Concept Paper

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Towards a Biological Definition of Parkinson's Disease

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Abstract: With the growing hope that disease-modifying treatments could target the molecular basis of neurodegenerative diseases even before the onset of symptoms, there is mounting pressure to define disease entities based on pathophysiology rather than on clinical syndromes. The Alzheimer's disease research community has recently transitioned from diagnostic criteria based on an amnestic syndrome to a purely biomarker-based disease definition, relying on the demonstration of amyloid-beta pathology, tau pathology, and neurodegeneration. In contrast, current diagnostic criteria for Parkinson's disease still rely on the presence of the well-described clinical syndrome of parkinsonism, with the addition of characteristic motor- and non-motor signs and symptoms. However, there is now unequivocal evidence that Parkinson's disease starts years before the onset of parkinsonism. Furthermore, neuropathologically defined Lewy body disease is clinically heterogeneous, combining a range of motor, non-motor, dopaminergic and non-dopaminergic features. Finally, clinically defined Parkinson's disease has diverse underlying etiologies most, but not all, associated with α-synuclein positive Lewy pathology. In light of recent scientific advances, we propose a biologically based definition for the diagnosis of Parkinson's disease, initially to be used for research purposes. The criteria use a three-component 'G-S-N' system. The first is documentation of defined gene variants ('G'), which cause or strongly predispose to PD as the most upstream component. The second is α-synuclein pathology ('S'), currently defined as pathological α-synuclein deposition in tissue or positive α-synuclein seeding assays. The third is evidence of underlying neurodegeneration ('N'), currently defined by specific neuroimaging procedures. The associated clinical syndrome ('C') is defined by a single high-specificity feature or multiple lower-specificity features. Initiating this transition will enable the field to fuel both basic and clinical research and move closer to the precision medicine required to develop clinically meaningful disease-modifying therapies. We acknowledge current limitations, ethical implications, and the need for prospective validation of this approach.

Keywords: Parkinson's disease; genetics; α-synuclein; neurodegeneration; clinical manifestation; diagnosis

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1. Introduction

A rapidly growing body of evidence is increasingly providing insights into the molecular etiology and pathogenesis of Parkinson's disease (PD), opening the realistic opportunity to develop clinically meaningful disease-modifying therapies.

Human genetics has taught us that PD, currently conceived as a clinicopathologic disease entity[1], may have various genetic or environmental causes that initiate the disease along different, only partially overlapping pathways[2-6].

Neuropathological findings have highlighted the pathogenic role of Lewy pathology (in Lewy bodies and Lewy neurites) and its major molecular component, the protein α-synuclein, which we refer to as "Parkinson's type synucleinopathy"[7,8]. The neuropathological perspective has helped to clarify the difference between PD and other synucleinopathies that lack Lewy pathology, such as multiple system atrophy (MSA), but has also challenged us to question the traditional boundaries between PD and dementia with Lewy bodies[9,10]. Importantly, neuropathology has also taught us that Lewy pathology is neither sufficient nor necessary for clinically-defined PD and there are cases with clinical and genetic PD diagnoses without this neuropathological feature[11].

Fluid, tissue, and imaging biomarkers are now sufficiently advanced to allow objective identification of genetic risk, pathological processes, and neurodegeneration, even before overt clinical symptoms have appeared.

Despite these advances, the currently accepted diagnostic criteria for PD are almost exclusively based on the identification of clinical features, and have been so for more than a century[1,10,12]. Notably, there is no single neurobiologically-based disease construct. Creating a biological diagnosis of PD as a disease entity with specific pathogenic mechanisms could serve as the basis for better preclinical and clinical objective diagnosis and staging, and for more accurate subdivision of PD according to active pathogenic mechanisms. In so doing, a biological diagnosis could advance research in multiple fields, including epidemiology, pathogenesis, biomarker discovery, and precision medicine development including disease-modifying therapies.

Here, we propose a biological definition of PD that includes genetic risk, the presence or absence of pathogenic α-synuclein in peripheral tissue or CSF, and the presence of characteristic features of neurodegeneration using selected imaging techniques. The establishment of a biological definition of PD recognizes that the biological processes that eventually lead to the development of the classic clinical features of PD are present long before the onset of these features, and that it is now possible to determine the presence of these changes much earlier than was previously feasible. We propose this definition and criteria initially for research purposes exclusively rather than as diagnostic criteria for clinical practice.

2. Development of the disease definition

Consensus method

References for this position paper were identified as described in the Search Strategy section. GUH and AEL initiated and coordinated the process. After initial discussions on the overarching structure of the biological definition of PD, working groups were formed for PD-specific pathogenic gene variants (CK, GUH), PD synucleinopathy (CHA, TFO, AEL), PD-associated neurodegeneration (WP, AJS), and PD-associated clinical status (DB, RP). The working groups reviewed the available evidence underpinning the use of the different biological constructs and were asked to propose solutions for each component, which were presented to the group in a series of face-to-face virtual rounds (total N=16 between June 2022 and March 2023) for critical discussion combined with follow-up emails until a unanimous consensus was reached on each component and its integrated interpretation.

