulating levels of ghrelin and impaired ghrelin responses to food [103], suggesting a dysregulated pathway. Further, exogenous administration of acyl-ghrelin is neuroprotective in an MPTP mouse model of PD by inhibiting microglial activation [104]. Additional neuroprotective functions of ghrelin include promoting synaptic remodeling and action potential firing in DA neurons[105]. Ghrelin may exert its neuroprotective effects, in part, by stimulating the AMP-activated protein kinase (AMPK) pathway [106], which promotes mitochondrial health [107].

Uroguanylin, produced in the small intestine, is another gut hormone currently being examined for potential neuroprotective qualities. Uroguanylin signals to guanylyl cyclase C (GUCY2C), an intestinal receptor recognized for its role in maintaining epithelial cell mitochondrial health and suppressing tumorigenesis [108, 109]. Recently, two discrete circuits of GUCY2C were mapped in the brain, including in the SNpc where it is expressed on DA neurons [110, 111]. The potential role that intestinal uroguanylin - neuronal GUCY2C signaling plays in preserving mitochondrial function in the midbrain and protecting DA neurons from degeneration is currently under investigation. Ultimately, understanding the gut-brain endocrine axis may offer accessible GI hormones as future effective biomarkers and therapeutic targets for PD.

Summary

PD is a prevalent, debilitating disease with a complex etiology weaving together the brain, gut, and immune system. Despite the clinical need to identify patients who are at risk for PD and could benefit from future disease-modifying therapies, there are no biomarkers approved for clinical management of this disease. The recent expansion in focus from the brain to peripheral systems signals an optimistic advance in unraveling the mystery of disease progression while opening the door to novel and accessible biomarkers. Among the most promising candidates for biomarkers are those that are present and affected across multiple systems, including a-syn, mitochondrial markers, and inflammatory mediators. Therefore, further insight into the relationship between the brain, gut, and immune system in PD will be useful in better understanding pathology and identifying appropriate targets for future biomarkers.

Future perspectives

There are no biomarkers currently approved for PD patients that identify individuals at risk, monitor the trajectory of disease, or predict and register responses to therapies. This may be a result of PD having long been considered a purely neurological disorder, limiting the investigation of biomarkers to changes in the brain, which actually may appear only in late stages of pathology. As the interdependent relationships between the PD gut, immune system and brain become better defined, we look forward to a broadening search for biomarkers that are increasingly accessible and reflective of different stages in the complex progression of PD across multiple systems. In particular, the gut has emerged as a major driver of PD. Studies correlating constipation and IBD with future PD development have widened the criteria for individuals who may benefit from early PD screening. Further, understanding the role of the gut in PD pathology has opened the door to colonic biopsies, stool samples, and blood draws to detect changes in inflammatory mediators, the microbiome, and GI hormone levels that may predict disease development. Additionally, the relatively novel autoimmune component of PD has opened more exciting opportunities for predicting pathology. As PD patients share several genetic loci common to other autoimmune disorders, individuals with a history of autoimmunity may benefit from PD screening. Moreover, the role that autoimmunity may play in mediating disease makes blood draws and downstream analyses of autoreactive T cells and cytokine signatures specific to PD viable options for accessible biomarkers. The future landscape for PD biomarkers will likely include a combination of analyses specific to the brain (neuroimaging, a-syn levels, and DA precursors and metobilites in the CSF), as well as peripheral markers (circulating exosomes, immunogical signatures, changes in the olfactory mucosa, gut biopsies). As impaired mitophagy is a common mechanism underlying familial and idiopathic PD, we expect that measuring this pathway, along with disrupted antioxidant mechanisms in fibroblasts and exosomes will be a promising readout of risk to developing PD. We also anticipate that assays quantifying pathological a-syn aggregation from peripheral sources, such as olfactory mucosa and blood samples, will provide accurate insight into susceptibility to Lewy body formation and PD progression. As our understanding of PD pathology widens to include potential sites of onset and progression outside of the brain, we look forward to the development of holistic biomarkers that reflect early changes preceding neurodegeneration across multiple systems.

Executive summary

- PD is the second most common neurodegenerative disorder affecting the aging population.
- There are currently no biomarkers approved to predict and diagnose PD patients.
- Emerging evidence suggests that dysregulated gut and immune mechanisms may underlie or precede PD development.
- Understanding PD pathogenesis opens the door to more accessible biomarkers, including those found in blood and CSF.
- Combining biomarkers of gut and immune health with indicators of mitochondrial and a-syn function may provide a holistic view of risk of disease and PD progression across multiple systems.

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Papers of interest (*)

Papers of considerable interest (**)

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