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## REVIEW

# Defining the Riddle in Order to Solve It: There Is More Than One “Parkinson’s Disease”

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**ABSTRACT: Background:** More than 200 years after James Parkinson described a clinical syndrome based on his astute observations, Parkinson’s disease (PD) has evolved into a complex entity, akin to the heterogeneity of

other complex human syndromes of the central nervous system such as dementia, motor neuron disease, multiple sclerosis, and epilepsy. Clinicians, pathologists, and basic science researchers evolved arrange of concepts

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and criteria for the clinical, genetic, mechanistic, and neuropathological characterization of what, in their best judgment, constitutes PD. However, these specialists have generated and used criteria that are not necessarily aligned between their different operational definitions, which may hinder progress in solving the riddle of the distinct forms of PD and ultimately how to treat them.

**Objective:** This task force has identified current inconsistencies between the definitions of PD and its diverse variants in different domains: clinical criteria, neuropathological classification, genetic subtyping, biomarker signatures, and mechanisms of disease. This initial effort for “defining the riddle” will lay the foundation for future attempts to better define the range of PD and its variants, as has been done and implemented for other heterogeneous neurological syndromes, such as stroke and

peripheral neuropathy. We strongly advocate for a more systematic and evidence-based integration of our diverse disciplines by looking at well-defined variants of the syndrome of PD.

**Conclusion:** Accuracy in defining endophenotypes of “typical PD” across these different but interrelated disciplines will enable better definition of variants and their stratification in therapeutic trials, a prerequisite for breakthroughs in the era of precision medicine. © 2023 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

**Key Words:** Parkinson’s disease; neurodegeneration; diagnostic criteria; neuropathology; biological definition; biomarker; Lewy body

## Introduction

Our understanding of Parkinson’s disease (PD) has evolved tremendously over the past 200 years, when it was initially described by James Parkinson, based on clinical observations.<sup>1</sup> Although the disease was initially catalogued as a movement/motor disorder, it is now widely accepted that its clinical manifestations extend far beyond the characteristic motor symptoms that are still the main required criteria for the clinical diagnosis of PD.<sup>2</sup> Progress in neuropathology and the advent of PD genetics<sup>3</sup> have greatly influenced our understanding of the biological mechanisms involved and have enabled us to interrogate the molecular underpinnings of the disease. However, despite great advances in the field, several traditional concepts continue to resist and be propagated, thereby often causing confusion and leading to the use of terminology that still does not distinguish idiopathic from genetic forms of PD. Ideally, these concepts should reflect our current best level of understanding of all the mechanisms that underlie the disease, thereby avoiding misconceptions and biases that may impede progress and distract the research community from questions that still need to be solved. For example, should we update the criteria defining PD? Should we continue to use motor symptoms as the basis for diagnosis? How do we best distinguish PD from other forms of parkinsonism? Should we continue to use the word “idiopathic” in forms of PD where genetic causes have been identified? How should we redefine and classify PD integrating the clinical features with the already-known underlying biology?

Here, we highlight several unresolved questions in the field with the aim of enhancing the dialogue between basic scientists and clinicians to revise and update definitions and terminology in the field of PD based on our best current knowledge.

Under the auspices of the International Parkinson and Movement Disorder Society (MDS), we assembled a task force named Biological Definition of Parkinson’s Disease. The major objectives of this task force were (1) to define typical PD on clinical grounds within a more precise definition of motor and nonmotor symptoms, (2) to delineate genomic sequence-based elements associated with complex versus monogenic forms of PD, (3) to update the neuropathological hallmark criteria of distinct variants that meet the clinical diagnosis of PD, and (4) to summarize the essential features of PD variants that should guide the development of useful laboratory models. Ultimately, our goal is to establish a novel basis for the discovery of (1) pathogenetic cause, (2) biomarkers of disease state, (3) markers of disease progression, and (4) targets for therapeutic interventions that best match mechanisms of disease in each patient.

## Basic Concepts

PD, as presently defined, represents the intersection of several different biological processes that, at a certain point, lead to axonal retraction and nigral degeneration of dopamine-producing neurons.<sup>4,6</sup> Although nigral degeneration alone is not the defining feature of PD, it is the basis for the efficacy of dopamine replacement therapy in these individuals.<sup>7</sup> The magnitude of symptom relief (even if temporary) by dopaminergic treatments for several motoric features of the disease is so overwhelming and unique for a neurodegenerative disease that it has introduced strong biases in our “nigrocentric” view of PD.

Currently, PD patients see a physician because mainly of their motor symptoms, receive a diagnosis based on clinical criteria (eg, UK Brain Bank Criteria<sup>8</sup>), receive

medication, join PD support groups, look up available disease information, and begin to understand the implications and likely treatment options as well as side effects from symptomatic therapies. Importantly, most patients meeting these criteria already have Lewy bodies (LBs) in their brains and do not display a simple Mendelian disease. Even within rare, Mendelian forms of the disease, most brains from patients will carry LBs, even though they may present with more variable phenotypes of the disease (Table 1).<sup>43</sup>

Added to this basic definition, there are many smaller groups of PD patients who present and evolve in a different manner, and we need to understand both the variability in the patients with LBs and the paths of those who do not display LB pathology (LBP). These smaller groups are important because they may provide pathomechanistic information on the neurodegeneration process and because they probably involve different molecular mechanisms. Therefore, these individuals should be involved in some more restricted clinical studies and may eventually be treated in different ways. Therefore, it is important that we are aware of these differences and be prepared to stratify the patients with the overall rubric. Additionally, we need to acknowledge that there are many individuals with dementia who have LBP but have a clinical phenotype that does not include parkinsonism. These individuals may also respond differently to dopamine replacement therapy but also to other pharmacological agents such as anticholinergics, monoamine oxidase B (MAO-B) inhibitors, Catechol-O-methyl transferase (COMT)-inhibitors, and glutamate/N-methyl-D-aspartate antagonists.

Thus, we are in a fluid situation where there is variability in terms of clinical phenotype, genetic markers, and pathological features (Fig. 1; Table 1). Any attempt to categorize the disease and define pathogenesis must always be considered a work in progress, which will need continuous updates, especially with regard to constant technological advances that improve our ability to detect relevant biomarkers.

