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Review

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Advances in biomarkers for Parkinson's disease: from molecular pathology to precision diagnostics

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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder, and continues to present significant challenges in early diagnosis, precise subtyping, and prognosis assessment. In recent years, the field of biomarker research has undergone a profound paradigm shift from static concentration measurements to functional activity detection. The most revolutionary breakthrough is the α-synuclein seed amplification assay (α-Syn-SAA), which enables ultrasensitive and specific detection of pathological α-Syn in both clinical and prodromal stages, thus providing an unprecedented window for early intervention. Substantial progress has also been made in the development of biomarkers such as neurofilament light chain (NfL), Alzheimer's disease-related biomarkers, genetic biomarkers, and detection technologies based on peripheral samples. The integrated application of cutting-edge technologies, such as RT-QuIC, high-resolution mass spectrometry, and high-field magnetic resonance imaging (MRI), is advancing the field into a new stage characterized by a focus on pathological activity, multi-omics integration, and non- or minimally invasive approaches. In this review, we explore recent advances in PD biomarkers, focusing on core pathophysiological markers. We examine the potential of multi-omics and artificial intelligence (AI) to enhance diagnostic, subtyping, and prognostic accuracy, while also outlining the pivotal role and future directions of biomarkers in advancing PD precision medicine.

Key words: Parkinson's disease; Biomarkers; Alpha-synuclein; Seed amplification assays; Multi-omics; Precision medicine

1 Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease (AD), affecting over 4 million people globally (Su et al., 2025). Currently, the clinical diagnosis of PD relies mainly on motor symptoms, which often leads to diagnosis at moderate to advanced stages when substantial loss of substantia nigra dopaminergic neurons has already occurred, thereby missing the window of opportunity for early detection and enrolment in disease-prevention trials. Furthermore, PD exhibits significant clinical and pathological heterogeneity and is frequently misdiagnosed as essential tremor (ET), drug-induced parkinsonism, vascular parkinsonism, or other atypical parkinsonian syndromes (APS), highlighting the urgent need for objective and precise biomarkers (Mitchell et al., 2021; Ye et al., 2023).

The field of biomarker research is undergoing a profound change from a focus on static protein concentrations towards the detection of functional activity. The pivotal advancement is the emergence of the

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alpha-synuclein seed amplification assay (α-Syn-SAA) technology. This technique enables direct monitoring of PD's core pathology by detecting the "seeding" activity of pathological α-Syn. Encouragingly, α-Syn-SAA not only identifies patients with clinical PD with over 90% sensitivity and specificity but can also detect pathological changes in prodromal individuals 10-20 years before the onset of motor symptoms, providing an unprecedented time window for early intervention (Russo et al., 2021). Concurrently, significant progress has been made in other types of biomarker research. Neurofilament light chain (NfL), a marker of axonal injury, has shown significant value in differentiating PD from APS (Marques et al., 2019). AD-related biomarkers in cerebrospinal fluid (CSF), such as amyloid-beta 42 (Aβ42), total tau (T-tau), and phosphorylated tau (p-tau) protein, aid in identifying co-pathology of AD in PD patients, providing critical insights for predicting and subtyping cognitive impairment (Montine et al., 2010). Furthermore, the rapid development of biomarker detection technologies based on peripheral samples like blood, saliva, and skin has opened avenues for minimally invasive or even non-invasive diagnosis and monitoring (Eller and Williams, 2009; Gibbons et al., 2024).

Technological innovations are collectively propelling the field forward. Protein misfolding cyclic amplification (PMCA) and real-time quaking-induced conversion (RT-QuIC) techniques have enabled the detection of minute quantities of pathological proteins to assist in diagnosis and the validation of drug pharmacology, although they are unsuitable for tracking disease progression. High-field strength magnetic resonance imaging (MRI) provides a new window for observing subtle structural changes in the substantia nigra. For example, dopamine transporter (DAT) imaging supports diagnosis and holds potential for progression tracking. However, it cannot verify the pharmacodynamic effects of disease-modifying therapies. High-resolution mass spectrometry and metabolomics technologies allow for the systematic analysis of small-molecule metabolites in bodily fluids, revealing disease-specific metabolic signatures. Simultaneously, single-cell extracellular vesicle detection technology facilitates the precise analysis of biomarkers derived from specific cell types. The integrated application of these technologies is driving PD biomarker research into a new developmental stage, characterized by a focus on pathological activity, multi-omics integration, and a shift towards non-invasive or minimally invasive approaches (Parnetti et al., 2019; Shao and Le, 2019). Particularly noteworthy is the deep integration of multi-omics data with artificial intelligence (AI), which is transforming our understanding of the disease (Dennis and Strafella, 2024). This integration shows immense potential not only in diagnostic applications but also in monitoring progression and assessing the therapeutic impact on disease trajectory. By integrating genomics, proteomics, and metabolomics data and combining them with clinical features and digital phenotypes, researchers can construct high-precision diagnostic and predictive models that transcend single-dimensional analysis. This integrated analytical approach not only enhances diagnostic accuracy but also promotes the fine subtyping of the disease, providing a scientific basis for the development of personalized treatment strategies.

In this review, we systematically elucidate the latest advances in PD biomarker research, with a focused examination of core biomarkers grounded in pathophysiological mechanisms. These biomarkers include α-Syn pathology, genetic variations, neuroimaging characteristics, biofluid indicators, as well as emerging digital and prodromal biomarkers. We further explore how the integration of multi-omics data with AI models can enhance diagnostic and subtyping precision. Finally, we offer perspectives on the pivotal role of biomarkers in advancing the future of precision medicine for PD.

2 Pathophysiological Basis of PD

The pathophysiology of PD is complex and multi-layered, centered on the formation of Lewy bodies (LBs) and Lewy neurites (LNs), and the progressive loss of dopaminergic neurons in the midbrain substantia nigra. These pathological changes not only constitute the neuropathological foundation of PD but also provide a biological explanation for its clinical manifestations.

2.1 LBs and Pathological Aggregation of α-Syn

LBs, the most characteristic pathological structures in PD, are spherical, eosinophilic neuronal cytoplasmic inclusions. Their main component is misfolded and aggregated α-Syn, but they also contain lipid membranes,

aberrant organelles (such as lysosomes and mitochondria), and hundreds of other proteins (Shahmoradian et al., 2019; Bregendahl et al., 2025). The pathological evolution of α-Syn is a multi-step process, beginning with normal soluble monomers that undergo conformational changes and post-translational modifications like phosphorylation, forming soluble oligomers, and ultimately assembling into insoluble amyloid fibrils that further aggregate into LBs (Peng et al., 2018; Kayed et al., 2020). Notably, pathological α-Syn exhibits "prion-like" properties, acting as a "seed" that can template the misfolding of endogenous α-Syn, thereby promoting the spread of pathology between cells and across different brain regions (Luk et al., 2012; Guo et al., 2020; Yang et al., 2023).

2.2 Loss of Nigral Dopaminergic Neurons and Basal Ganglia Circuit Dysfunction

Another core pathological event in PD is the progressive, selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) of the midbrain. This process leads to a severe deficiency in dopaminergic innervation of the nigrostriatal pathway, consequently causing a functional imbalance between the direct and indirect pathways of the basal ganglia. This imbalance is the direct cause of the core motor symptoms in patients, such as bradykinesia, rigidity, resting tremor, and postural instability (Kalia and Lang, 2015). Note that by the time of clinical diagnosis, a significant proportion of nigral dopaminergic neurons have already been lost, and synaptic and axonal dysfunction likely occurs even earlier, suggesting that axonal damage is a key pathological process in the early stages of PD (O'keeffe and Sullivan, 2018).

2.3 Spatial Progression of Pathology and Braak Staging

The pathological progression of PD exhibits a distinct spatiotemporal pattern within the nervous system. The Braak staging system proposes that α-Syn pathology typically begins in the peripheral nervous system, such as the anterior olfactory nucleus or the autonomic plexuses of the gut (Stage 1), subsequently spreads upwards along axons to sequentially involve the medulla oblongata, pons, and other brainstem regions (Stage 2), then reaches the midbrain substantia nigra (Stage 3), and finally spreads to the limbic system and neocortex (Stages 4–6) (Braak et al., 2003). This "bottom-up" spatiotemporal pattern of pathological spread provides a theoretical basis for explaining prodromal symptoms (such as hyposmia originating from olfactory bulb pathology and isolated rapid eye movement sleep behavior disorder (iRBD) originating from brainstem nuclei pathology) (Postuma et al., 2015; Postuma et al., 2019; Berg et al., 2021). These findings also support the rationale for aiding early diagnosis by detecting α-Syn pathology in peripheral tissues (e.g., via skin or salivary gland biopsy) (Tsukita et al., 2019).

However, PD exhibits significant pathological heterogeneity. Nearly half of cases may not strictly follow the classic Braak pattern, instead showing a "top-down" progression where pathology begins in the brain (e.g., the limbic system) (Jellinger, 2019). Based on multimodal imaging studies, two disease subtypes have been proposed: "body-first" and "brain-first." The body-first subtype aligns with the Braak hypothesis, while the brain-first subtype, potentially driven by genetic factors, begins in the nigrostriatal system (Jellinger, 2019).

In summary, the pathophysiological basis of PD is a complex process involving protein misfolding and aggregation, selective neuronal loss, the spread of pathological proteins along neural pathways, and multi-system involvement. This multi-layered, dynamically evolving pathological framework not only deepens our understanding of the disease's essence but also lays a solid foundation for developing biomarkers and therapeutic strategies targeting the core pathological processes.

3 Classification and Cutting-Edge Detection Methods for PD Biomarkers

3.1 Core Biomarkers Based on α-Syn Pathology

The pathological misfolding and aggregation of α-Syn, leading to Lewy body formation, is the core

pathological hallmark of PD and other synucleinopathies. Consequently, the direct detection of pathological α-Syn is regarded as a crucial goal for achieving a precise diagnosis of PD.