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3. Biological definition of Parkinson's disease

3.1. PD disease concept

Selected PD-specific pathogenic gene variants may serve as the earliest definable upstream cause or predisposition to a Parkinson's type synucleinopathy, which is believed to result in PD-associated neurodegeneration, that eventually leads to corresponding clinical abnormalities in a wide range of domains. However, variations from this sequence are extremely common, such as the absence of PD-specific pathogenic gene variants in most cases of Parkinson's type synucleinopathy, or pathological changes lacking Parkinson's type synucleinopathy in a minority of cases with a definable genetic cause or predisposition. Moreover, the pattern of PD-associated neurodegeneration (and its subsequent clinical correlates) can vary considerably between individuals. Therefore, we have sought an overarching biological approach that encompasses this variability. We recognize that the clinical abnormalities associated with PD are part of a continuum that reflects disease stage and variability, but under a biologic definition, they will not be considered defining features of the disease. We propose that a biological disease definition provides an umbrella under which it is possible to harmonize different disease concepts that have been derived from a predominantly clinical perspective, including preclinical PD[13], pre-motor PD[13], prodromal PD[12,14], defined non-motor syndromes (REM sleep behavior disorder (RBD)[15], postganglionic pure autonomic failure (PAF)[16]) motor PD[10,17,18], PD with dementia (PDD)[19], and dementia with Lewy bodies (DLB)[19-21].

3.2. Genetics

A monogenic pathogenic variant predisposing to PD can be detected in ~15% of all patients [22]. Confirmed types of monogenic typical PD include four dominantly inherited forms (*SNCA*-PD, *LRRK2*-PD, *VPS35*-PD, *CHCHD2*-PD) and three recessively inherited forms (*PRKN*-PD, *PINK1*-PD, *PARK7*-PD)[23]. The likelihood of developing clinical PD depends strongly on the PD gene involved and, in the case of *SNCA* and *GBA*[24], also on the type of the pathogenic variant. With respect to *GBA*-PD, for the biological definition of PD, we only include those pathogenic *GBA* variants that greatly increase the risk of manifesting PD and, thus, can be viewed as acting in a dominant fashion with highly reduced (age-dependent) penetrance[25]. Reduced penetrance, i.e. the conditional probability of being affected by a disease given a particular pathogenic genotype, is well-documented in inherited disorders.[26] Detailed genotype and phenotype information on these conditions is available[27,28], and www.mdsgene.org, except for *GBA* and *CHCHD2*, which are in progress).

In the current context, we propose different levels of pathogenic effect (**Table 1**; **Supplement S1** for details):

- The first level comprises the fully penetrant variants: *SNCA* triplications, *SNCA* missense variants, as well as bi-allelic *PRKN*, *PINK1*, and *PARK7* missense, nonsense, small indels, and copy number variants.
- The second level comprises variants that confer a strong predisposition to PD but not complete penetrance, including *SNCA* duplications and pathogenic variants in *LRRK2*, *VPS35*, and *CHCHD2*.
- The third level, which results in an intermediate predisposition with highly reduced penetrance, consists of carriers of highly pathogenic *GBA* variants. *GCH1* monoallelic pathogenic variants [29,30] and 22q11.2 deletion syndrome[31] may also fall into this category, but are currently considered investigational due to limited knowledge.

A second aspect to consider with regard to the PD-specific pathogenic gene variants is the degree of predisposition for a Parkinson's type synucleinopathy (**Table 1**):

- Variants in *SNCA*[32] and *GBA*[25] unequivocally predispose to Parkinson's type synucleinopathy.
- *LRRK2* monoallelic (or biallelic) pathogenic variants predispose to Parkinson's type synucleinopathy in most cases, whereas neurodegeneration without synucleinopathy occurs in a minority[30].

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- Biallelic variants in *PRKN* predispose to a Parkinson's type synucleinopathy in approximately 20% of the cases[33].
- Only very few post-mortem reports are available for variants in *PINK1*[34,35], *PARK7*[36], and *CHCHD2*[37], and the 22q11.2 deletion syndrome[31], some associated with Parkinson's type synucleinopathy and others not, i.e. on a very low level of evidence.
- For variants in *VPS35* and *GCH1*, the predisposition for a Parkinson's type synucleinopathy is currently unknown[30].

We recommend reporting the PD-genetic status of a person as positive (G_F^+ or G_P^+, respectively), when a **f**ully penetrant pathogenic variant or a pathogenic variant with strong or intermediate **p**redisposition is confirmed (**Table 1**). All other conditions (pathogenic gene variants with low predisposition for PD or polygenic risk scores, or absent or unknown genetic contributions) are considered as G^- "genetically indeterminate".

Table 1. PD-specific pathogenic gene variants.