Here, we present various operational definitions of PD, according to different perspectives, to illustrate the complexity of the issue and to promote a scientific discussion that should lead to a unified and comprehensive definition of the disease.

For the task force, it was advisable that an update in the definition of PD did not change the name of the disease, to avoid adding confusion in an already-diverse field.

## Clinicopathological Definition of PD

The most frequently written clinicopathological definition of PD, widely accepted by the clinical and scientific community, describes this clinical entity as a slowly

progressive neurological disorder with parkinsonism without features suggestive of an alternative diagnosis, responding to dopaminergic treatment, and associated with loss of *substantia nigra* neurons and the presence of LBs in some of the remaining neurons.<sup>7</sup> Although this definition is easy to explain and widely used, its application in clinical practice and research allowed for PD to be a heterogeneous neurologic disorder that varies widely in clinical manifestations and progression.

## Definition of Idiopathic PD

In general, when referring to “typical” PD, the word “idiopathic” has been used to differentiate it from other forms of parkinsonism with different phenomenology and, usually, worse prognosis (atypical parkinsonian syndromes) or with different causes (secondary parkinsonism). Although, in medicine, the term “idiopathic” refers to any disease with an unknown cause, the monogenic forms of PD that account for up to 5% of the sporadic cases are still included in the idiopathic PD (iPD) forms.<sup>44</sup> Therefore, this is clearly a topic that needs further consideration.

## Other Terms Applied to iPD

Other commonly used terms for the clinical entity PD include juvenile-onset PD, which refers to patients with an age of onset before 21 years, and young-onset PD/early-onset PD (EOPD) when the age of onset is between 21 and 50 years.<sup>45-47</sup> When we analyze the pathophysiological basis for this classification, we realize that the proposed cutoff age to separate between juvenile- and early onset is based on a higher risk of familial parkinsonism in patients with onset before 21 years and the absence of known hereditary factors identified over the age of 21.<sup>45</sup> However, these terms were introduced in 1987, whereas the first gene mutation responsible for PD was identified only in 1997.<sup>3</sup>

The term “EOPD” has also been used to describe the disease with different cutoff ages from 40 to 60 years.<sup>47,48</sup> In fact, recognizing this is an area that deserves additional attention (the MDS established a task force dedicated to this topic) to better define EOPD.<sup>47,49</sup>

## Definition of Atypical Parkinsonism

Although the term “parkinsonism” is widely used, there is no established definition. It usually refers to a clinical syndrome characterized by bradykinesia and two of three features: a 4- to 6-Hz resting tremor, muscular rigidity, and/or postural instability.<sup>50</sup> PD is the most common neurodegenerative cause of parkinsonism. Other neurodegenerative conditions associated

**TABLE 1** Neuropathological characteristics of selected genetic forms of PD

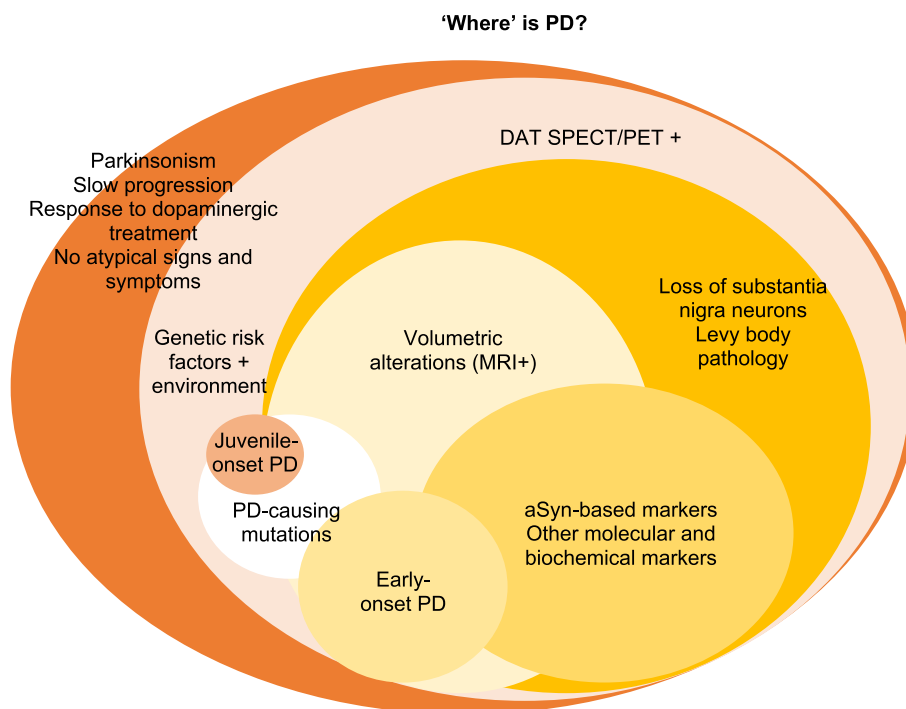
| Gene         | Epidemiology  | Parkinsonian endophenotype   | Neuropathology  |
|--------------|---|--|---|
| <i>LRRK2</i> | G2019S (4% of familial and 1% of sporadic PD, most common in Ashkenazi Jewish and North African Berber populations) <sup>9–12</sup><br><br>R1441G (found in up to 46% of autosomal dominant forms of PD in Basque populations) <sup>18</sup><br><br>G2385R and R1628P (variants found in 10% and 3% of PD patients in Asian populations, respectively) <sup>19,20</sup> | G2019S phenotype is similar to idiopathic PD with a slower rate of motor decline <sup>13,14</sup>  | Lewy body pathology is found in about 50% of <i>LRRK2</i> -PD brains <sup>15–17</sup>   |
| <i>SNCA</i>  | 0.2% of sporadic and 1%–2% of familial PD cases <sup>13</sup>   | Dose–effect seen with duplications and triplications: duplication phenotype is more similar to sporadic PD with earlier presentation and may feature depression; <i>SNCA</i> triplication carriers and point mutation carriers are more likely to be associated with earlier onset, rapid progression, more pronounced dysautonomia and dementia <sup>21</sup> | More diffuse and extensive burden of aSyn than idiopathic PD; depositions in brainstem and throughout cerebrum <sup>13</sup>  |
| <i>PRKN</i>  | Most common cause of autosomal recessive EOPD <sup>22</sup>   | EOPD resembles sporadic PD; levodopa responsive with notable features, including dystonia, slow progression, hyperreflexia, frequent dyskinesias and paucity of cognitive impairment <sup>23</sup>   | Lewy bodies are found in approximately one-third of <i>PRKN</i> -linked PD cases, and neuronal loss is most prominent in the ventral <i>substantia nigra</i> <sup>24,25</sup> |
| <i>PINK1</i> | Second most frequent cause of autosomal recessive EOPD <sup>26,27</sup>   | Slowly progressive, levodopa-responsive EOPD with symptoms developing before age 40 years <sup>27,28</sup>   | Few existing <i>PINK1</i> neuropathology reports suggest its deficiency causes <i>substantia nigra</i> cell loss but with the development of Lewy bodies <sup>29,30</sup>     |
| <i>DJ-1</i>  | Third most frequent cause of autosomal recessive EOPD; 0.4%–1% of AR PD cases <sup>26</sup>   | Motor phenotype similar to <i>PRKN</i> and <i>PINK1</i> -linked PD with a notable prevalence of dystonia (46% of cases) and dyskinesias (23% of cases) <sup>26</sup>   | Few <i>DJ-1</i> neuropathological studies exist; aSyn and Lewy body formation is seen consistently <sup>31,32</sup>   |
| <i>VPS35</i> | 0.4% of PD cases <sup>33</sup>  | Similar to idiopathic PD with earlier onset at age 50–60 years <sup>33,34</sup>  | Not available yet   |
| <i>GBA1</i>  | Variants occur in 2%–10% of PD subjects among non-Ashkenazi Jews; as high as 31% in Ashkenazi PD patients <sup>35–38</sup>  | Motor symptoms progress more quickly, and nonmotor features such as autonomic dysfunction, cognitive impairment, hyposmia and REM sleep behavior disorder are more common than in typical PD <sup>39–41</sup>  | Widespread Lewy body pathology is seen in virtually all cases of <i>GBA1</i> -linked PD <sup>24</sup>   |