3.1.1 From Static Quantification to Functional Activity

In PD research, α-Syn in CSF has consistently held a central position, and its research history clearly illustrates the evolution from static quantification to functional activity. Early studies focused mainly on measuring total α-Syn (T-α-Syn) levels in CSF. Although meta-analyses consistently found these levels to be significantly reduced in PD patients, its limited diagnostic accuracy and substantial overlap with other neurodegenerative disorders has prevented them from becoming a reliable standalone diagnostic marker (Gao et al., 2015; Eusebi et al., 2017). To improve specificity, the research focus shifted towards more pathology-relevant specific forms, including oligomeric α-Syn (o-α-Syn) and phosphorylated α-Syn (p-α-Syn, particularly at Ser129). Studies generally found relative increases in the levels of these potentially neurotoxic forms in the CSF of PD patients (Atik et al., 2016; Majbour et al., 2016). However, immunoassays based on protein concentration showed considerable variability across studies, and their diagnostic performance when used alone remained suboptimal; diagnostic accuracy significantly improved only when combined with other biomarkers like tau protein, highlighting their potential as part of a panel (Zhou et al., 2015; Majbour, et al., 2016). A fundamental breakthrough in α-Syn detection came with the emergence of the α-Syn-SAA technology, which shifted the detection target from "quantity" to "functional activity", namely the propensity for pathological aggregation. By leveraging the ability of pathological aggregates to act as "seeds" for amplification in vitro, the SAA technique directly detects seeding-competent α-Syn in CSF with ultra-high sensitivity and specificity. This method not only identifies patients with clinical PD with near-perfect accuracy but can also detect pathological seeds in prodromal individuals (such as those with iRBD years before the onset of motor symptoms, which represents the forefront of early diagnosis (Shahnawaz et al., 2017; Miglis et al., 2021; Concha-Marambio et al., 2023b; Agin-Liebes et al., 2025). This conceptual and technological leap, shifting the focus from static "quantity" to dynamic "activity", marks the official entry of PD biomarker research into a new era targeting the core pathology.

3.1.2 α-Syn-SAA Assays

The α-Syn-SAA is a groundbreaking diagnostic technique originating from prion disease research. It uses pathological α-Syn present in patient biofluids or tissues as "seeds" to induce the misfolding and formation of amyloid fibrils from a large amount of recombinant normal α-Syn monomers in vitro, thereby achieving exponential signal amplification. This process is typically implemented using mainstream technologies like RT-QuIC or PMCA, with real-time monitoring often using the thioflavin T (ThT) fluorescent dye (Concha-Marambio et al., 2023a). RT-QuIC offers a shorter assay duration (24-48 h) and simpler operation, making it suitable for routine clinical testing. In contrast, PMCA boasts higher amplification efficiency and greater sensitivity for detecting low seed concentrations, albeit with a longer detection time, rendering it more applicable for research or intensive screening of high-risk populations. The resulting fluorescence kinetic curve, comprising lag, exponential, and plateau phases, not only provides a qualitative "positive/negative" readout but also contains rich information regarding seed concentration and conformation **(Figure 1)**. Leveraging its high sensitivity for detecting pathological seeds, α-Syn-SAA technology significantly enhances the sensitivity and specificity of PD diagnosis, particularly in early-stage detection and differential diagnosis.

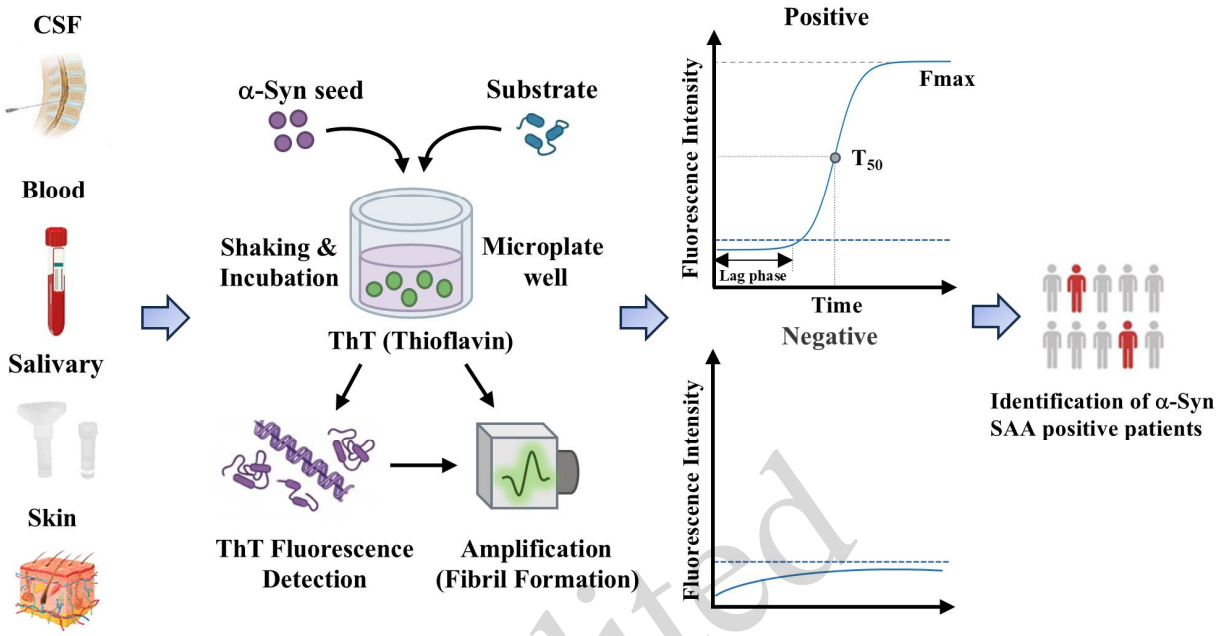

Figure 1. Schematic illustration of the α-synuclein seed amplification assay (α-Syn-SAA) workflow. Biological samples including cerebrospinal fluid (CSF), blood, saliva, and skin biopsies are incubated in microplate wells containing recombinant α-Syn substrate. The presence of pathological α-Syn seeds triggers a conformational conversion and amplification process through shaking and incubation, leading to fibril formation. The addition of thioflavin T (ThT) enables fluorescence detection of amyloid formation. Real-time ThT fluorescence monitoring generates kinetic curves: positive samples exhibit a characteristic sigmoidal curve with a lag phase, T_{50} (time to 50% maximum fluorescence), and Fmax (maximum fluorescence intensity), while negative samples show minimal fluorescence increase. This assay allows identification of individuals with pathological α-Syn aggregates.

3.1.3 Application and Performance Across Different Biospecimens

CSF currently represents the most extensively studied and consistently reliable biospecimen. Large multicenter studies, such as the Parkinson's Progression Markers Initiative (PPMI), have confirmed the exceptionally high diagnostic accuracy of CSF α-Syn-SAA, demonstrating superior sensitivity (87.7%) and specificity (96.3%) in distinguishing PD from healthy controls (Siderowf et al., 2023). Notably, this research further revealed the intrinsic heterogeneity of PD. The SAA positivity rate among *LRRK2* mutation carriers (67.5%) was significantly lower than that in sporadic PD cases (93.3%), a finding consistent with post-mortem observations that a subset of this genetic subtype lacks typical Lewy body pathology. This finding enables the potential for in vivo molecular subtyping of PD. Furthermore, α-Syn-SAA can detect pathological seeds in the prodromal phase, years before the onset of clinical symptoms (e.g., in patients with iRBD), with positivity rates as high as 75%-93%, underscoring its substantial potential for early diagnosis and risk stratification (Concha-Marambio, et al., 2023b; Iranzo et al., 2023).

α-Syn-SAA not only demonstrates exceptionally high diagnostic performance in CSF but also shows promising prospects for application to peripheral samples such as saliva, skin, and oral mucosa (Bsoul et al., 2025). Skin biopsy combined with α-Syn-SAA for the detection of seeding activity has shown high sensitivity (94%) and specificity (98%) in diagnosing PD (Wang et al., 2020). Furthermore, the combined detection of α-Syn-SAA in serum and saliva can achieve a sensitivity of 95.83%, a specificity of 96.15%, and an area under the curve (AUC) as high as 0.98, which is comparable to the diagnostic efficacy of CSF testing, highlighting the significant potential for minimally invasive diagnosis (Wang et al., 2024b). Additionally, breakthroughs in α-Syn-SAA detection using saliva and oral mucosa samples provide a new pathway for completely

non-invasive screening (Zheng et al., 2024).

3.1.4 Differential Diagnosis and Disease Subtyping

Building upon its capability for precise qualitative diagnosis, research focus has deepened to the extensive analysis of quantitative parameters derived from α-Syn-SAA reaction kinetics. These parameters, including lag time (T50), time to threshold (TTT), maximum fluorescence intensity (Fmax), and AUC, are thought to reflect the conformation, propagation efficiency, or in vivo burden of pathological "seeds," thereby providing crucial information for uncovering disease heterogeneity (Shahnawaz et al., 2020; Singer et al., 2020).

Studies have revealed significant differences in α-Syn-SAA kinetic parameters among PD patients with different clinical subtypes or genetic backgrounds. For instance, patients with *LRRK2*-associated PD exhibit a longer T50 and TTT, and a smaller AUC, whereas sporadic PD patients with concomitant dysautonomia (DysA) show a shorter T50 and a larger AUC (Grillo et al., 2025). More importantly, prospective longitudinal studies have elevated the prognostic value of SAA to a new level. Coughlin et al. (2025), in a landmark study on a large prodromal PD cohort, showed for the first time that baseline qualitative SAA results and quantitative parameters are powerful predictors of conversion to clinical PD or Lewy body dementia. Their study found that prodromal individuals with a positive SAA result had a significantly higher risk of converting to clinical disease compared to SAA-negative individuals. Among SAA-positive individuals, amplification kinetics served as an additional risk stratification factor—those classified as "fast amplifiers" based on median TTT exhibited the highest conversion risk, establishing a clear risk gradient: "fast amplifiers" > "slow amplifiers" > "SAA-negative". These differences not only reflect heterogeneity in α-Syn aggregation mechanisms underlying different subtypes but also provide novel insights for using α-Syn-SAA parameters in disease subtyping and prognosis assessment.

3.1.5 Challenges and Corresponding Solutions in the Clinical Translation of α-Syn-SAA

Despite the rapid advancement of α-Syn-SAA technology, its widespread application still faces several challenges. Firstly, its detection sensitivity for specific genetic subtypes, such as those with *LRRK2* mutations, remains suboptimal, resulting in a relatively high false-negative rate. Secondly, peripheral samples like saliva are susceptible to interference from factors like diet and oral microbiota, and the lack of standardized pre-processing protocols compromises result reproducibility. Finally, the absence of uniform testing reagents and operational standards across laboratories hinders the comparability of results between different centers, impeding the broad adoption of this technology.

In summary, as a core biomarker for PD, the detection methodology for α-Syn is undergoing a fundamental shift—from traditional concentration measurements toward functional and conformational assessments. The breakthrough in SAA technology, in particular, has enabled the detection of pathology years or even decades before the onset of clinical symptoms, significantly advancing the progress of early diagnosis and disease-modifying therapies for PD. However, despite these rapid advancements, transforming α-Syn-SAA from a specialized research tool into a widely adopted clinical application remains constrained by several translational bottlenecks.