Designation	Biomarker Status	Abnormality	Pathogenic effect	Presdisposing for
G_F^+	Recommended	*SNCA* monoallelic triplication[30,32]	fully penetrant	Parkinson's type synucleinopathy
G_F^+	Recommended	*SNCA* monoallelic pathogenic single nucleotide variants[30,32]	fully penetrant	Parkinson's type synucleinopathy
G_F^+	Recommended	*PRKN* biallelic pathogenic variants[30,33]	fully penetrant	Neurodegeneration without synucleinopathy in ~80% and Parkinson's type synucleinopathy in ~20% of the cases
G_F^+	Recommended	*PINK1* biallelic pathogenic variants[30,34,35]	fully penetrant	Parkinson's type synucleinopathy in a single case and neurodegeneration without synucleinopathy in another
G_F^+	Recommended	*PARK7* biallelic pathogenic variants[30,36]	fully penetrant	Parkinson's type synucleinopathy in the single case published
G_P^+	Recommended	*SNCA* monoallelic duplication[30,32]	strong predisposition	Parkinson's type synucleinopathy
G_P^+	Recommended	*LRRK2* monoallelic (or biallelic) pathogenic variants[30]	strong predisposition	Parkinson's type synucleinopathy in most cases; neurodegeneration without synucleinopathy in a minority
G_P^+	Recommended	*VPS35* monoallelic pathogenic variants[30]	strong predisposition	unknown
G_P^+	Recommended	*CHCHD2* monoallelic pathogenic variants[30,37]	strong predisposition	unknown
G_P^+	Recommended	*GBA* monoallelic severely pathogenic variants[25,30]	medium predisposition	Parkinson's type synucleinopathy
G^-	Investigational	*GCH1* monoallelic pathogenic variants[30]	medium predisposition	Parkinson's type synucleinopathy in the single case published

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G^-	Investigational	22q11.2 deletion syndrome[30,31]	medium predisposition	Neurodegeneration without synucleinopathy in 1/3 and Parkinson's type synucleinopathy in 2/3 published cases

G_F^+: fully penetrant pathogenic gene variants, G_P^+: pathogenic gene variants with strong or intermediate predisposition. See **Supplement S1** for further details.

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3.3. Synucleinopathy

We recommend designating the Parkinson's type synucleinopathy status of a person as positive (S^+), when a pathological test specified in **Table 2** is confirmed. All other conditions are considered as S.

Table 2. Parkinson's type synucleinopathy.

Designation	Biomarker Status	Abnormality	Sensitivity*	Specificity*
S^+	Recommended	α-syn SAA in CSF	High	High
S^+	Recommended	α-syn SAA in skin	High	High
S^+	Recommended	α-syn ICH/ICF in skin	Moderate	High
S^+	Investigational	α-syn SAA in neuronal exosomes from plasma	Insufficient evidence	Insufficient evidence
S^+	Investigational	α-syn SAA in plasma	Insufficient evidence	Insufficient evidence
S^+	Investigational	α-syn SAA in submandibular gland	Insufficient evidence	Insufficient evidence
Exclusion criterion ruling out S^+	For S^+ testing unable to differentiate PD from MSA (e.g., selected SAA methodologies in CSF and SAA in skin to date)	Elevated Neurofilament Light chain (NfL)	High for atypical parkinsonism (e.g., MSA)	High for MSA but low for specific diagnosis (e.g., also elevated in PSP but these cases would be S^- in the absence of copathology)
Exclusion criterion ruling out S^+	For S^+ testing unable to differentiate PD from MSA*	Neuroimaging features of MSA (e.g., characteristic changes in the putamen, cerebellum and pons)	Moderate	High

*high> 80%; moderate >70 < 80%; low < 70%. α-syn: α-synuclein, CSF: cerebrospinal fluid, IHC: immunohistochemistry, IHF: immunohistofluorescence; MSA: multiple system atrophy, PD: Parkinson's disease, PSP: progressive supranuclear palsy, SAA: seeding amplification assay. See **Supplement S2** for further details.

The pathology of PD, with selected exceptions (see below), is defined by the presence of widespread aggregated α-synuclein as Lewy bodies and Lewy neurites in the central and peripheral nervous systems. It is widely believed that the deposition and spread of pathologic forms of misfolded

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α-synuclein are key aspects in the development and progression of the neurodegenerative process[38]. Therefore, we propose that pathological α-synuclein should be the defining attribute of the biochemical category of the disease definition (i.e., an α-synuclein positive or negative ($S^{+/-}$) designation). All individuals classified in a new biological definition as having "sporadic" PD must be S^{+} while some genetic causes will be S^{-} as they may lack α-synuclein aggregation (e.g., particularly most *PRKN*-PD (i.e. biallelic gene variant carriers)[33] and a proportion of *LRRK2*-PD cases[39]. Thus, we propose to include α-synuclein-negative forms of PD (S^{-}) in the biological definition of PD, if genetic markers with sufficient high penetrance are present.

Multiple methods have evaluated the presence of presumed pathogenic α-synuclein *in vivo* in biological fluids (CSF, saliva, blood, tears) and tissues (e.g., skin, salivary glands, gastrointestinal tract, olfactory mucosa) (**Supplement S2**). Currently, the evidence does not support using measurements of α-synuclein levels in biological fluids as a molecular marker of a biological diagnosis of PD[40,41]. Immunohistochemistry (IHC) and immunohistofluorescence (IHF) have been applied to multiple tissues. Currently, the data suggest that only skin biopsies, with specific methods, provide adequate sensitivity and specificity to be recommended for use as part of the biological definition of PD. The pattern and distribution of α-synuclein in the biopsy should be considered in differentiating PD from MSA.