Source: Adapted from Wise and Alcalay.<sup>42</sup>

Abbreviations: AR, autosomal recessive; aSyn, α-synuclein; EOPD, early-onset PD; PD, Parkinson’s disease; REM, rapid eye movement.

with parkinsonism are grouped together under the term “atypical parkinsonism” (eg, multiple system atrophy [type P], progressive supranuclear palsy [PSP], and corticobasal degeneration). Typically, they do not respond as well to dopaminergic treatments, develop less dyskinesia after chronic levodopa therapy, and have a more

rapid progression and thus a worse prognosis (ie, shortened life span) when compared to most cases with typical PD.<sup>51</sup> The motor syndrome can also manifest as a result of various vascular, drug-related, infectious, toxic, structural, or other causes that lead to “secondary parkinsonism.”



**FIG.. 1.** The biological definition of PD: where is Parkinson's disease? The schematic represents the various layers that form the biological basis for defining PD. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

## Criteria for PD

The proposed clinical criteria for the diagnosis of PD were mainly formulated to facilitate the differential diagnosis of PD from other parkinsonian syndromes. Among the most frequently applied criteria are the Gelb's criteria<sup>52</sup> and the UK Parkinson's Disease Society Brain Bank Diagnostic (UK Brain Bank Criteria).<sup>50</sup> Although these criteria have been used as diagnostic tools in clinical practice, they were mainly developed for research purposes.

The proposed Gelb's clinical diagnosis criteria were based on a review of the literature and propose three levels of diagnostic confidence: definite, probable, and possible. The diagnoses of definitive PD required histopathologic confirmation. However, it was made explicit in the same publication that there were no universally accepted histopathologic criteria for the diagnosis of PD, and the proposed criteria just summarized what had been used in the limited number of available clinicopathological series.<sup>52</sup>

The UK Brain Bank Criteria<sup>50</sup> were proposed based on a clinicopathological correlation study of histopathological findings from 100 patients diagnosed with iPD. Its application had a specificity of 98.6% and a sensitivity of 91.1%, and up to 10% of the diagnoses made in living patients were reclassified at postmortem examinations. Its use in earlier stages had even more severe limitations, as the criteria itself included as supportive

factors, aspects that are dependent on the progression of motor symptoms and response to levodopa.<sup>53,54</sup>

A task force of MDS proposed new Clinical Diagnostic Criteria for PD in 2015.<sup>2</sup> These criteria kept the core of the UK Brain Bank Criteria and incorporated and attributed more relevance to some nonmotor features of the disease. Although they were mainly developed for use in clinical research, they can also be applied in clinical practice. The criteria defined two levels of diagnostic certainty, (1) clinically established PD and (2) probable PD, and proposed a diagnostic approach based on three categories of diagnostic features: absolute exclusion criteria (which rule out PD), red flags (which must be counterbalanced by additional supportive criteria to allow diagnosis of PD), and supportive criteria (positive features that increase confidence in PD diagnosis). A retrospective application of these new criteria in a large cohort of PD patients suggested that they enable a slightly better separation of patients with atypical parkinsonism or secondary parkinsonism when compared with what is achieved using the UK Brain Bank Criteria.<sup>55</sup>

## Criteria for Prodromal PD

Prodromal disease has been defined as the disease stage where early symptoms or signs of neurodegeneration are present, but clinical diagnosis based

on motor parkinsonism is not yet possible. Anticipating the need to better define and classify the “premotor” and early stages of PD due to the clinical development of disease-modifying therapies, the MDS convened a task force to develop research criteria for prodromal PD. These criteria were developed based on the likelihood of prodromal disease being present and proposed a new approach for the assessment of the individual probability of prodromal PD. They include motor and nonmotor clinical symptoms, clinical signs, and ancillary diagnostic tests.<sup>56,57</sup> These criteria recognize as high-risk markers for prodromal PD the following factors: a sibling with PD with age onset below 50 years, *substantia nigra* hyper-echogenicity, probable rapid eye movement sleep behavior disorder (RBD) as identified by a polysomnogram, abnormal dopaminergic positron emission tomography/single-photon emission computed tomography (PET/SPECT), possible subthreshold parkinsonism (ie, the presence of one clinical sign, eg, bradykinesia), and olfactory loss.<sup>56,57</sup>

The formal inclusion of the *substantia nigra* hyper-echogenicity and dopamine transporter (DAT) neuroimaging as risk markers for PD constitutes the first association between clinical criteria and a potential physiopathological marker.