Addressing these challenges requires integrated strategies across methodology, clinical validation, and regulatory pathways: 1) Methodological standardization and quantification: the most pressing issue is inter-laboratory heterogeneity. The absence of uniform testing reagents and operational standards across laboratories severely hinders the comparability of results between different centers. Variations in core assay components, such as recombinant α-Syn substrate preparation, buffer composition, and shaking kinetics, compromise result reproducibility. Furthermore, the current qualitative nature of the assay limits its use for tracking disease progression. Overcoming this requires establishing global standard operating procedures (SOPs) and third-party quality control systems, ultimately pivoting from laboratory-developed tests toward

fully automated, FDA/NMPA-certified commercial kits capable of providing quantitative readouts (Bellomo et al., 2022). 2) Diagnostic sensitivity, specificity, and subtyping: while highly sensitive for idiopathic PD (iPD), SAA detection sensitivity for specific genetic subtypes, particularly those with *LRRK2* mutations, remains suboptimal, resulting in a relatively high false-negative rate (Siderowf, et al., 2023). Furthermore, standard SAA cannot reliably differentiate PD from multiple system atrophy (MSA) or dementia with Lewy bodies (DLB), as it detects pathological α-Syn common to all synucleinopathies. To resolve these diagnostic dilemmas, future iterations must exploit conformational differences in α-Syn strains to develop subtype-specific assays (Shahnawaz, et al., 2020). In the interim, incorporating SAA into multimodal panels (e.g., combined with blood NfL or DAT-PET (positron emission tomography)) will significantly mitigate false-negative and false-positive risks. 3) Sample accessibility and peripheral innovations: currently, the most robust SAA results rely on invasive CSF sampling. Exploring peripheral alternatives is highly attractive but faces significant technical hurdles. Breaking this bottleneck necessitates optimizing pre-analytical extraction methods for peripheral biofluids and integrating high-sensitivity platforms (e.g., microfluidics, single-molecule technologies) to enhance the signal-to-noise ratio in these complex matrices (Okuzumi et al., 2023; Gibbons, et al., 2024). 4) Clinical implementation and trial utility: unclear regulatory pathways and high costs impede routine adoption. To justify its clinical application, robust cost-effectiveness models must be established, prioritizing SAA for cases of ambiguous early diagnosis and rigorous clinical trial enrollment (Simuni et al., 2024). In therapeutic trials, using longitudinal quantitative SAA as a direct indicator of target engagement, combined with digital and imaging biomarkers, will significantly improve trial efficiency and accelerate precision therapy development.

3.2 Genetic Biomarkers

Genetic biomarkers are crucial for both familial and early-onset PD (EOPD), not only for clarifying etiology but also for complementing pathological biomarkers for precise molecular subtyping. Among the dozens of identified risk loci, *LRRK2*, *GBA1, SNCA*, *PINK1*, and *PRKN* represent the most extensively studied and clinically significant targets (Blauwendraat et al., 2020).

Pathogenic variants in the *LRRK2* gene represent the most common genetic cause of autosomal dominant PD, with a particularly high prevalence in populations of North African, Ashkenazi Jewish, and Basque descent (Lesage et al., 2006; Ozelius et al., 2006; González-Fernández et al., 2007). The most common mutation, G2019S, results in late-onset PD with clinical features indistinguishable from iPD. This mutation causes hyperactivation of the leucine-rich repeat kinase 2 protein encoded by *LRRK2*. Clinically, mutation carriers often exhibit slower disease progression and a lower risk of cognitive impairment. Furthermore, elevated LRRK2 kinase activity in their peripheral blood mononuclear cells (PBMCs) , such as through increased levels of phosphorylated RAB12 (pSer106), can serve as an endogenous biomarker for monitoring the efficacy of targeted therapies, such as *LRRK2* inhibitors (Cortés et al., 2025). Given these distinct characteristics, we recommend prioritizing *LRRK2* genetic testing for patients with a family history consistent with autosomal dominant inheritance, those from high-prevalence ethnic backgrounds (e.g., North African, Ashkenazi Jewish, or Basque), or individuals exhibiting atypical clinical phenotypes characterized by preserved olfaction and slow disease progression, regardless of the age at onset.

Mutations in the glucocerebrosidase gene 1 (*GBA1*), such as N370S and L444P, represent the strongest known genetic risk factor for PD. *GBA1* mutation-associated PD (*GBA1*-PD) patients typically experience an earlier age of onset, a more aggressive disease course, and a significantly higher risk of cognitive impairment and dementia (Shao and Le, 2019). Research has found that specific glycosphingolipids in the plasma of patients with *GBA1*-PD, such as the C24:1 and C24:0 isoforms of glucosylceramide (GluCer), are present at significantly higher levels than in patients with iPD and healthy controls. Importantly, the measurement of these lipids in plasma shows low intra-day and inter-day variability, offering more stability than measurements in PBMCs (Den Heijer et al., 2023). This suggests that plasma GluCer isoforms could serve as potential and stable biomarkers for *GBA1*-PD, facilitating precise molecular subtyping of patients in clinical trials and future

clinical practice. Consequently, *GBA1* screening is strongly indicated for PD patients with early cognitive decline, rapid motor progression, or a relevant family history, irrespective of other clinical features.

SNCA, the first gene discovered to be associated with familial PD, is significant not only due to its rare pathogenic missense mutations and multiplications but also due to the role of its common epigenetic regulation (Polymeropoulos et al., 1997). Notably, *SNCA* multiplications show a clear gene-dosage effect and are strongly associated with early onset and rapid progression to dementia. Thus, copy number variation (CNV) analysis is warranted in severe EOPD cases. Studies have found that the methylation level of the promoter in intron 1 of the *SNCA* gene in the blood of PD patients is significantly lower than in healthy controls, and this hypomethylation state is associated with disease risk. More importantly, the SNP rs3756063 has been identified as a methylation quantitative trait locus (mQTL), showing consistent allelic effects in both blood and brain tissues (Pihlstrøm et al., 2015). This suggests that the *SNCA* methylation pattern in peripheral blood may, to some extent, reflect the epigenetic landscape in the central nervous system, offering a potential peripherally accessible epigenetic biomarker for PD and bridging genetic variation, epigenetic regulation, and environmental factors.

Beyond single-gene diagnostics, the integration of genetic biomarkers enables distinct pathological and therapeutic subtyping in sporadic PD. For instance, *LRRK2* mutation carriers often exhibit preserved olfactory function, include a higher proportion of females, and have a lower α-Syn-SAA positivity rate, suggesting potentially distinct underlying pathological mechanisms (Siderowf, et al., 2023). *GBA1* mutations, conversely, are associated with a higher risk of cognitive impairment and more rapid disease progression (Real et al., 2023). Additionally, EOPD caused by *PRKN* or *PINK1* mutations is often accompanied by mitochondrial dysfunction and enhanced inflammatory responses, with serum IL-6 and circulating cell-free mtDNA levels significantly elevated in this subtype, providing potential targets for immunomodulatory therapy (Borsche et al., 2020). For EOPD patients (especially those with onset <40 years) without a dominant family history, or those exhibiting excellent and sustained response to levodopa, slow progression, and dystonia at onset, sequencing of *PRKN* and *PINK1* should be the first-line genetic test. Notably, similar to certain *LRRK2* cases, classic Lewy body pathology may be absent in a subset of *PRKN* mutation carriers. With the increasing availability and decreasing cost of genetic testing, these biomarkers are transitioning into routine clinical practice. For example, routine screening of EOPD patients for *LRRK2, PINK1*, and *PRKN* helps clarify etiology, while selective screening of sporadic PD patients for *GBA1* is performed based on ethnic background. Ultimately, these genetic biomarker screenings not only aid in early diagnosis and risk assessment but also provide the molecular foundation for targeted therapies and patient stratification in the era of precision medicine.

3.3 Neuroimaging Biomarkers

The accurate diagnosis of PD, particularly its differentiation from APS, represents a central challenge in clinical practice. Recent rapid advancements in neuroimaging techniques have given rise to a series of biomarkers with high sensitivity and specificity, providing crucial support for precise diagnosis. The current research landscape shows a clear trend evolving from unimodal assessments towards multimodal integration (**Figure 2**).

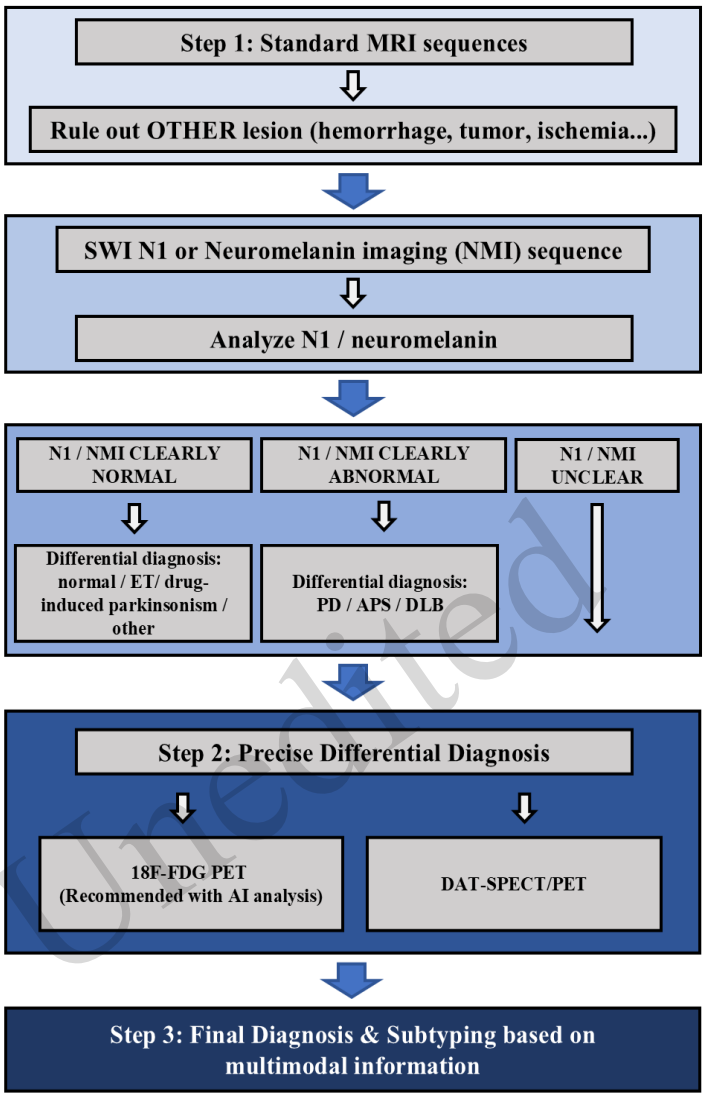

Figure 2. Optimized diagnostic pathway for parkinsonian syndromes using multimodal imaging. This flowchart presents a stepwise algorithm for diagnosing parkinsonian syndromes by integrating advanced MRI biomarkers with molecular imaging. The process begins with a comprehensive MRI protocol to exclude secondary causes and evaluate nigral integrity through Nigrosome-1 (SWI) and/or neuromelanin-sensitive imaging. Findings are categorized as clearly abnormal, clearly normal, or indeterminate. All cases proceed to the differential diagnosis phase, where cerebral glucose metabolism (^{18}F-FDG PET, ideally with AI analysis) or dopaminergic integrity (DAT-SPECT/PET) is assessed. Final diagnosis and subtyping are achieved through developing integrated diagnostic models that combine imaging (such as NM imaging, DAT-PET), body fluid biomarkers (such as α-Syn-SAA, p-tau217, NfL), and genetic information.