The development of α-synuclein seeding amplification assays (SAAs) has revolutionized the potential for widespread application of a biological diagnosis of PD[42,43] (**Supplement S2**). SAA α-synuclein positivity has been found in multiple biological samples, with skin and CSF having the highest sensitivities (0.92(0.87–0.95) and 0.90 (0.86–0.93) respectively)[44]. SAAs may be positive in patients at very early stages of the disease process (RBD and PAF)[45,46]. Many caveats must be considered with respect to different analysis methods, especially related to the differentiation of PD from MSA (potentially requiring additional exclusionary measures such as plasma neurofilament light chain levels or neuroimaging techniques) (**Table 2** and **Supplement S2**). Rapid advances are expected, with an eventual evolution from the current binary (+/-) diagnostic test results to methods of monitoring disease status and progression.

Many biological pathways are postulated to be involved in PD. Numerous studies have evaluated other candidate biomarkers for PD, including markers of neurodegeneration, neuroinflammation, protein aggregation, or proteostasis network components (**Supplement S2**) but none reliably distinguish PD from controls, or PD from other neurodegenerative parkinsonian disorders. Given the biological heterogeneity, technological complexity, inter-laboratory variability, and the need for cross-validation by different laboratories, these approaches are not ready for use as diagnostic biomarkers[47,48].

We recommend that the $S^{+/-}$ component of the biological definition of PD document the presence of pathological α-synuclein using validated IHC/IHF and/or SAA methods in skin biopsies or SAA assay in CSF while other tissues and fluids are still being investigated (**Table 2**).

3.4. Neurodegeneration

We recommend that evidence of *any* of the findings below is sufficient to define neurodegeneration in biologically suspected PD (**Table 3, Supplement S3**). However, the specificity of currently available methods to differentiate between PD and other neurodegenerative forms of parkinsonism is imperfect and restricted to a limited number of neuroanatomical systems.

A principal confirmation of PD-associated neurodegeneration is that of dopaminergic denervation. Reduced striatal uptake (typically asymmetric and with a caudal to rostral pattern of abnormality) can be detected using markers for the dopamine transporter (DAT), vesicular monoamine transporter 2 (VMAT2) or aromatic acid decarboxylase (F-dopa). While sensitivity is high, similar findings are seen in MSA, including the rostral-caudal gradient, and in PSP (which tends to affect caudate and putamen equally)[49]. Findings incompatible with PD, and more typical of these other neurodegenerative parkinsonisms, would include radioisotopic evidence of marked post-synaptic dopamine receptor loss (e.g. [^{11}C]raclopride PET or [^{123}I]iodobenzamide SPECT)[50].

A second indication of PD-associated neurodegeneration is altered glucose metabolism, evidenced by FDG PET. While changes in glucose metabolic networks (Parkinson Disease Related Pattern; PDRP)

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are only *indirectly* related to loss of nigrostriatal DA neurons, the findings are so typical as to provide presumptive evidence of denervation to define PD. Similar changes are seen in prodromal disease (RBD)[51], but have also been reported with neuroleptic use[52]. Specificity of this finding within degenerative forms of parkinsonism is high, as atypical parkinsonisms such as MSA, PSP or CBS are associated with different characteristic patterns[53].

A third line of evidence indicating PD-associated neurodegeneration is cardiac sympathetic denervation. Reduced tracer uptake on delayed MIBG SPECT or F-dopamine PET provide sufficient evidence of peripheral cardiac sympathetic denervation to define the presence of neurodegeneration in PD, and may be seen in prodromal disease (RBD, PAF), but not in all cases of early PD[54]. Specificity is high, but imperfect, as abnormalities have been reported in PSP and especially MSA, and interpretation can be challenging.

Non-dopaminergic molecular imaging of other neurotransmitter systems using serotonin or noradrenaline transporter PET ligands such as ^{11}C-DASB or ^{11}C-MeNER respectively, or PET ligands of acetylcholine esterase to demonstrate peripheral cholinergic denervation are of interest but not sufficiently validated yet to serve as anchors for the definition of neurodegeneration[55-57].

MR imaging of substantia nigra pathology using iron-sensitive MRI, free water or neuromelanin imaging are promising potential future imaging markers of neurodegeneration but currently still considered investigational[49] (**Table 3**).

We recommend reporting the PD-associated neurodegeneration status of a person as positive (N^+), when a pathological test specified in **Table 3** is confirmed. All other conditions are considered as N.

Table 3. PD-associated neurodegeneration.