Recent results showed that the proposed criteria have a high specificity in identifying prodromal PD (mainly because of the high specificity of RBD as an included nonmotor symptom) but that the identified patients will meet the clinical criteria for PD only at later and uncertain time points.<sup>58,59</sup> Notwithstanding, we posit that, in the future, the boundaries of prodromal PD will evolve if we include nonmotor problems as “cardinal signs,” making it possible to diagnose PD before the onset of the typical motor features.

## PD Subtypes

Many PD subtype classifications have been proposed based on predominant clinical features and data-driven analysis. However, there is still no consensus on the most useful PD subtype classification in clinical practice and research and also on the one that better reflects the different trajectories in physiopathological mechanisms of the disease.

The oldest concept has been defined based on the predominant motor signs at an early stage and has defined the tremor-dominant and akinetic-rigid dominant clinical subtypes.<sup>60</sup> At later stages, another subtype has been proposed with relevant axial signs, including postural instability and gait disturbance (postural instability gait disorders [PIGD]). The PIGD subtype is also characterized by more severe disease manifestations at diagnosis and greater cognitive progression, more frequent hallucinations, and psychosis.<sup>61</sup>

In a prospective cohort study from Montreal, three subtypes were defined, namely motor/slow progression, diffuse/malignant, and intermediate. Patients with the diffuse/malignant phenotype were more likely to have mild cognitive impairment, orthostatic hypotension, and RBD at baseline. At follow-up, they showed a more rapid progression of cognitive decline, the presence of other nonmotor symptoms, more severe motor symptoms and signs, and a worse global composite outcome.<sup>62</sup>

In another study, using data from the Parkinson’s Progression Markers Initiative (PPMI), patients were classified again as having a “mild motor-predominant,” “diffuse malignant,” or an “intermediate” form. A hierarchical cluster analysis was performed using as key classifiers a motor summary score and three nonmotor features (cognitive impairment, RBD, and dysautonomia). Patients with the diffuse malignant subtype progressed faster in overall prognosis, with greater decline in cognition and in dopamine functional neuroimaging after an average of 2.7 years. Patients with diffuse malignant PD also had more atrophy in the brain network and the lowest level of cerebrospinal fluid (CSF) amyloid- $\beta$  and amyloid- $\beta$ /total-tau ratio (AD-like CSF profile).<sup>63</sup> In another study using PPMI data, five distinct motor subtypes were identified based on the motor assessment items: tremor dominant, axial dominant, appendicular dominant, rigidity dominant, and postural and instability gait disorder dominant.<sup>64</sup> Interestingly, a study that analyzed all published studies of data-driven PD subtype classification failed to demonstrate reproducibility in a cohort created for clinical research purposes—the Longitudinal and Biomarker Study in PD systems. These results question the validity and widespread use of these data-driven PD subtype classification systems.<sup>65</sup> An alternative option would be to shift to a semi-mechanistic model of PD based on progression of functional disability rather than individual clinical scales, defining the respective pathogenetic/genetic fingerprints in each cohort and determining discrete strategies to tackle the underlying pathology.

## Neuropathological Definition of PD—“It Is Complicated”

The main morphological correlate of the clinical Parkinson’s syndrome is the loss of nigral dopaminergic neurons that project to the basal ganglia or their profound dysfunction (such as in the case of neuroleptic exposure or normal-pressure hydrocephalus). The ventrolateral neurons are particularly vulnerable, whereas the dorsal and medial neurons are more resistant.<sup>16</sup> In PD, this neuronal loss is associated with LBP, but there are numerous other diseases that can lead to nigral neuronal cell loss with parkinsonism.<sup>66</sup>

PD is part of the spectrum of LB disease (LBD), which also includes PD dementia (PDD) and dementia with LB (DLB). Neuropathologically, these diseases are all

characterized by LBP, which refers to the presence of  $\alpha$ -synuclein (aSyn) aggregates in neuronal cell bodies and neuronal processes, termed LB and Lewy neurites, respectively. According to the Braak stages for LBP progression in PD,<sup>67</sup> LBP initially occurs in the dorsal motor nucleus of the vagal nerve (medulla oblongata) and anterior olfactory nucleus (olfactory bulb), followed by locus coeruleus (pons) and substantia nigra, where it leads to severe cell loss and parkinsonism. In later stages, LBP may spread to limbic and neocortical areas and be associated with clinical dementia. However, recent evidence indicates that aSyn aggregates can be observed in different peripheral tissues already during diagnosis, challenging the concept of spreading as initially hypothesized by Braak for PD.<sup>68,69</sup> The distinction between DLB and PDD is based on clinical findings: in PDD the onset of parkinsonism should precede dementia by at least 1 year, and in DLB cognitive impairment manifests before, during, or within 1 year of the onset of parkinsonism, suggesting that the Braak stages for LBP progression are not valid for DLB, where severe LBP may be initially seen in limbic and neocortical areas.<sup>70</sup> In fact, the Braak stages were proposed based on the study of PD and PDD patients, not DLB.

PD and DLB are the most common causes of parkinsonism (>50%).<sup>66</sup> Other diseases associated with parkinsonism, for example, multiple system atrophy (MSA), predominantly display aSyn aggregates in the form of glial inclusions (Papp Lantos bodies). aSyn inclusions are often also present in tauopathies (PSP, corticobasal degeneration, Guam Parkinson's dementia complex, and chronic traumatic encephalopathy) and in TAR DNA-binding protein (TDP)-43 proteinopathies (frontotemporal lobar degeneration TDP and Perry syndrome).