As summarized in **Table 1**, different APS exhibits distinct and characteristic imaging patterns based on conventional MRI, nigrosome-1/neuromelanin-sensitive MRI, and DAT imaging, establishing a theoretical foundation for differential diagnosis. Note that these imaging biomarkers are not absolutely specific. For example, although DAT imaging is highly specific for distinguishing PD from conditions without dopaminergic deficits (such as essential tremor), it is abnormal in PD as well as in other degenerative parkinsonisms like MSA and PSP, and thus cannot precisely differentiate between them. Building upon this, various cutting-edge imaging techniques, such as nigrosome-1 MRI targeting subtle substantia nigra structures, neuromelanin-sensitive MRI reflecting neuronal population integrity, DAT-SPECT (single- photon emission

computed tomography)/PET assessing dopaminergic system function, and ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET revealing whole-brain metabolic networks, have unique core technologies and specific advantages and limitations, which make them best suited for different clinical application scenarios.

Therefore, systematically reviewing and comparing the diagnostic efficacy of these imaging methodologies is essential for constructing an efficient and accurate diagnostic pathway for PD.

3.3.1 Molecular Imaging (PET/SPECT) Biomarkers

3.3.1.1 DAT Imaging

DAT imaging using ^{123}I-ioflupane SPECT (commonly known as DaTscan) is the gold standard for assessing the integrity of the nigrostriatal pathway. It is capable of detecting dopaminergic dysfunction years before the onset of clinical symptoms, standing as one of the most reliable imaging modalities for supporting a clinical diagnosis of PD (Iranzo et al., 2011). Research has shown that DAT tracer uptake decreases exponentially and linearly with disease severity (Hoehn and Yahr stage) and disease duration, revealing that about half of the dopamine uptake capacity is already lost by the time clinical symptoms appear (Badoud et al., 2016). This technique shows reduced striatal tracer uptake (particularly in the posterior putamen) in both PD and APS, while uptake is typically normal in non-degenerative conditions such as essential tremor or drug-induced parkinsonism. Therefore, beyond its established role in diagnostic support, the linear decline of tracer uptake makes DAT imaging a potentially useful biomarker for tracking long-term disease progression. However, it is crucial to note its limitations in the context of clinical trials. Because DAT imaging reflects mainly the structural integrity and compensatory state of presynaptic dopaminergic terminals rather than the underlying core pathology, it is generally not suitable for demonstrating the pharmacological effects or target engagement of disease-modifying compounds. This functional distinction is critical when selecting imaging biomarkers for specific trial endpoints.

With the application of deep learning, the diagnostic efficacy of DAT imaging has been significantly enhanced. Studies show that deep learning can decode profound information directly from DAT images that surpasses traditional quantitative analyses (such as specific binding ratios in defined regions of interest), enabling high-accuracy discrimination between iPD, MSA, and progressive supranuclear palsy (PSP), with AUC values as high as 0.953, 0.948, and 0.900, respectively (Zhao et al., 2022).

3.3.1.2 Cerebral Glucose Metabolism Imaging: ^{18}F-FDG PET

^{18}F-FDG PET, combined with statistical parametric mapping (SPM) or network analysis, reflects the activity of whole-brain neural networks. PD is characterized by a specific PD-related pattern, which manifests as hypermetabolism in the putamen, globus pallidus, thalamus, pons, and cerebellum, coupled with hypometabolism in the premotor and parietal cortices (Eidelberg et al., 1994; Schindlbeck and Eidelberg, 2018). ^{18}F-FDG PET shifts the focus of differential diagnosis from a single neurotransmitter system to dysfunction across entire brain networks, making it one of the most powerful tools for distinguishing PD from APS, such as MSA and PSP (Tang et al., 2010). A meta-analysis showed that AI-assisted ^{18}F-FDG PET can achieve a pooled AUC as high as 0.97 for discriminating PD from APS (Wang et al., 2024a).

3.3.2 MRI Biomarkers

3.3.2.1 Conventional (Standard) MRI

Conventional MRI serves as a diagnostic "safety net", aimed mainly at excluding other pathologies that can mimic PD symptoms but are fundamentally different in nature, such as structural lesions including brain tumors, cerebral hemorrhage, cerebral infarction, and hydrocephalus (Haller et al., 2025).

3.3.2.2 Nigrosome-1 (N1) and the "Swallow Tail Sign"

Using high-resolution susceptibility-weighted imaging (SWI) or quantitative susceptibility mapping (QSM), a characteristic hyperintense "swallow tail" appearance can be observed in the posterior substantia nigra. In PD, due to neuronal loss and abnormal iron deposition, this normal "swallow tail sign" disappears. Its diagnostic sensitivity and specificity for PD both exceed 90% (Schwarz et al., 2014; Mahlknecht et al., 2017). Crucially, similar to DaTscan, the N1 sign is typically abnormal in PD and most APS (e.g., MSA, PSP), while it usually remains normal in essential tremor, drug-induced parkinsonism, and vascular parkinsonism, making it a powerful tool for differential diagnosis (Sung et al., 2016).

3.3.2.3 Neuromelanin-Sensitive MRI

Neuromelanin is a pigment found within dopaminergic neurons. Neuromelanin-sensitive MRI uses specific magnetization transfer or T1-weighted sequences to visualize the neuromelanin-rich SNc as a region of high signal intensity. In PD patients, degeneration of these neurons leads to a marked reduction in both the signal intensity and volume of this region, providing a quantitative tool for assessing neuronal degeneration. This technique enables the direct quantification of dopaminergic neuronal loss in the substantia nigra, showing high specificity for the diagnosis of PD (He et al., 2021). Similar to the N1 sign, it also aids in differentiating PD from essential tremor, drug-induced parkinsonism, and vascular parkinsonism. Its advantage lies in its potential to combine with techniques like QSM, allowing for the simultaneous assessment of iron deposition and neuromelanin loss within a single imaging session.

Table 1 Imaging Features of Different Diseases on Conventional MRI, Nigrosome 1/Neuromelanin MRI, and DAT-SPECT/PET.

Disease	Conventional MRI	Nigrosome 1/ Neuromelanin MRI	DAT-SPECT/PET	Key Imaging Characteristics
PD	**Usually normal**	**Abnormal** (signal reduction/loss)	**Abnormal**	-
MSA	**Abnormal** (e.g., "Hot cross bun sign" in the pons, putaminal abnormalities)	**Usually abnormal**	**Abnormal**	Differentiation from PD relies on specific atrophy patterns on conventional MRI.
PSP	**Abnormal** (Midbrain atrophy, "Hummingbird sign")	**Usually abnormal**	**Abnormal**	Metrics like the midbrain-to-pons ratio aid in differentiation.
ET	**Normal**	**Normal**	**Normal**	Key differentiator from PD: Both N1 MRI and DAT -SPECT/PET are normal.
Drug-Induced Parkinsonism	**Normal**	**Normal**	**Normal**	Key differentiator from PD, suggests symptoms are reversible.
Vascular Parkinsonism	**Abnormal** (Evidence of cerebrovascular disease)	**Normal**	**Normal**	Imaging shows a vascular cause, with no dopaminergic deficit.

3.4 Fluid Biomarkers

Among various categories of biomarkers, fluid biomarkers represent one of the most intensively

researched areas due to their ability to directly or indirectly reflect pathological changes in the central nervous system. Based on the invasiveness of sample collection, they can be categorized into CSF, blood, and other bodily fluids (such as saliva and urine). Characteristic biomarkers in the CSF, blood, and saliva of PD patients are illustrated in **Figure 3**.