Designation	Biomarker Status	Examination	Interpretation	Sensitivity*	Specificity*
N^+	Recommended	DAergic PET/SPECT	Striatal dopaminergic deficit	High	Low
N^+	Recommended	Metabolic FDG PET	PD related brain metabolic pattern	High	High
N^+	Recommended	Cardiac MIBG SPECT	Sympathetic cardiac denervation	Moderate to high	Moderate
N^+	Investigational	Neuromelanin MRI	Limited test-retest stability	Moderate to high	Low
N^+	Investigational	Iron-sensitive MRI	Sophisticated method restricted to specialized centers, may not directly prove neurodegeneration	Moderate to high	Low
N^+	Investigational	Substantia nigra free water MRI	Sophisticated method restricted to specialized centers, may not directly prove neurodegeneration	High	Moderate to high if applied to extra-nigral sites
N^+	Investigational	Structural MRI (T1)	Sophisticated method restricted to specialized centers	Low	Moderate to high
N^+	Investigational	Diffusion tensor imaging	Sophisticated method restricted to specialized centers	Low to moderate	High
N^+	Investigational	Multimodal MRI	Sophisticated method restricted to specialized centers	Moderate to high	High
Exclusion criterion ruling out N^+	Recommended	Structural MRI	Findings characteristic of atypical parkinsonism: e.g. PSP (midbrain/superior cerebellar atrophy); e.g. MSA (pontine atrophy, hot-cross bun sign, cerebellar atrophy, increased basal ganglia iron with putaminal rim), e.g. CBS (parietal atrophy)	Moderate, stage dependent	High
Exclusion criterion ruling out N^+	Recommended	FDG PET	PSP and MSA each have characteristic patterns that are distinct from PD	High	High
Exclusion criterion ruling out N+	Investigational	^{11}C-raclopride PET or ^{123}I-iodobenzamide SPECT	Major reductions in dopamine receptor binding in early disease are more suggestive of PSP or MSA (modest reductions can be seen in treated PD)	Moderate	High

*high> 80%; moderate >70 < 80%; low < 70%. See **Supplement 3** for further details. CBS: corticobasal syndrome, FDG: fluoro-deoxy-glucose, MIBG: metaiodbenzylguanidin, MRI: magnetic resonance imaging, MSA: multiple system atrophy, PD: Parkinson's disease, PET: positron emission tomography, PSP: progressive supranuclear palsy, SPECT: single-photon emission computerized tomography.

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3.5. Biological designations

The biological designations resulting from different combinations of biomarkers are listed in **Table 4**. Any interpretation needs to consider the possibility of false negative findings in the categories G, S, and N due to current technical limitations.

Table 4. Biological designations in biomarker-positive individuals.

Gene pathogenic variant	Synucleinopathy	Neurodegeneration	Biological designation
G^-	S^+	N^+	**Sporadic PD**
		N^-	**Sporadic Parkinson's type synucleinopathy**
	S^-	N^+	**Non-PD neurodegeneration** (or false negative S test)
		N^-	**No evidence for PD**
G_{F^+}	S^+ or S^-	N^+ or N^-	**Genetic PD** (e.g. *SNCA*-PD)
G_{P^+}	S^+	N^+	**Genetic PD** (e.g. *GBA*-PD)
		N^-	**Genetic Parkinson's type synucleinopathy** (e.g. *GBA*-Parkinson's type synucleinopathy)
	S^-	N^+ (gene predisposing for either Parkinson's type synucleinopathy or non-synucleinopathy)	**Genetic synuclein-negative PD** (e.g. *LRRK2*-PD, *PRKN*-PD)
		N^+ (gene consistently predisposing for Parkinson's type synucleinopathy)	**Non-PD neurodegeneration** (or false neg. S-test)
		N^-	**Genetic predisposition for PD** (e.g. *GBA*-predisposition for PD)

G_{F^+}: fully penetrant pathogenic gene variants, G_{P^+}: pathogenic gene variants with strong or intermediate predisposition, G^-: pathogenic gene variants with low predisposition for PD or polygenic risk scores, or absent or unknown genetic contributions are considered as "genetically indeterminate". See **Supplement S4** for critical appraisal.

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Conscious of the fact that monogenic conditions may have a long preclinical period starting as early as birth or even conception, the field of hereditary neurodegenerative disease is beginning to adapt its classifications to consider this phase as the earliest disease stage[58]. In line with this reasoning, we recommend that carriers of fully penetrant pathogenic gene variants qualify for a diagnosis of **genetic PD**, based on the presence of this variant per se (**Figure 1, Table 4**). Pathogenic gene variants with reduced penetrance would qualify as **genetic predisposition for PD**, but require additional evidence of neurodegeneration for a diagnosis of **genetic PD**. Pathogenic gene variants with low predisposition for PD, polygenic risk scores, or absent or unknown genetic contributions are considered as **genetically indeterminate** in the current construct.