In addition, some genetic diseases show nigral neuronal cell loss and parkinsonism without specific protein accumulation (associated with mutations in PINK1, PRKN, POLG, and some forms of LRRK2). As explained earlier, parkinsonism may also be associated with nondegenerative factors such as vascular (vascular parkinsonism), toxic (manganese poisoning), drug induced (antipsychotic medications), and infectious (post-encephalitic parkinsonism or even SARS-CoV2).<sup>66,71,72</sup>

Although LBP is the hallmark pathology of LBD, other age-associated neuropathological changes may be seen in addition, and their prevalence in LBD increases with advancing age, reflecting respective findings in the general population.<sup>73-75</sup> Not surprisingly, hyperphosphorylated tau (eg, neurofibrillary tangles) and amyloid- $\beta$  (eg, amyloid plaques) pathology, which are the hallmark pathologies of Alzheimer's disease (AD), are particularly frequent in LBD, with up to 50% showing severe additional AD pathology<sup>76,77</sup> and over 20% showing limbic-predominant age-related TDP-43 encephalopathy.<sup>76,78</sup>

Although these pathologies are more frequent and more severe in DLB than in PD/PDD,<sup>79</sup> they have—together with cerebrovascular disease—an impact on clinical symptoms and biomarker profiles, thereby providing a neuropathological explanation for the heterogeneity of the PD phenotype.

It is important to highlight that the term “Parkinson's disease” is sometimes broadly used to refer to parkinsonism as such, irrespective of the underlying pathology (eg, “genetic PD”). However, the current neuropathological definition of PD refers to parkinsonism with nigral neuronal loss and LBP, whereas parkinsonism in general may have a plethora of different causes and associated pathologies.

### Genetic Forms of PD

The adoption of unbiased, genome-wide association approaches (GWAS [genome-wide association study]) yielded, so far, about 90 genomic loci containing common variability that influences the risk of developing PD.<sup>80</sup> These variants are present in the population but are of small effect size (odds ratios typically  $\sim$ 1.05–1.3). Whereas each variant on its own contributes little to PD risk, algorithms considering multiple risk variants in a calculated polygenic risk score (PRS) can be highly informative. In particular, PRS has thus far been associated with PD risk, age of onset, and rate of progression (in motor function and cognition).<sup>80-82</sup> Furthermore, even among high-effect-size variants (also called Mendelian forms of PD, see later), PRS is associated with disease status, that is, penetrance, in LRRK2<sup>83</sup> and in the expressivity of Gaucher disease (that is caused by biallelic variants at the GBA1 locus).<sup>84</sup>

On the contrary, unbiased genome-wide studies in rare, large families with multiple cases of PD (mostly clinically diagnosed) yielded a number of genes, which, if mutated, can cause monogenic (Mendelian) forms of disease (for recent reviews see Puschmann<sup>85</sup>). Although these forms are usually very rare, their identification has provided novel and important insights into molecular mechanisms and pathways implicated in the neurodegenerative process, and they have informed and fueled the current waves of biological studies, which, in turn, might translate into new targets for disease modification.<sup>86</sup>

### Mendelian Forms of PD

From the perspective of “a biological definition of PD,” the Mendelian forms challenge the validity of the current clinical and pathological definitions of this disease.

First, in some families with mutations in one of the most established PD-causing genes, such as SNCA or LRRK2, some individuals who carry the disease-



causing variant display clinical phenotypes that differ from those present in “classical PD” (eg, a dementing illness resembling LBD, or frontotemporal dementia [FTD], or atypical parkinsonism, resembling PSP).<sup>87,88</sup> Mutations in the SNCA gene encoding for  $\alpha$ -syn represent the prototypical forms of genetic PD. These are autosomal dominant forms and include point mutations (eg, A30P, E46K, G51D, and A53T/E), as well as gene multiplications—duplications or triplications. Apart from the p.A53T mutation, which is not uncommon in subjects of Greek descent, the other point mutations are quite rare, and it is thus difficult to have a comprehensive view of their phenotypic spectrum. Patients with the p.A53T mutation typically present with a classical motor onset of PD, although cases with a DLB-like or FTD-like presentation have been reported.<sup>87,89,90</sup> This generally presents a more severe form compared to iPD, with earlier onset and more rapid progression and with prominent nonmotor features of neuropsychiatric disturbances, dysautonomia, and dementia.<sup>89-91</sup> SNCA triplication cases are also quite severe, perhaps even more so than p.A53T cases,<sup>92</sup> whereas duplication cases have a more variable presentation and evolution.

Neuropathologically, genetic SNCA cases represent typical widespread synucleinopathies, with added features that may include predominant affectation of the neurites, oligodendroglial  $\alpha$ Syn deposition, and enhanced tau pathology (Table 1). Looking at the big picture, these cases, apart from some exceptions, do not provide a conceptual problem. They are, from a clinical and neuropathological perspective, typical synucleinopathies and represent a very fertile ground of research to understand how a genetic defect in the SNCA gene leads to PD.

Second, mutations in LRRK2 are the most common cause of a monogenic form of clinically typical PD although the proportion of asymptomatic carriers developing symptoms may be lower than initially predicted.<sup>93</sup> Yet, strikingly, LBs are not detectable in a substantial fraction of patients with LRRK2 mutations analyzed postmortem, and this pattern has been confirmed in patients with several disease-causing mutations in this gene (Table 1).<sup>15,94</sup> In some of these LRRK2 patients, tau-positive pathology is found.<sup>95</sup> In others, only nigral neuronal loss is present in the absence of distinctive protein aggregation.<sup>96,97</sup> Obviously, this broad pleomorphism challenges the current pathological definition of PD. Taking LRRK2 as a model for iPD would imply that LBs are not an invariable nor a necessary pathological feature associated with cell death and suggest that some of the patients with late-onset PD may not have LBP.