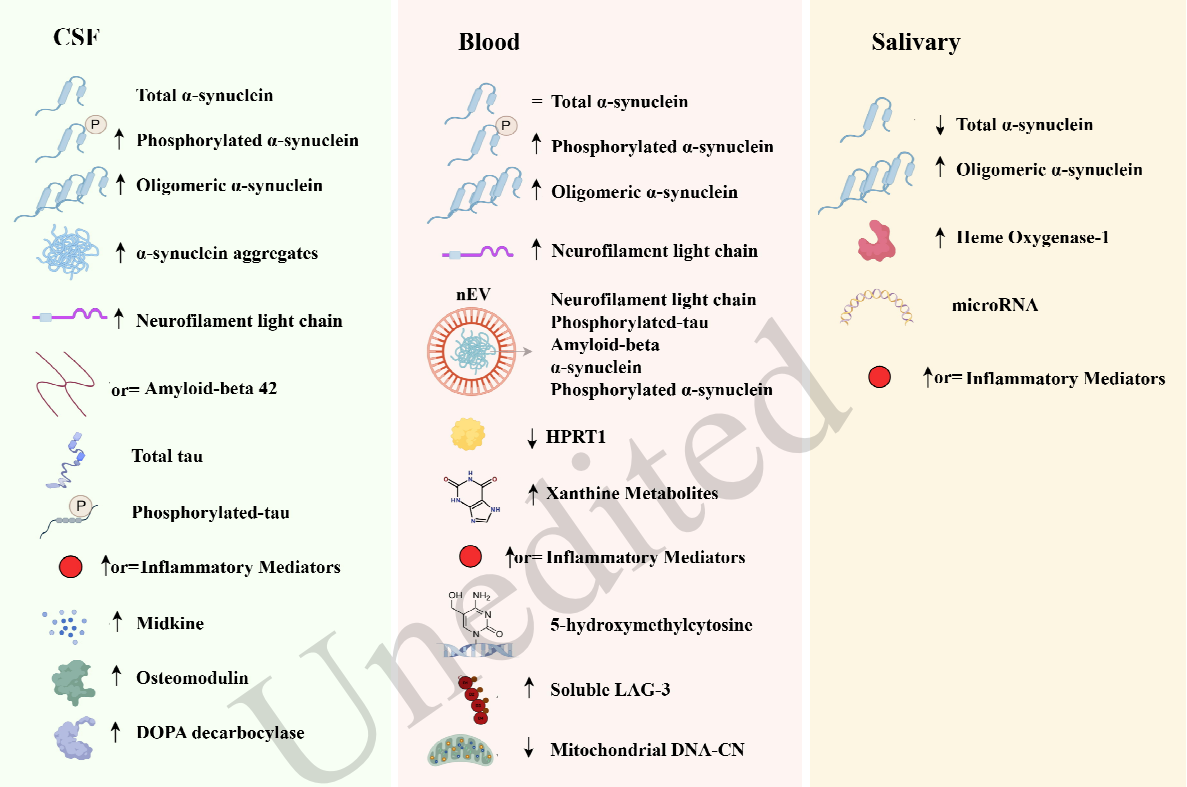

Figure 3. Biomarker profiles in cerebrospinal fluid, blood, and saliva in PD. This schematic summarizes reported alterations in biomarker levels across three biofluids in PD compared to healthy controls. Arrows indicate significant increases (↑) or decreases (↓); equals signs (=) denote no significant change.
In CSF, key changes include decreased total-α-synuclein; increased phosphorylated-α-synuclein, oligomeric-α-synuclein, α-synuclein aggregates, phosphorylated-tau, midkine, osteomodulin, and DOPA decarboxylase; variable or unchanged levels of neurofilament light chain, total-tau, and Aβ42; and elevated inflammatory mediators.
In blood, notable alterations include unchanged total-α-synuclein; elevated phosphorylated-α-synuclein, oligomeric-α-synuclein (particularly in neuronal-derived extracellular vesicles, nEVs), HPRT1, xanthine metabolites, 5-hydroxymethylcytosine, soluble LAG-3, and mitochondrial DNA copy number; unchanged neurofilament light chain; and variable inflammatory mediators. nEVs carry neurofilament light chain, phosphorylated-tau, β-amyloid, and α-synuclein species.
In saliva, total-α-synuclein is decreased, while oligomeric-α-synuclein, heme oxygenase-1, microRNA, and inflammatory mediators are increased or unchanged.

3.4.1 CSF

CSF, being in direct contact with the central nervous system, has long held a pivotal role in biomarker research for neurodegenerative diseases and is widely regarded as a reference standard in the PD field. Beyond α-Syn as previously discussed, the classical AD-related biomarkers in CSF, such as Aβ42, T-tau, and p-tau, provide significant supplementary value for the assessment and subtyping of cognitive impairment in PD (Bartl et al., 2022). However, note that these markers are not specific to PD. For instance, reduced CSF Aβ42 levels are most characteristic of AD, and their presence in PD patients suggests co-existing AD pathology, which explains why about one-third of PD patients with mild cognitive impairment exhibit an AD-like CSF biomarker

profile. These markers not only aid in evaluating the risk of cognitive impairment in PD but also help identify the presence of concomitant AD pathology. Numerous studies have confirmed that PD patients with cognitive impairment (PD-CIND) or those who have developed dementia (PD-D) exhibit significantly reduced CSF Aβ42 levels, with some patients also showing elevated p-tau. Notably, about one-third of PD patients with mild cognitive impairment and nearly half of those with PD dementia display a CSF biomarker profile reminiscent of AD (i.e., decreased Aβ42 accompanied by elevated p-tau) (Montine, et al., 2010). This finding has substantial clinical implications, suggesting that AD-like pathology may be superimposed on the disease course in a subset of PD patients, collectively exacerbating their cognitive decline. Longitudinal studies have further deepened our understanding; tracking of early PD patients revealed that the rates of decline in CSF Aβ42 and p-tau over three years were more pronounced than those of healthy controls. Furthermore, lower baseline Aβ42 levels were predictive of future subtle decline in cognitive, motor, and autonomic functions (Irwin et al., 2020). Additionally, research indicated that the concentration of the soluble triggering receptor expressed on myeloid cells 2 (sTREM2) was significantly elevated in a PD subgroup with abnormally increased CSF tau protein, but not in those with only Aβ abnormalities. sTREM2 levels correlated positively with both T-tau and p-tau181, and were already elevated in PD patients with normal cognitive function, suggesting its potential as an immune biomarker reflecting tau-related neuronal injury and microglial activation. This helps identify PD subtypes with AD co-pathology (Wilson et al., 2020). These findings not only underscore the potential of CSF biomarkers in prognostic assessment but also reveal the complexity of PD disease mechanisms, indicating that interventions targeting Aβ might be beneficial for specific PD subgroups with particular biomarker profiles.

NfL is a structural component of the neuronal axonal cytoskeleton. It is released into the extracellular space and ultimately enters the CSF and blood upon axonal damage, making it an excellent marker reflecting neuroaxonal injury. Large-scale cohort studies have confirmed that CSF NfL (cNfL) is a highly valuable biomarker for neurodegeneration and prognosis in the early stages of PD. Research has found that in the initial phases of the disease, higher cNfL levels are significantly associated with more severe motor symptoms (particularly postural instability/gait difficulty and bradykinesia, rather than tremor), more pronounced striatal dopaminergic denervation, and white matter microstructural damage (Bäckström et al., 2020). Studies have shown that both CSF and serum NfL levels are elevated in PD patients, but their discriminatory power when used alone is limited. However, when cNfL is combined with the p-α-Syn/T-α-Syn ratio and the o-α-Syn/T-α-Syn ratio into a biomarker panel, the AUC for distinguishing PD patients from healthy controls can reach 0.92, with significantly improved sensitivity and specificity (Oosterveld et al., 2020).

Other markers in the CSF, such as functional enzymes and metabolic small molecules, reveal the pathophysiological processes of PD from different dimensions, providing powerful new tools for early and differential diagnosis. For instance, dopa decarboxylase (DDC), a key enzyme in the dopamine synthesis pathway, shows significantly elevated levels in the CSF of PD patients. This change is already present in the prodromal stage and in newly diagnosed patients, demonstrating high value for early diagnosis (AUC 0.79-0.94). Its reliability as an independent indicator is further strengthened by being unaffected by Levodopa treatment (Appleton et al., 2024).

3.4.2 Blood

Despite the significant value of CSF biomarkers, sample collection is invasive and not easily repeatable, which severely limits their widespread adoption in routine clinical practice, long-term disease monitoring, and large-scale screening. Blood, being a minimally invasive and easily repeatable sample source, presents an ideal alternative. However, major challenges remain: the baseline concentration of α-Syn in blood is extremely low, and samples are highly susceptible to interference from factors such as hemolysis (leading to hemoglobin contamination) and platelet activation. This has resulted in contradictory and poorly reproducible findings in early studies based on total plasma or serum α-Syn levels (Barbour et al., 2008).

To enhance the central nervous system specificity of blood biomarkers, extracellular vesicles (EVs),

particularly those of neuronal origin, have become a research focus (Xylaki et al., 2023). Exosomes are nano-sized lipid bilayer vesicles secreted by cells, capable of carrying bioactive molecules like proteins and nucleic acids from their parent cells and crossing the blood-brain barrier. Therefore, their cargo can better reflect the pathological state of the CNS. Using specific antibodies (e.g., against the neuronal surface marker L1CAM) for immunocapture, researchers can "enrich" neuron-derived exosomes from the complex composition of blood, providing a crucial pathway for the non-invasive detection of CNS pathology. Studies show that the levels of α-Syn within these neuron-derived exosomes from the serum of PD patients and prodromal individuals (e.g., those with iRBD) are significantly elevated (about 2-fold), demonstrating tremendous potential for early identification and risk stratification. The AUC for distinguishing high-risk individuals from healthy controls reached 0.91 (Yan et al., 2024).

Going a step further, research using single extracellular vesicle detection technology has found that α-Syn can attach to the membrane surface of $L1CAM^+$ EVs. This membrane-bound form of α-Syn shows excellent performance for diagnosing both the prodromal and clinical stages of PD (AUC values of 0.93 and 0.95, respectively) (Yan et al., 2025). Longitudinal studies on plasma EVs have also found that levels of α-Syn, tau, and β-amyloid within them show an increasing trend over time, and their dynamic changes correlate significantly with the deterioration of patients' activities during daily living (as measured by UPDRS-II scores) (Hong et al., 2025). Similarly, the detection of p-α-Syn (Ser129), NfL, and microRNAs in exosomes has also shown good diagnostic and differential diagnostic capabilities (Jiang et al., 2020; Gilboa et al., 2024; Yu et al., 2024a; Yu et al., 2024b). In summary, exosome technology addresses the core challenges of blood-based detection by enabling specific "enrichment" of CNS-derived biomarkers through immunocapture. The unique internal protein environment of exosomes also favors the stability of modified proteins, offering an elegant solution to the central problem of blood-based detection.

Blood NfL (bNfL), as an easily accessible biomarker, holds important value in the differential diagnosis of PD. bNfL exhibits good stability and a high correlation with cNfL concentrations (Lin et al., 2019). Research indicates that bNfL levels are significantly higher in patients with APS, such as MSA and PSP, than in those with iPD. It can differentiate between these conditions with high accuracy (AUC 0.81–0.91), and this discriminatory ability remains robust even in the early stages of the disease (disease duration ≤ 3 years) (Hansson et al., 2017). Furthermore, bNfL levels correlate positively with the severity of motor symptoms and the rate of cognitive decline. High baseline NfL levels can predict the future risk of dementia, suggesting its potential as a prognostic biomarker (Mollenhauer et al., 2020; Aamodt et al., 2021).

Although NfL is not a PD-specific marker, it serves as a universal indicator reflecting the "intensity" of neurodegeneration. While this lack of specificity limits its utility as a standalone diagnostic tool, it provides crucial objective information for differential diagnosis and tracking long-term disease progression. Furthermore, NfL holds significant potential for demonstrating the macroscopic pharmacological effects of disease-modifying therapies, even though it cannot verify specific target engagement like α-Syn. Collectively, these features make it a highly practical tool for both routine clinical management and clinical trial monitoring. Additionally, other blood-based markers such as soluble lymphocyte activation gene-3 (sLAG-3), C-reactive protein (CRP), apolipoprotein A1 (ApoA1), surface markers on PBMCs, and various metabolites (e.g., uric acid and xanthine metabolites) have been found to be associated with PD diagnosis, subtyping, or disease progression, although their specificity requires further validation (Cui et al., 2019; Lawton et al., 2020; Konstantin Nissen et al., 2022; De Lope et al., 2024; Liu et al., 2025).