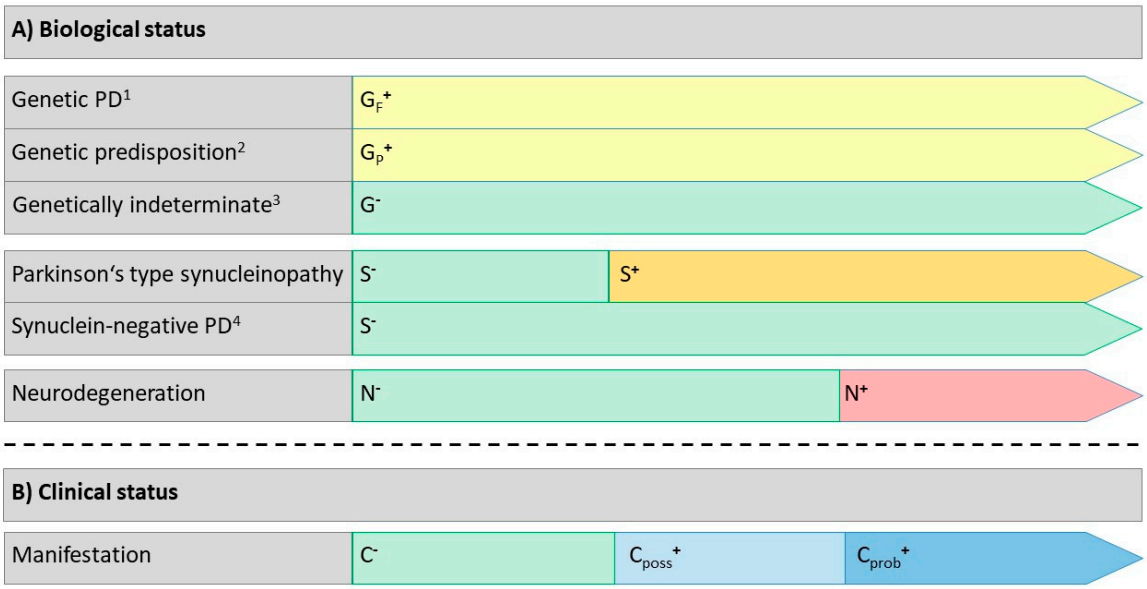

Figure 1. Research framework of PD biological definitions. Temporal sequence and variability of the components contributing to the biological definition of PD. Green bars indicate a physiological condition, other colours indicate pathological conditions. [1] Fully penetrant pathogenic gene variants (G_F^+) qualify for diagnosis of „genetic PD", based on G positivity per se. [2] Pathogenic gene variants with strong or intermediate predisposition (G_P^+) for PD qualify as „genetic predisposition"; they require additional N positivity for a diagnosis of PD. [3] Pathogenic gene variants with low predisposition for PD or polygenic risk scores, or absent or unknown genetic contributions are considered as "genetically indeterminate" (G^-); they require additional S and N positivity for a diagnosis of PD. [4] Synuclein-negative PD may be diagnosed in absence of S positivity in cases with pathogenic gene variants that do not consistently predispose for a Parkinson's type synucleinopathy (e.g. G_{LRRK2}^+ S^- N^+). G^+ or S^+ or N^+ individuals must be further sub-classified by their clinical status, regardless of their N status, since signs and symptoms might arise already due to neuronal dysfunction, preceding overt neurodegeneration. Note that the graph does not imply any temporal alignment of the occurrence of the different C states in relation to the G, S, or N states.

S^+ qualifies for **Parkinson's type synucleinopathy**, as long as N positivity is not yet present. S^+ is an essential prerequisite for a biological **PD** diagnosis in G^- individuals, but requires further evidence of N^+ (since currently we have no other biological marker of neuronal dysfunction preceding neurodegeneration). As indicated above, genetic causes of PD are variably associated with Parkinson's type synucleinopathy. In selected cases, including some designated as "genetic PD" (e.g., *SNCA* gene variants or triplications), G^+ is expected to be associated with a S^+ state as the biological process becomes established, while in others S positivity is not expected (e.g., most *PRKN* gene variants). Thus, in G^+ individuals, the biological diagnosis of **PD** may also be established in S^-

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conditions, if the specific gene does not unequivocally predispose to a Parkinson's type synucleinopathy.

N^+ generally indicates the transition from Parkinson's type synucleinopathy to biologically defined **PD** (or to genetic **synuclein-negative PD** in rare instances).

Conditions not compatible with a diagnosis of PD are also reported in **Table 4**. A critical appraisal of these allocations is presented in **Supplement S4**.

3.6. Clinical manifestations

G^+ or S^+ individuals must be further sub-classified by their clinical status, regardless of their N status, since signs and symptoms might arise due to neuronal dysfunction preceding neurodegeneration, or due to neurodegeneration that defies current methods of visualisation or measurement. This involves defining the presence of associated clinical symptoms or signs (C^+), and determining whether any clinical signs or symptoms can be attributed to their biologically defined PD. We propose to apply these clinical criteria to any individual designated as G^+, S^+ or N^+.

A. The concept of C^+

Some important considerations should be noted in defining the concept of a C^+ state (**Supplement S5**):

1) Early clinical symptoms of PD are diverse, may fluctuate in severity and are often predominantly non-motor, thereby reflecting pathology outside the area that currently defines clinical PD on standard diagnostic criteria (i.e., the substantia nigra). Although non-motor features frequently precede motor ones, generally there is no uniform order of appearance (precluding definition of a specific unitary 'nonmotor-then-motor' staging). Moreover, non-motor features and subtle motor features commonly co-exist. Therefore, a clinical status designation should be agnostic to the order and nature of appearance of clinical features.