Third, LBs are detectable in an expanding list of monogenic neurodegenerative diseases, which are clinically far from the current clinical definition of PD, such as juvenile onset, rapidly progressive parkinsonism,

dystonia parkinsonism, or parkinsonism dementia, sometimes also associated with severe brain iron accumulation, such as patients with PLA2G6-associated neurodegeneration,<sup>98</sup> mitochondrial membrane protein-associated neurodegeneration (caused by c9orf12 mutations),<sup>99</sup> RAB39B mutations,<sup>100</sup> and VPS13C mutations.<sup>101</sup>

Of note, several variants in the glucocerebrosidase (GBA1) gene<sup>102</sup> and a single variant in LRRK2 (G2019S)<sup>103</sup> appear to be conceptually different, in that they represent strong genetic determinants of disease which, however, display intermediate frequency and effect size between the classical rare, highly penetrant Mendelian variants, on one end, and the common small-effect risk variants (GWAS), on the other end of the spectrum. In some populations (North African Berbers or Ashkenazi Jews) the LRRK2-G2019S variant is present in about 30% to 40% of the typical iPD patients, including familial and sporadic forms.<sup>104</sup>

Other monogenic forms of PD also present diverse motor features and LBP, hinting at complex underlying biology (Table 1).

If the wide clinical variability associated with the monogenic forms would be true for the disease in general, we should consider that PD, and at least some cases presenting as different neurodegenerative diseases (eg, DLB, PSP), might actually share the underlying biology. Understanding this may actually enable us to tackle the disease more accurately.

## Current “Diagnostic” Biomarkers

### Molecular Imaging Biomarkers

Molecular imaging biomarkers were first applied in PD over 30 years to assess dopaminergic denervation as well as changes in postsynaptic striatal dopamine receptors and brain metabolism related to disease progression and cognitive decline.<sup>105,106</sup> These first studies utilized PET with three key tracers (18F-fluorodopa, 11C-raclopride, and 18F-fluorodeoxyglucose) and were performed in limited cohorts, mainly for research purposes. However, the possibility to highlight defects in dopamine metabolism in both premotor and early PD has provided the groundwork for more extensive use of molecular imaging in clinical practice. The introduction of DAT imaging of the striatal DAT with SPECT has extended the availability of molecular imaging as a biomarker for clinical diagnosis. The diagnostic accuracy of DAT-SPECT imaging is very high, with over 90% sensitivity and specificity, but may differentiate only between degenerative and nondegenerative parkinsonism, as loss of DAT in the striatum is common to most conditions affecting the basal ganglia (including vascular lesions and atypical PD).<sup>107,108</sup> A number of studies have reported the impact of DAT-SPECT imaging on clinical

utility, suggesting that its use in the diagnostic workup is cost-effective, can shorten time to diagnosis, and change therapeutic management.<sup>109,110</sup> DAT-SPECT imaging has also been considered as a surrogate progression biomarker in clinical trials testing neuroprotection or disease modification (eg, recent studies on immunotherapies). To support this potential application, European medicines agency (EMA) just qualified DAT-SPECT to be used as an enrichment biomarker in PD clinical trials assessing dopamine deficiency consistent with parkinsonism as a tool to aid in subject selection.<sup>111</sup> Nevertheless, the significance of prospective changes in the DAT is challenged by the possibility of variable modulation of this transporter by the investigational products affecting tracer binding and by the limited relationship between the magnitude of loss and functional disability. In addition, the relationship between DAT-SPECT binding decrements and nigral cell degeneration is not linear. In particular, during onset of motor symptoms, loss of DAT may occur with relatively preserved nigral cell body function.<sup>112</sup>

Recently, PET imaging has been used to detect concomitant amyloid pathology in PD patients to assess its contribution to cognitive decline.<sup>113</sup> As many as 30% to 50% of PDD and DLB patients demonstrated diffuse deposits similar to those detected in AD, indicating that amyloid pathology plays an important but not exclusive role in cognitive decline in synucleinopathies<sup>114</sup> and/or reflecting co-occurrence of PD and the more frequent AD.

### Structural and Functional Magnetic Resonance Imaging

Compared to molecular imaging, magnetic resonance imaging (MRI) allows acquisition of multiple complementary biomarkers of brain functions for both clinical and research purposes in PD. Specific changes in the basal ganglia, brainstem, and cerebellum are now considered good biomarkers for early identification of atypical parkinsonism versus PD.<sup>115</sup>

Several studies have reported nigral changes in PD reflecting increased iron content using quantitative iron-sensitive techniques.<sup>116,117</sup> However, other studies have also reported overlap with healthy controls (HC), and a few found no changes between PD patients and HCs.<sup>118</sup> Increased iron content was also demonstrated in both asymptomatic and symptomatic LRRK2 and PRKN mutation carriers. Asymptomatic carriers had values in the range of those of PD patients, suggesting that iron deposition is an early event possibly occurring in premotor PD.<sup>119</sup> Independently of the role of iron in the substantia nigra and its contribution to the pathophysiology of PD, a recent trial showed that although it is possible to reduce the nigrostriatal iron content using a chelator (deferiprone), it was associated with an aggravation of parkinsonism questioning the potential

benefits of such an intervention and the true role of iron in the pathogenesis of PD.<sup>120</sup>

An alternative approach is to investigate nigral pars compacta changes profiting from paramagnetic properties of neuromelanin and using high-resolution T1-weighted images.<sup>121</sup> This approach has shown better diagnostic accuracy than iron nigral signal in separating PD from essential tremor and HCs,<sup>122</sup> and also in LRRK2 patients.<sup>123</sup>

Finally, volumetric and functional resting-state MRI techniques have been used extensively as biomarkers of progression, particularly to detect changes related to cognitive decline. Dopamine depletion and spread of the pathology to the cortex lead to regional gray matter thinning, decreased coupling in the corticostriatal sensorimotor network and between the striatum and the brainstem, and global changes in brain dynamics. Currently, application of MRI biomarkers should be mainly considered explorative, because reproducibility and harmonization of acquisition and methods of analyses across different scanners remain a relevant limiting factor.<sup>124</sup>

### Biochemical and Molecular Markers

As described earlier, the heterogeneity of PD, the complexity of underlying causes, and the gradual progressive decline of various functions indicate that a single, nonimaging-based biomarker is unlikely to be sufficient for the diagnosis of PD. Instead, a combination of different biomarkers, or perhaps even an algorithm, will be required to diagnose each variant at different stages of illness.