3.4.3 Other Non-Invasive Bodily Fluids

Beyond CSF and blood, fully non-invasive bodily fluids such as saliva and urine are gaining increasing attention due to their unique advantages. Although related studies are relatively small in scale, they have shown encouraging application prospects.

Saliva, as a completely non-invasive sample, shows potential in PD biomarker research. Saliva not only

contains a rich diversity of molecules but the salivary glands are densely innervated by the autonomic nervous system, making it an ideal source for neurogenic biomarkers (De Bartolo et al., 2024). As mentioned in section 3.1.2, highly sensitive detection of α-Syn-SAA in saliva has shown good discriminatory power in early PD (Zheng, et al., 2024). Furthermore, salivary metabolite profiles (analyzed via deep learning models), heme oxygenase-1 (HO-1), cortisol, TNF-α, microRNAs, and others are also being explored as potential markers (Song et al., 2018; Xu et al., 2023; De Bartolo, et al., 2024).

Urine offers another non-invasive sample type. Studies have found that levels of α-Syn aggregates in urine are significantly elevated in PD and iRBD patients. Analysis using surface-based fluorescence intensity distribution technology showed moderate sensitivity and specificity in distinguishing patients from controls (Müller et al., 2025). However, similar to α-Syn in CSF, urinary α-Syn aggregates are a common marker for synucleinopathies and are not specific to PD.

Overall, research on salivary and urinary biomarkers remains in its early stages, facing challenges such as insufficient standardization of sample processing, diurnal fluctuations, and numerous interfering substances. Future large-scale, multicenter studies are needed to validate their reliability.

3.5 Multi-omics Data and AI Models

While the individual categories of biomarkers previously discussed, such as pathological, genetic, imaging, and fluid-based, each have their strengths, they struggle to fully capture the high heterogeneity of PD. The integration of multi-omics data and the analytical power of AI technologies offer a new pathway for synthesizing multi-dimensional information to enhance diagnostic and subtyping accuracy.

Among these approaches, metabolomics involves the systematic analysis of endogenous small-molecule metabolites, directly reflecting the biochemical characteristics of the organism under specific physiological or pathological states. In PD research, this technology has already revealed widespread disturbances across multiple pathways, including amino acid and lipid metabolism, energy metabolism, and gut microbiome-brain axis communication. For instance, a plasma metabolomics analysis based on a large Luxembourg cohort discovered that various xanthine metabolites (such as inosine, xanthosine, xanthine, and hypoxanthine) were significantly upregulated in the blood of PD patients, particularly in treatment-naïve, newly diagnosed individuals. Further mechanistic exploration, integrating transcriptomics data, suggested that downregulated expression of the HPRT1 enzyme might be a key mechanism underlying these metabolic disturbances and the associated reduction in cellular ATP production (De Lope, et al., 2024). This indicates that HPRT1 and the related xanthine metabolism pathway hold promise not only as potential PD biomarkers but also as novel therapeutic targets.

Beyond the findings mentioned above, untargeted lipidomics studies have revealed significant dysregulation of ceramide, triacylglycerol, and sphingomyelin metabolism in the serum and CSF of PD patients, implicating sphingolipid metabolism and mitochondrial dysfunction as core pathological processes in PD (Galper et al., 2022). Simultaneously, abnormal elevations in gut microbiota-derived metabolites, such as bile acids and harmful substances like phenylacetylglutamine, highlight the potential role of gut microbes in PD pathogenesis (Shao et al., 2021).

Methodologically, metabolomics research is deeply integrating with advanced data analysis strategies. A study proposing the interpretable machine learning framework CRANK-MS demonstrated the ability to directly analyze full metabolomics data without relying on pre-selected features, achieving exceptionally high predictive performance for PD diagnosis (AUC > 0.995). Notably, this model not only confirmed endogenous metabolites closely associated with PD (such as triterpenoids and diacylglycerols) but also unexpectedly identified an exogenous per- and polyfluoroalkyl substance (PFAS), suggesting its potential association with PD risk years before clinical symptoms appear (Zhang et al., 2023). Similar integration strategies have also succeeded in predicting PD cognitive impairment (PDCI). By fusing plasma proteomics and metabolomics data, researchers identified key markers, including glycocholic acid, and constructed high-performance prediction

models (AUC up to 0.981) (Yang et al., 2024). Furthermore, sampling strategies in metabolomics are expanding towards non-invasiveness. Analysis of volatile metabolites based on skin sebum, enabled by mass spectrometry, allows for non-invasive screening of PD (AUC of 0.77). Intriguingly, its findings even align with clinical observations of "super-smellers," offering a novel approach for community-based early screening (Poewe, 2019). In summary, this body of research showcases the powerful potential of metabolomics combined with interpretable AI. It can not only build predictive models but also extract biologically meaningful hypotheses from vast datasets.

Unlike metabolomics, proteomics focuses on the systematic discovery and validation of biomarkers at the protein level. For instance, through large-scale screening of the CSF proteome using proximity extension assay technology, studies have identified novel supportive diagnostic biomarkers for PD, such as Midkine (MK) and DDC, in multi-center cohorts (Paslawski et al., 2023; Rutledge et al., 2024). These proteins exhibit changes in the early or even prodromal stages of PD, holding significant diagnostic value. Among them, MK is significantly elevated in PD patients and has shown good diagnostic performance (AUC 0.84) upon validation by ELISA (Paslawski, et al., 2023). Another in-depth mass spectrometry-based proteomics study analyzed CSF samples from over 200 individuals, quantifying more than 1,700 proteins, and found that proteins like osteomodulin (OMD) and CD44 were significantly upregulated in PD. Subsequently, machine learning models screened a panel of promising biomarker candidates, including prolactin (PRL), VGF nerve growth factor inducible (VGF), and mannosidase alpha class 2B member 1 (MAN2B1), for distinguishing patients from healthy controls (Karayel et al., 2022). In summary, proteomics directly captures changes in proteins, the functional executors, and the biomarkers it provides are often closer to the effector level of the disease, holding direct translational significance.

Beyond proteins and metabolites, epigenomics, particularly DNA methylation and hydroxymethylation, provides a dynamic molecular bridge connecting genetic factors and environmental influences (Wang et al., 2019). A study based on the PPMI cohort included the first large-scale longitudinal DNA methylation analysis of whole blood from sporadic PD patients, identifying 5,178 differentially methylated positions, of which 579 changed significantly over three years. It also pinpointed key genes related to neuronal function, such as *CYP2E1*, *NDRG4*, and *CTSH* (Gonzalez-Latapi et al., 2024). This indicates that the blood methylome not only reflects PD-associated epigenetic alterations but also evolves with disease progression.

More cutting-edge research has developed diagnostic models based on 5-hydroxymethylcytosine (5hmC) epigenetic signatures in blood DNA, demonstrating excellent performance for early PD detection. The area under the curve reached 0.923 in both training and test sets, and the model scores significantly correlated with the severity of motor symptoms (Wang et al., 2025). While multi-omics-based predictive models show strong performance, many of the identified metabolites, proteins, and epigenetic alterations may reflect general mechanisms of neurodegeneration, rather than pathogenic processes unique to PD. Disrupted purine metabolism and activated inflammatory pathways are typical examples. These candidate markers therefore require rigorous validation in large cohorts with appropriate neurodegenerative disease controls to confirm their diagnostic specificity before clinical application. Epigenetic biomarkers, as dynamic and reversible modifications, not only aid in early diagnosis but may also reveal the impact of environmental exposures on PD risk and offer new possibilities for monitoring the effects of interventions.

The deep integration of multi-omics data with machine learning models has significantly enhanced the capability for early prediction and precise subtyping of PD, marking a transition in research from single-modality, static descriptions to multi-modal, dynamic, and predictive approaches. However, clinical translation in this field still faces challenges such as data standardization, model interpretability, and generalizability. Overcoming these bottlenecks and ultimately advancing precision medicine for PD will require the construction of large-scale, longitudinal, multi-center multi-omics databases along with the development of more robust and interpretable AI algorithms.

3.6 Clinical and Digital Biomarkers

While molecular and imaging biomarkers offer precision, they often depend on specialized equipment. In contrast, the combination of clinical phenotypes and digital technology provides complementary solutions for community-level screening and long-term monitoring of PD, characterized by low cost and high accessibility. Beyond molecular and imaging biomarkers, clinical features derived from practice, which are easily obtainable, and digital phenotypes based on modern sensing and data analysis technologies show substantial practical value and broad application prospects in the early screening, diagnostic support, and long-term disease management of PD.

3.6.1 Prodromal Clinical Phenotype Markers

Although not "molecular biomarkers" per se, these markers can efficiently identify high-risk populations and provide clues for subsequent pathological biomarker testing.

3.6.1.1 Hyposmia

Hyposmia is one of the most common prodromal symptoms of PD. The University of Pennsylvania Smell Identification Test (UPSIT), as a standardized tool, is widely used to screen high-risk individuals. Research has found that loss of smell is significantly associated with the risk of synucleinopathy. Furthermore, in patients with iRBD, abnormal olfactory test results can predict conversion to PD or DLB (Miglis, et al., 2021). For example, a study based on the PPMI cohort showed a high concordance between hyposmia and positive CSF α-Syn-SAA results, particularly in sporadic PD (Siderowf, et al., 2023). Olfactory testing is cost-effective, simple to perform, and suitable for primary care settings and large-scale screening.