2) Clinical symptoms differ in their specificity. In the context of defined biologic PD, some will be almost pathognomonic (e.g., core motor features, RBD, neurogenic orthostatic hypotension). However, many are common in the general population, and will remain non-specific even after diagnosing biologic PD.

3) Many clinical features are also early-phase markers of other synucleinopathies, including MSA and DLB without PD. Although clinical clues can help distinguish these conditions (e.g., normal olfaction suggesting possible MSA, MCI suggesting DLB) it is currently impossible to reliably determine which clinical condition will develop by using only clinical markers.

4) The definition of a C^+ state should not be confused with a stage of disease. Markers of early PD include those that may have a very long average latency to full clinical PD (olfaction, autonomic dysfunction) and others that typically become abnormal only proximate to full clinical PD (cognitive changes, motor exam). The C^+ state includes all stages of disease. Moreover, in this definition there is no distinction between 'prodromal' and later 'defined' disease stages. Rather, in parallel, clinical status could also be stratified according to its impact on activities of daily living (mild, moderate, severe functional impairment).

In summary, the core definition of a 'C^+' state is that clinical symptoms and/or signs of PD have occurred as a consequence of the progressive biological process.

B. Methodology for diagnosing the C^+ state.

We recommend that the clinical status should be reported in a three-component system, i.e. asymptomatic (C^-), presence of clinical abnormalities possibly or probably related to PD (C_{poss}^+, C_{prob}^+).

The criteria for the C^+ states are provided in **Table 5**. For each of these clinical features, it should be presumed that no other, more probable, explanation exists for the symptom (according to best clinical judgement). For example, if a subject has subthreshold parkinsonism while on medications capable of causing parkinsonism, or has urinary dysfunction likely explained by prostatism, the feature should not be scored as present. Moreover, the development and evolution of the feature should be consistent with early PD (e.g. a static symptom with onset before age 30 would generally

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be excluded). Also note that criteria are to be applied in persons with biologic evidence of PD (i.e. G^+ or S^+ or N^+). If not, either the MDS prodromal PD criteria (which use a much lower pretest likelihood, and therefore a higher threshold for diagnosis)[12,14] or MDS clinical PD criteria[10] can be applied.

Table 5. Clinical manifestations in patients with biologic evidence of PD.

A. Clinical abnormalities possibly related to PD (C_{poss}^+)
- Option 1: If S^+ or N^{+*}: at least 1 feature from 1 of the following categories **- Option 2:** If isolated G^+ (S^- and N^-): at least 1 feature from 2 of the following categories
1. Motor
a. A single cardinal manifestation of parkinsonism (i.e. expert-examined bradykinesia, or rest tremor) b. Abnormal quantitative motor testing (>1 standard deviation below age-adjusted normal motor speed)
2. Sensory
a. Olfactory loss
3. Autonomic
a. Chronic constipation
b. Urinary dysfunction
c. Severe erectile dysfunction (onset at an age <60)
d. Likely neurogenic orthostatic hypotension (i.e. heart rate increase <0.5 bpm/mmHg systolic BP drop)[60]
4. Sleep
a. History of REM sleep behavior disorder (without polysomnographic confirmation)
b. Excessive daytime somnolence
5. Affective / Cognitive
a. Depression
b. Mild cognitive impairment
B. Clinical abnormalities probably related to PD (C_{prob}^+)
- Option 1: If S^+ or N^{+*}: at least 1 feature from at least 2 of the categories of **A**, or **- Option 2:** If isolated G^+ (S^- and N^-): at least 1 feature from at least 3 of the categories of **A**, or **- Option 3:** If G^+, S^+, or N^+: at least 1 of the following features:
a. ***Parkinsonism[10]*** (bradykinesia plus one of rigidity or rest tremor)
b. ***Dementia***
c. ***REM sleep behavior disorder*** (polysomnography-confirmed)
d. ***Neurogenic orthostatic hypotension*** (≥20/10 mmHg blood pressure drop within 3 minutes of standing or head-up tilt test)[60]

These clinical features are to be documented in individuals designated as G^+ or S^+ or N^+ using the criteria outlined in the text. Unless otherwise noted, the definition of each feature from the MDS Prodromal Criteria[12,14] applies. Note that for each feature, it should be verified that there is no other more likely explanation based on best clinical judgment and that the temporal evolution of the symptom is consistent with PD.

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*N+ would only be considered when combined with G+ or S+.
See **Supplement 5** for further details.

4. Discussion

Here, we propose a biological definition of PD that comprises PD-specific pathogenic gene variants, Parkinson's type synucleinopathy, and PD-associated neurodegeneration. Recent advances, most notably the establishment of sensitive and specific *in vivo* biomarkers for the presence of underlying α-synuclein pathology[42-44], have placed the field in the critical position of evolving from largely clinically based diagnostic approaches to an emphasis on the biological underpinnings of a disease that affects the central and peripheral nervous systems decades before our clinical approaches permit diagnostic consideration. A biological definition of PD is mandatory for the next stage of a broad range of basic and clinical research studies as well as serving as a framework for future biomarker-based subclassification and staging that may be essential pre-requisites for successful implementation of precision medicine approaches to disease modification.