Despite the aforementioned variability, the accumulation of misfolded  $\alpha$ -syn in LBs is still considered a principal pathological hallmark of typical PD. Therefore, the focus of intense research activity in the field for more than 15 years has been the quantification of  $\alpha$ -syn for biomarker purposes. In particular,  $\alpha$ -syn is currently considered to be released and to spread between cells, possibly via the extracellular space, reaching the CSF and interstitial fluid. In fact, the presence of full-length  $\alpha$ -syn has been convincingly shown in extracellular matrices, such as plasma, conditioned cell media, and CSF.<sup>125,126</sup> It is currently considered that only a minute amount of the abundant intracellular aSyn protein is physiologically released into the extracellular space. Most studies suggest a 10% to 15% reduction in total  $\alpha$ -syn in the CSF in PD patients, consistent with findings in other  $\alpha$ -syn-aggregation disorders, such as DLB and MSA.<sup>127-129</sup> However, recent evidence indicates that the characteristics of CSF aggregates of aSyn that are associated with PD and MSA may correspond to different conformational strains of aSyn and can discriminate between the two disorders.<sup>130</sup> Studies identifying and quantifying posttranslationally modified

forms of aSyn in CSF (eg, oligomerization or phosphorylation) have been reported.<sup>131,132</sup>

Importantly, recent technological developments are enabling the detection of other forms of  $\alpha$ -syn, not only in the CSF but also in other body fluids.<sup>133</sup> Optimized aSyn seeding amplification assays, as informed by exciting advances in the biomarker field of prion proteins, have shown high sensitivity and specificity to diagnose PD.<sup>134-136</sup> These findings create excitement and hope that this platform could be used diagnostically in the future. At the present moment, four disadvantages of these assays still remain but are on the verge of being overcome: (1) time-consuming execution, (2) lack of a quantitative (rather than qualitative) readout that would enable the assessment of response to disease-modifying treatment over time, (3) requirement for routine standardization (ie, beyond the current small number of laboratories that can perform them), and (4) identification of the mechanism(s) by which the assays separate PD from other synucleinopathies. Although the effect of templating of PD-derived CSF samples has been clearly achieved through exogenously added, recombinant, full-length aSyn, the nature of the “templating agent,” that is, aSyn itself versus non-aSyn-based CSF constituent(s), has remained elusive. Interestingly, short RNA-sequencing revealed PD-characteristic patterns in both the CSF and blood.<sup>137</sup> These methods also need to be developed for higher throughput. Whether the slope of aggregation *in vitro* can separate between early PD and late PD as well as between typical PD and other forms of parkinsonism remains to be determined.

Besides aSyn, other marker candidates will likely be added to a diagnostic algorithm. With inflammation being a risk factor for PD,<sup>138,139</sup> inflammatory markers like interleukin 6, tumor necrosis factor- $\alpha$ , and others may be interesting candidates.<sup>140,141</sup> The critical question will be whether markers of cellular injury in the CNS (eg, neurofilament, tau-protein, glial fibrillary acidic protein) and circular RNAs that are not expressed by nonneuronal cells in the periphery could emerge as markers of PD progression. Neurofilament light chain as a marker for axonal damage has been shown to be significantly elevated in CSF and blood in various neurodegenerative disorders, including multiple sclerosis and amyotrophic lateral sclerosis.<sup>142</sup> In PD, neurofilament levels are slightly higher versus HCs and enable a good separation from other differential diagnoses (with markedly higher levels in atypical parkinsonian syndromes), even in serum, and show longitudinal correlation with disease progression.<sup>143</sup> Other CSF and blood markers have been studied in different cohorts and are currently being validated systematically in cohorts like the PPMI, which, as a matter of fact, suffer from the limitations of the clinical definitions.<sup>144</sup>

Apart from the more etio-pathological-related biochemical markers, it should not be forgotten that the recent update on the research criteria for prodromal PD recognized diabetes and low plasma urate levels in men as new prodromal markers, although it remains unclear if the association is causal.<sup>57</sup>

Tissue biomarkers (eg, aSyn in skin biopsies) are also currently being explored and could enable us to support the clinical diagnosis of PD, and/or of variants, in the future.<sup>145</sup>

## Redefining PD: Implications for Cell and Animal Models

### The Value of Model Systems

Cell and animal models of different diseases can often lack direct relevance for the human disease and for predicting clinical outcomes of investigational therapeutic strategies. Yet preclinical studies in cells and animals are usually required before clinical trials can proceed, and animal models are needed to identify and test pathophysiological mechanisms that cannot be studied in humans.<sup>146,147</sup> Because of the reasons discussed earlier, modeling a disease like PD has not proven to be trivial as it is not always clear which aspects of the disease should be modeled.<sup>147</sup>

### Classical Toxin-Based Models of PD

The classical animal PD models, which have been used since the 1960s, are based on the destruction of the nigrostriatal dopaminergic neurons with toxins, administered either locally or systemically. The most commonly used toxins are 6-hydroxydopamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, and, to a lesser extent, rotenone and paraquat. These toxin-based models have been useful for the identification and testing of treatments for the cardinal motor deficits of PD. As such, these models have contributed to the development of dopamine agonists and deep brain stimulation and also provide useful tools for developing treatments for levodopa-induced dyskinesia. However, they have failed to predict clinical success of neuroprotective strategies, as such models are unlikely to reproduce the pathophysiological mechanisms of the disease.

### Genetic-Based Models: The Need for Doing Better

The difficulties in defining PD clearly indicate that no model can be seen as a “perfect” model of such a complex disease. At the very least, a “good” model should be based on a mechanism that is known, from studies in humans, to be relevant to at least some forms of the disease.

Mechanisms uncovered by rare genetic forms turn out to contribute to our understanding of sporadic

PD. Therefore, genetic models become highly relevant for testing hypotheses and neuroprotective treatments. The difficulty remains to define which aspect(s) of PD need to be present for the model to qualify as a “good” model of the disease.