3.6.1.2 iRBD

iRBD is currently recognized as one of the strongest prodromal markers for PD. Patients exhibit dream-enactment behaviors during REM sleep, such as shouting, punching, or kicking, due to the loss of the normal muscle atonia that characterizes this sleep stage. Long-term prospective cohort studies have confirmed that individuals with iRBD represent the highest-risk group for converting to defined synucleinopathies, such as PD or DLB. Blood biomarker research provides strong biological corroboration for this. For instance, one study showed that pathogenic α-Syn can be detected in the blood 1 to 10 years before a clinical PD diagnosis, with all subsequently diagnosed patients testing positive on blood α-Syn-SAA prior to symptom onset (Kluge et al., 2024). Studies have found that 75.3% of iRBD patients have misfolded α-Syn in their CSF, and 38.1% converted to PD or DLB after a follow-up of 2.48 years. α-Syn positivity, mild cognitive impairment, and abnormal DAT-SPECT were identified as independent risk factors predicting this conversion (Muñoz-Lopetegi et al., 2024). Additionally, p-α-Syn deposits in skin biopsies and positive RT-QuIC assays show high positivity rates in iRBD, offering complementary, less invasive diagnostic avenues (Kuzkina et al., 2021). These biomarkers aid in identifying high-risk individuals during the prodromal phase, providing a critical time window for potential neuroprotective therapies. As a clinical marker, iRBD successfully enriches a high-risk prodromal PD population, offering an ideal cohort for clinical trials targeting disease-modifying therapies. It also serves as a crucial indicator for clinicians to initiate long-term follow-up monitoring of these patients.

3.6.2 Digital Biomarkers

The rise of digital phenotyping in recent years has brought significant changes to the objective assessment of PD. It uses wearable sensors (e.g., smartwatches, wrist-worn accelerometers) and/or ambient sensors to continuously, non-invasively, and frequently quantify various motor symptoms such as tremor, gait abnormalities, bradykinesia, and postural instability. A key study published in Nature Medicine, based on

large-scale population data from the UK Biobank, perfectly illustrates this. The research showed that daytime activity acceleration data collected by wearable devices, when analyzed by machine learning models, could effectively identify individuals who would later develop PD up to seven years before clinical diagnosis. The predictive performance of this model was significantly superior to that of traditional models based on genetics, lifestyle, blood biochemical markers, or known prodromal symptoms (Schalkamp et al., 2023). This highlights the technology's immense potential as a low-cost, non-invasive screening tool for early PD warning and for enrolling high-risk individuals into neuroprotective clinical trials.

Another study went a step further by integrating digital gait sensor data, metabolomics, and clinical data, using machine learning to assess the ability to diagnose PD, predict motor severity, and identify comorbidities. The results showed that gait data alone could effectively distinguish PD patients from controls (AUC as high as 92%) and predict motor symptom severity (AUC of 75%). Furthermore, multimodal data fusion significantly enhanced the predictive performance for detecting complex comorbidities like hallucinations (Brzenczek et al., 2024).

Digital biomarkers offer the advantages of objectivity and the ability to be collected continuously in real-world environments, providing high clinical relevance and effectively capturing subtle changes often missed during brief clinic examinations. According to their functional utility, while digital biomarkers currently have limited capability for making a definitive clinical diagnosis compared to molecular markers, they offer irreplaceable value in monitoring natural disease progression. By continuously capturing high-frequency, real-world motor and non-motor data, they are exceptionally well-suited for demonstrating the symptomatic efficacy and tracking the pharmacological impact of treatments on disease trajectory, particularly for therapies targeting highly fluctuating symptoms. However, despite their significant advantages, this technology currently still has limitations. These include susceptibility to interference from individual activity habits (e.g., lower daily activity in older adults), challenges with patient adherence due to the need for long-term device wear, and a lack of unified standards for data analysis and interpretation.

Integrating digital metrics derived from wearable devices with molecular and imaging biomarkers is emerging as a powerful strategy to advance precision medicine in PD. For instance, combining continuous gait and tremor data captured by wearables with results from α-syn-SAA or cerebrospinal fluid neurofilament light chain levels can provide an integrated view, encompassing both molecular pathology and its impact on real-world function. Similarly, integrating digital motor fluctuation data with DAT-SPECT or neuromelanin-sensitive MRI enables a more refined understanding of the relationship between nigrostriatal degeneration and daily motor variability. Such a multimodal integration approach not only improves diagnostic accuracy and prognostic stratification but also holds promise for enriching clinical trial cohorts by enrolling patients with both biomarker-verified pathology and quantifiable digital endpoints, thereby enhancing the sensitivity to detect therapeutic effects in disease-modifying clinical trials.

4 Summary and Outlook

The landscape of PD biomarkers has rapidly evolved from static concentration measurements to functional and multimodal assessments. Recent progress is driven mainly by three core advances: the ultrasensitive detection of pathological seeding activity via α-Syn-SAA, deep molecular subtyping enabled by multi-omics and AI, and enhanced clinical accessibility through peripheral biofluids.

To translate these analytical insights into a tangible clinical roadmap, we propose a biomarker-driven precision medicine workflow for PD (**Figure 4)**. This framework integrates the multimodal biomarkers discussed throughout this review into a sequential four-stage process, ranging from prodromal screening to targeted intervention, thereby operationalizing the transition from molecular pathology to precision diagnostics.

However, to successfully translate these tools into precision medicine, we must move beyond generalized observations. The utility of each biomarker strictly depends on the specific clinical or research objective. As systematically summarized in **Table 2**, we provide targeted recommendations for selecting appropriate biomarkers based on distinct goals.

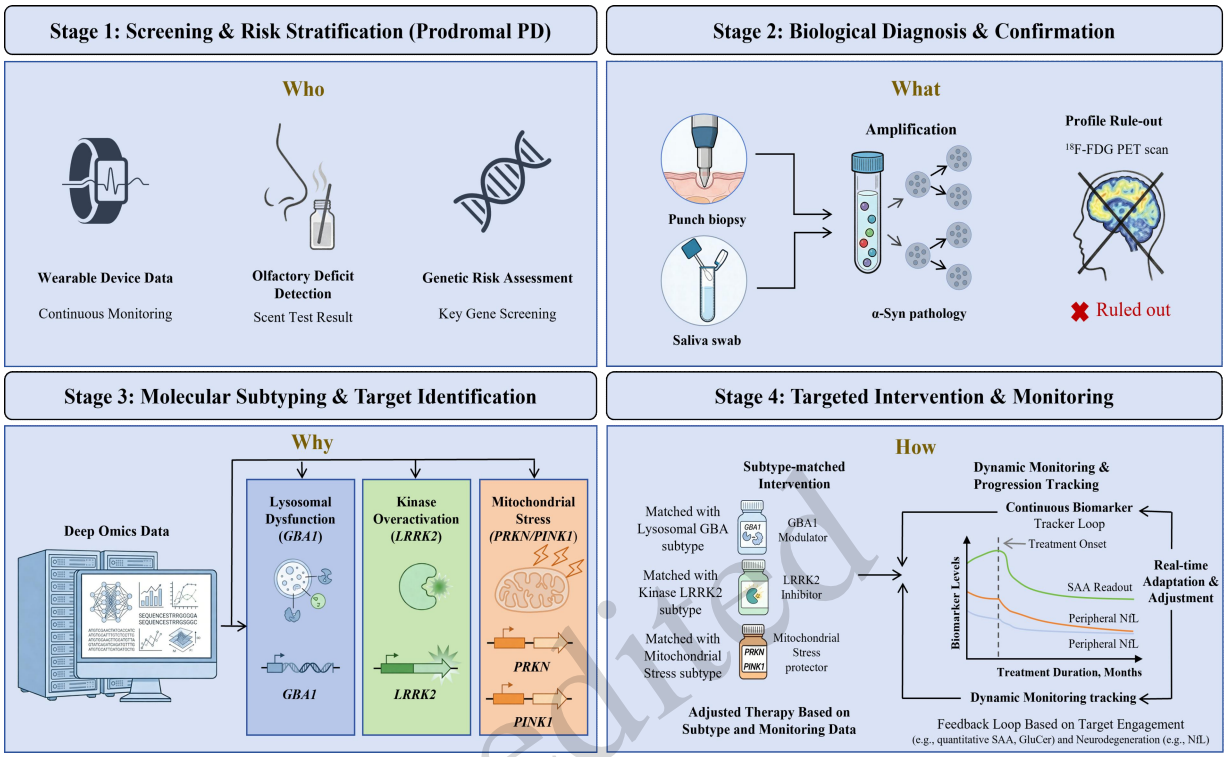

Figure 4. Proposed Clinical Workflow for Precision Medicine in PD. To transition the concept of precision medicine from theoretical frameworks into routine practice, we propose an ideal, biomarker-driven clinical workflow comprising four sequential stages:

Stage 1: Prodromal Screening and Risk Stratification (The "Who"). In the primary care setting, cost-effective and non-invasive tools should be deployed first. Wearable digital biomarkers (detecting subtle motor slowing or sleep disturbances like iRBD) combined with olfactory testing and genetic screening for common variants (*GBA1*, *LRRK2*) will serve to stratify high-risk populations long before the onset of classic motor symptoms.

Stage 2: Biological Diagnosis and Differential Confirmation (The "What"). Once a high-risk individual is identified, definitive molecular confirmation is required. The current ideal workflow utilizes minimally invasive peripheral α-Syn-SAA (e.g., skin biopsy or saliva) to confirm underlying synucleinopathy. Concurrently, blood NfL testing and ^{18}F-FDG PET imaging are then applied to rule out APS, ensuring diagnostic purity.

Stage 3: Molecular Subtyping and Target Identification (The "Why"). Upon confirming PD, the workflow shifts to deep phenotyping. Rather than categorizing patients solely by clinical symptoms (e.g., tremor-dominant), patients undergo comprehensive genomic sequencing and fluid multi-omics profiling. This defines their intrinsic biological subtype—identifying whether their disease is primarily driven by lysosomal dysfunction (e.g., *GBA1*), kinase overactivation (e.g., *LRRK2*), or mitochondrial/inflammatory stress (e.g., *PRKN*).

Stage 4: Targeted Intervention and Dynamic Monitoring (The "How"). In the therapeutic phase, treatment is precisely matched to the molecular subtype (e.g., assigning a *GBA1*-PD patient to a glucosylceramide synthase inhibitor trial). Long-term monitoring pivots away from subjective clinical rating scales towards objective, dynamic biomarkers: utilizing quantitative longitudinal α-Syn-SAA to assess anti-aggregation efficacy, target-specific markers (e.g., plasma GluCer or PBMC p-RAB12) to verify pharmacological target engagement, and continuous digital tracking paired with NfL to evaluate overall disease progression and neuroprotection.