New criteria that incorporate biological components have been proposed for other neurodegenerative diseases, most notably Huntington disease (HD)[58] and Alzheimer's disease (AD)[59], and these are contributing to ongoing clinical research advances in their respective fields.

Acknowledging that 15% of PD is monogenetic[22] we distinguish between genetic and non-genetic cases and acknowledge a category of *genetic PD* lacking other biological criteria in individuals who have high penetrance gene variants. In analogy to the recommendations in HD[58], this might be defined as a 'Stage 0' PD. One could consider PD patients with incompletely penetrant genetic predisposition (i.e. *LRRK2* and *GBA*) as comparable to HD at-risk individuals with 36-39 CAG repeats. Beyond this, our approach is distinctly different from the Integrated Staging System proposed for HD[58]. In contrast to staging of the disease, we are proposing a Clinical State component with the understanding that other efforts are ongoing to establish a staging system for PD. It is not currently possible in PD to determine many of the background data used to develop the HD staging system (e.g. progressive neuroimaging changes such as caudate atrophy) prior to the development of overt clinical symptoms. However, the wide application of a biological definition of PD as we are proposing would enhance and permit such research to be conducted.

Our biological definition of PD using three binary classes (G/S/N) is comparable but not identical to the Amyloid/Tau/Neurodegeneration (A/T/N) approach proposed for AD[59]. Like the ATN system, which was particularly triggered by the development of tau biomarkers, the PD biological definition has been made possible by the development of tools to detect α-synuclein pathology *in vivo*.

There are similarities and clear differences between these two approaches. For example, ATN is agnostic to temporal ordering while GSN implies an order to the three components. ATN does not specify clinical disease status; our proposed approach includes a clinical component layered onto the binary GSN components.

The incorporation of a S- designation is critical to our definition. This acknowledges that α-synuclein is not necessary for the development of clinical PD and is notably absent in a proportion of patients with selected genetic forms of PD, including an important minority of *LRRK2* cases[30] and the majority of patients with biallelic *PRKN* pathogenic variant[33]s. However, the majority of patients with *LRRK2* gene variants are S+, and some patients with biallelic pathogenic *PRKN* gene variants do demonstrate classical Lewy body pathology, as do most patients with clinical parkinsonism carrying *PRKN* heterozygous pathogenic variants. There is insufficient information about what to expect with biallelic pathogenic *PINK1* and *PARK7* gene variants, although both have been reported to be associated with α-synuclein-positive Lewy body pathology[34-36]. Given this knowledge, we believe that defining PD exclusively as a "synucleinopathy" misrepresents our current understanding of the pathology and pathogenesis of PD and the inclusion and formal acknowledgment of S- cases will advance our understanding of PD.

Of note, this biologic definition makes no distinctions between prodromal and clinical stages, and makes no distinctions between clinical PD and DLB. This should not be interpreted as an invalidation of diagnostic criteria for these states. Rather, the biologic and clinical definitions

overlap, and serve different purposes. The criteria for prodromal PD[12] and DLB[21] useful for identifying patients in whom the biological criteria are not met, for situations in which there is no opportunity to identify markers for G/S and N, and for identifying patients with MCI or neuropsychiatric symptoms who have early-stage DLB. The criteria for clinical PD[10] remain important in making accurate clinical diagnoses of PD as the cause of parkinsonism, while the criteria for DLB[21] remain important for identifying DLB as the underlying cause of dementia. The criteria for the biological definition of PD, in contrast, recognize the unifying biologic factors underlying these conditions.

5. Limitations / Implementation / Future directions

We believe that establishing a biological definition of PD will advance research on a number of fronts including epidemiology, natural history, neuroimaging, clinical trials, the development of newer biomarkers, to name just a few. However, we acknowledge important limitations and concerns raised by this approach. Although we propose the biological definition of PD for the exclusive purposes of advancing research, we recognize that some may obtain testing and apply these criteria to asymptomatic individuals in a non-research setting. This has important ethical implications, particularly given our limited understanding of the natural history of individuals in the various biological categories we propose and our current inability to prevent progression of PD from its early stages. Prospective studies must validate the evolution of these biological categories and provide more reliable methods predicting the underlying biological processes in asymptomatic individuals. We expect continuous advances in our understanding of genetic (including polygenic risk scores) and of environmental risk factors, and, therefore, these may be incorporated into future iterations or revisions of this system. We also expect further optimization of S and N biomarkers that will improve the sensitivity and specificity of the currently recommended testing. For example, we expect the development of successful S imaging ligands that can easily be incorporated once available. We expect that newer tools will successfully identify relevant disease mechanisms in subgroups of patients, allowing their incorporation into the biological scheme (e.g., a marker of inflammation or mitochondrial dysfunction). Finally, other classification components could be combined with GSN designations where appropriate (e.g., an additional ATN designation[59] in patients with cognitive dysfunction). We emphasize that the current proposal is a first step in the critical process of moving the field from a purely clinical to a biological approach to the disease.

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