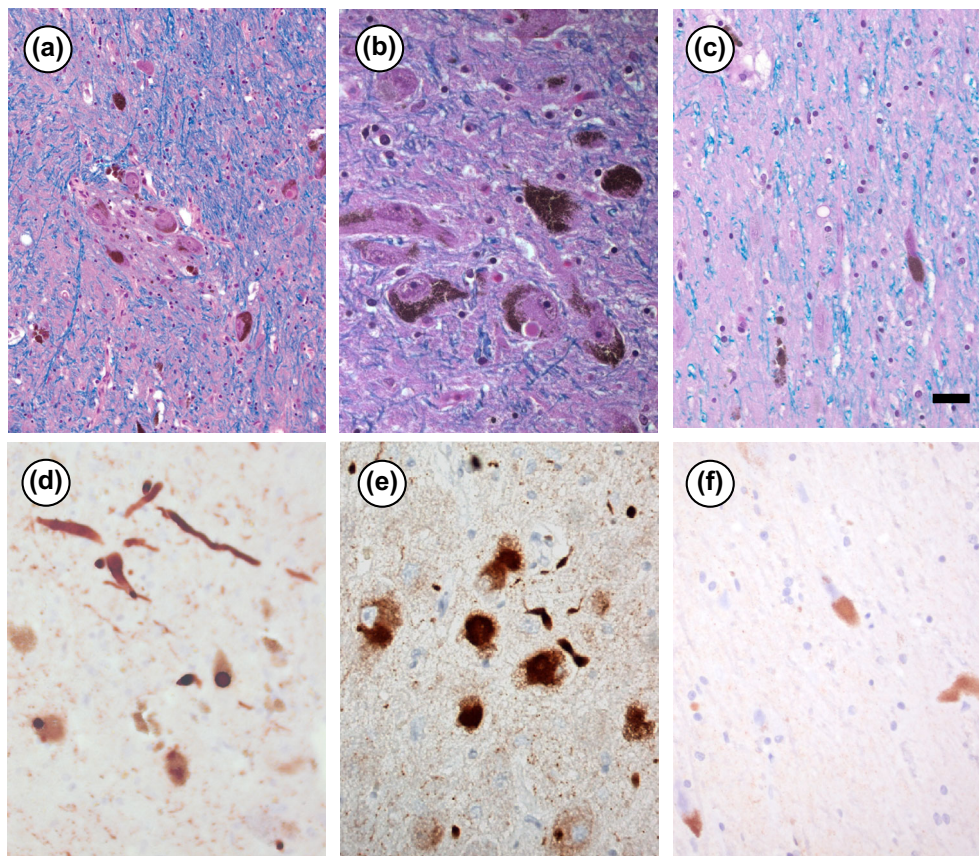
A diverse range of animal models are extremely important for addressing specific aspects of the disease. For example, simple and versatile model organisms such as the invertebrates *Caenorhabditis elegans* and *Drosophila* are useful to elucidate genetic interactions and mechanisms; and mammalian models in mice, rats, and, in some cases, nonhuman primates are critical to reproduce circuit-level alterations.

In conclusion, the “best” model of PD is one that best enables us to address the question being asked, rather than an elusive animal model that would reproduce all phenotypical aspects of PD. Because those aspects are not necessarily present in all patients or forms of the disease, a useful model should rather reproduce mechanisms that are disease relevant and match the intended drug target. Thus, the current difficulty in providing a simple and

universal definition of PD illustrates the need for multiple models and for critical evaluation of key pathologies and phenotypes in each model. Which specific aspect(s) of the disease does it reproduce? How meaningful and robust are the endpoints? To what extent can the findings in this particular model be generalized to span other forms of PD? Similar to the clinician who needs to recognize PD behind diverse clinical presentations and possible causes, basic and translational scientists should be open to recognize how a particular model is suited for the study of any particular aspect of this complex disorder. All models that are genetically relevant to PD should therefore be viewed as useful for the identification and mechanistic understanding of central and peripheral symptoms and pathologies found in disease.

### Cell Models of PD

Cell models of autosomal dominant forms of genetic PD, relying on the expression of wild-type or mutant forms of the proteins, are very useful for assessing



**FIG.. 2.** Neuronal loss in the substantia nigra pars compacta does not correlate with Lewy body (LB) pathology. (A–C) Luxol hematoxylin eosin (LHE) staining (as described in Agin-Liebes et al<sup>148</sup>) of (A) PD case showing loss of pigmented neurons, presence of macrophages, and LB-containing neurons; (B) incidental LB disease case showing LB-containing neurons; (C) LRRK2 case (G2019S mutation) showing severe loss of pigmented neurons and no LB-containing neurons. Scale bar: 20 mm. (D–F) Immunostaining against aSyn ( $\alpha$ -synuclein, as described in Agin-Liebes et al<sup>148</sup>) of the same cases as in the images on top. (D) PD case showing LB- and LN (Lewy neurites)-containing neurons. (E) Incidental LB disease case showing LB- and LN-containing neurons. (F) LRRK2 case (G2019S mutation) showing no LB- or LN-containing neurons. Magnification 400 $\times$ . [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

specific molecular alterations, despite limitations due to their simplicity.<sup>148</sup> Importantly, cell-based models offer the possibility of still much-needed, large-scale efforts often referred to as “fishing expeditions,” in search of the molecular mechanisms underlying the PD pathophysiology.

### Concluding Remarks

iPD is a heterogeneous entity presenting with diverse clinical features that encompass motoric and nonmotor-based signs. Despite tremendous progress in our understanding of the pathological mechanisms and of clinical features involved, the underlying cause(s) of iPD is/are unknown in almost 90% of the cases. Strikingly, almost all diagnostic criteria applied in clinical practice and research are based on expert-based consensus, case series, and limited clinicopathological correlations. There are currently no established disease subtype classifications, which question the adequacy of the current clinical and biological factors used for the definition of progression models. Therefore, current subtype classifications are mainly generated using clinical data analysis with limited cohort sizes and lack of extensive neuroimaging, neurophysiological, and wet-laboratory markers, including genetics as well as autopsy confirmation. This results in weak correlation with biological markers and problems with replicability. Interestingly, new prodromal criteria strengthen the association with potential biological markers but do not reliably indicate the fact, the direction (ie, iRBD developing into MSA, PD, DLB), or the time of conversion. Protective factors (eg, resilience or genetic factors) have also not been studied extensively in this regard.

In conclusion, although there is still a tremendous need to improve our understanding of the underlying biology leading to PD, we are confident that we are now at a point where clinical, genetic, imaging, detailed pathological examinations and biological data should enable us to better define the “riddle” of PD to ultimately identify novel diagnostics and therapeutic strategies. ●

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Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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