Table 2 Comparison of Research Methods for PD Biomarkers: Clinical, Scientific and Informational Value with Core Application Scenarios

Biomarker Category	Core Technology / Target	Key Biomarkers	Main Strengths	Main Limitations	Clinical Value	Research Value	Info Value	Core Application Scenarios
α-Syn Pathology	SAA (RT-QuIC, PMCA), immunoassays	α-Syn-SAA, p-α-Syn, o-α-Syn, T-α-Syn	Directly detects core pathology; ultra-high sensitivity/specificity (>90%); detects prodromal phase; quantitative parameters enable risk stratification	Lower sensitivity in certain genetic subtypes (e.g., *LRRK2*); peripheral samples lack standardization; limited diagnostic efficacy of T-α-Syn	+++	+++	+++	Diagnosis (especially prodromal); Pharmacological effects (pharmacodynamic marker for α-Syn-targeted therapies)
Genetic (*SNCA*)	DNA sequencing, genotyping, methylation analysis	*SNCA* point mutations, multiplication, methylation levels (blood)	First identified PD causative gene; clear gene-dosage effect; high penetrance for point mutations; epigenetic modifications linked to sporadic PD	Rare point mutations; extremely rare multiplication; high clinical heterogeneity; peripheral blood expression affected by cell composition	++	+++	+++	Diagnosis (rare familial PD); Progression (multiplication indicates early-onset/rapid decline); Pharmacological effects (genetic stratification for trials)
Genetic (GBA1)	DNA sequencing, genotyping	GBA1 mutations (e.g., N370S, L444P)	Most common genetic risk factor; clearly associated with rapid progression and high cognitive risk; plasma GluCer serves as a pharmacodynamic marker	Incomplete penetrance; interpretation requires family history; targeted therapies still under development	+++	+++	+++	Diagnosis; Progression (warns of rapid cognitive decline); Pharmacological effects (GluCer as marker, enrollment in targeted trials)
Genetic (*LRRK2*)	DNA sequencing, genotyping	*LRRK2* mutations (e.g., G2019S)	Major cause of autosomal dominant PD; relatively benign disease course; p-RAB12 serves as a target engagement marker	Incomplete penetrance; ethnic-specific prevalence; lower α-Syn-SAA positivity rate	++	+++	+++	Diagnosis (early-onset, specific ethnicities); Progression (relatively benign course); Pharmacological effects (p-RAB12 monitoring)
Genetic (*PRKN/PINK1*)	DNA sequencing, genotyping	*PRKN, PINK1* mutations	Major cause of autosomal recessive early-onset PD; clear mitochondrial dysfunction; serum IL-6 and mtDNA serve as markers	High clinical heterogeneity; Lewy pathology absent in some cases; targeted therapies not yet mature	++	+++	+++	Diagnosis (early-onset, recessive families); Progression (slow progression); Pharmacological effects (exploratory for mitochondrial therapies)
Structural MRI (Nigrosome-1)	High-resolution SWI / QSM	Nigrosome-1 ("swallow tail" sign)	Non-invasive; high spatial resolution; directly visualizes nigral microstructural changes (e.g., iron deposition)	Dependent on scanning parameters and expertise; interpretation requires training; normal anatomical variations may affect specificity	+++	++	++	Diagnosis (Differential diagnosis: distinguishing PD from APS)

Structural MRI (Neuromelanin)	Neuromelanin-sensitive MRI	NM-MRI signal, SN/LC volume	Non-invasive; specifically visualizes dopaminergic neuronal populations; directly reflects neuronal loss	Lacks standardization across clinical centers; lower accessibility than conventional MRI	++	++	++	Diagnosis (adjunctive for early PD); Progression tracking (longitudinal assessment of neuronal loss)
Molecular Imaging (Dopamine)	DAT-SPECT / PET	DAT-SPECT / PET binding ratio	"Gold standard" for nigrostriatal integrity; objectively visualizes functional deficits; high specificity	Radiation exposure; high cost; "floor effect" in moderate-to-advanced stages; cannot differentiate PD from APS	+++	++	++	Diagnosis (early diagnosis, differentiation from ET/drug-induced); Progression tracking (early stages)
Molecular Imaging (Metabolism)	^{18}F-FDG PET	PD-related spatial covariance pattern (PDRP), APS-specific patterns	Exceptional efficacy in differentiating PD from APS (AUC ~0.97); reveals disease-specific metabolic network patterns	Radiation exposure; high cost; metabolic changes are not intrinsically PD-specific; requires pattern recognition or AI	+++	+++	++	Diagnosis (precise differentiation of PD from MSA, PSP); Pharmacological effects (PDRP as an efficacy endpoint)
Fluid (CSF)	Immunoassays, mass spectrometry, SAA	α-Syn species, NfL, Aβ42, p-tau, DDC, sTREM2	Directly reflects CNS biochemical state; well-validated; enables robust multi-biomarker panels	Invasive (lumbar puncture); not easily repeatable; unsuitable for population screening	+++	+++	+++	Diagnosis (confirms pathology); Progression tracking (dynamic changes); Pharmacological effects (target engagement)
Fluid (Blood)	Immunoassays, exosome isolation, metabolomics	NfL, T-α-Syn, nEV-derived α-Syn/p-tau/Aβ, GluCer isoforms, metabolites	Minimally invasive; easily repeatable; ideal for longitudinal monitoring; exosomes enable CNS enrichment	Low CNS-specific protein concentrations; hemolysis interference; requires specialized processing	++	+++	++	Screening (high-risk populations); Progression tracking (continuous follow-up); Pharmacological effects (e.g., GluCer)
Fluid (Peripheral)	Saliva/urine assays, skin biopsy	Salivary α-Syn-SAA, skin p-α-Syn, HO-1, microRNAs; urinary aggregates	Non-invasive (saliva/urine); minimally invasive (skin); high patient acceptability	Diurnal variations; dietary/microbiome interference; less standardized than CSF/blood	++	++	++	Screening (early/prodromal); Diagnosis (adjunctive, combined with clinical data)
Multi-omics + AI	Metabolomics, proteomics, epigenomics, machine learning	Metabolite panels, protein signatures, DNA methylation/hydroxymethylation	Captures disease heterogeneity; enables discovery of novel pathways; high predictive performance (AUC >0.95)	Requires large datasets; lacks standardization; interpretability challenges; computationally intensive	+	+++	+++	Diagnosis (future multi-marker panels); Progression tracking (dynamic profiling); Pharmacological effects (multidimensional efficacy)
Digital + Clinical	Wearables, olfactory tests, sleep studies	Gait/activity metrics, UPSIT, iRBD status	Low-cost; non-invasive; continuous real-world data collection; captures prodromal features	Susceptible to behavioral confounders; adherence challenges; lacks unified standards	+++	++	++	Screening (population stratification); Progression tracking (continuous real-world assessment); Pharmacological effects (functional outcomes)

Notes: +++, High / Very High (Primary application); ++, Medium / Medium-High (Secondary application); +, Emerging / Limited (Exploratory application). Info Value, Informational Value.

Abbreviations: APS, atypical parkinsonian syndromes; CSF, cerebrospinal fluid; DAT, dopamine transporter; DDC, DOPA decarboxylase; ET, essential tremor; GluCer, glucosylceramide; HO-1, heme oxygenase-1; LC, locus coeruleus; MSA, multiple system atrophy; nEV, neuron-derived extracellular vesicles; NfL, neurofilament light chain; NM, neuromelanin; PET, positron emission tomography; PMCA, protein misfolding cyclic amplification; PSP, progressive supranuclear palsy; QSM, quantitative susceptibility mapping; RT-QuIC, real-time quaking-induced conversion; SAA, seed amplification assay; SN, substantia nigra; SPECT, single-photon emission computed tomography; sTREM2, soluble triggering receptor expressed on myeloid cells 2; SWI, susceptibility-weighted imaging; UPSIT, University of Pennsylvania Smell Identification Test.

Based on current evidence, we propose the following framework for optimal biomarker deployment. First, for diagnosing clinical PD and differentiating it from APS, relying on a single modality is insufficient. While α-Syn-SAA is unparalleled for confirming underlying synucleinopathy, it cannot reliably distinguish PD from MSA. Therefore, a robust diagnostic panel should combine CSF or peripheral α-Syn-SAA with blood NfL (which is selectively elevated in APS) and/or ^{18}F-FDG PET (Hansson, et al., 2017). This combination successfully verifies the core molecular pathology while effectively ruling out atypical variants. Second, for identifying prodromal PD, the optimal strategy relies on combining clinical enrichment with molecular confirmation. Cost-effective digital biomarkers and clinical phenotyping (e.g., identifying iRBD or hyposmia) should serve as first-line screening tools to stratify high-risk populations (Siderowf, et al., 2023). Subsequently, applying minimally invasive peripheral α-Syn-SAA (e.g., skin or saliva) to these enriched cohorts provides definitive molecular confirmation years before motor onset. Third, for tracking disease progression, traditional diagnostic markers often exhibit a 'floor effect' or lack quantitative capacity over time. We expect that a multimodal approach will constitute the gold standard for monitoring disease trajectory, combining digital biomarkers (for continuous, objective assessment of real-world functional decline), longitudinal neuromelanin-sensitive MRI (for direct quantification of dopaminergic neuronal loss), and biofluid NfL (reflecting the ongoing intensity of neurodegeneration) (Tolosa et al., 2021). Finally, for demonstrating the pharmacological effects of disease-modifying therapies, target engagement must be verified by mechanism-specific functional markers. For therapies targeting α -synuclein aggregation, analyzing the quantitative kinetic parameters of longitudinal α-Syn-SAA represents the most direct approach. Conversely, for genetic subpopulations, target-specific readouts, such as plasma GluCer for *GBA1*-targeted interventions or PBMC p-RAB12 for *LRRK2* kinase inhibitors, are essential for confirming mechanistic efficacy (Giladi et al., 2023; Cortés, et al., 2025).

Building upon this framework, the field urgently requires rigorous technological standardization and large-scale validation in prospective, multi-center cohorts. Establishing standardized protocols and diagnostic thresholds for cutting-edge assays will ensure reliable and reproducible results (Simuni, et al., 2024). Through sustained efforts to match specific biomarkers with their optimal application scenarios, we can translate these analytical tools into tangible power for improving patient outcomes, guiding PD management fully into the era of precision medicine.

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Author contributions

Xiang LI and Honglin CHEN wrote the manuscript. Xu WANG and Yuhe HU contributed to the literature collection. Zhaofei YANG and Min WEI provided the original idea, revised the manuscript, and contributed to the funding acquisition. All Authors have read and approved the final manuscript.

Compliance with ethics guidelines

All authors declare that they have no conflict of interest. This review does not contain any studies with human or animal subjects.

Declaration on the use of generative AI tools

DeepSeek was employed as a supplementary language refinement tool to identify and rectify grammatical inaccuracies and enhance textual coherence during the preparation of the manuscript. Subsequently, all authors critically reviewed, revised, and finalized the work.

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