

# Towards biomarker-based diagnosis of Parkinson disease

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## Abstract

The current clinical diagnostic criteria for Parkinson disease (PD) have limitations and are inherently insensitive to the earliest stages of disease, when classical motor signs can be absent. Imaging and genetic tests are currently used to support or establish a diagnosis of PD, but no validated biomarker-based diagnostic framework currently exists. Substantial progress has been made in the field of molecular disease markers, most notably with the development and validation of seed amplification assays (SAAs), which enable detection of very low levels of pathological  $\alpha$ -synuclein in the cerebrospinal fluid and other biofluids and tissue. In this Review, we discuss the potential of  $\alpha$ -synuclein SAAs and other biomarkers to improve diagnostic accuracy and enable earlier diagnosis of PD. We consider biological disease definitions that have been proposed on the basis of these biomarkers, highlighting their merits, limitations and implications for PD research and clinical management. Research is ongoing to determine the predictive value of PD biomarkers in healthy people and people with prodromal PD and to develop markers that are sensitive to disease progression, both of which are key for implementation of trials involving drugs designed to modify or prevent disease. Integrating clinical, genetic, molecular and imaging biomarkers should enable earlier, more accurate diagnosis of PD and characterization of PD subtypes, thereby enabling personalized treatment to slow or even prevent PD.

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## Key points

- The development of seed amplification assays (SAAs), which can detect very low levels of pathological  $\alpha$ -synuclein in biofluids and tissue, has led to a shift in the field of Parkinson disease (PD) diagnosis.
- Use of  $\alpha$ -synuclein SAAs improve accuracy of PD diagnosis and have the potential to enable identification of prodromal and preclinical stages of PD.
- Frameworks have been proposed for the biological definition and classification of PD, based on combinations of molecular biomarkers, neuroimaging markers and PD-associated genetic variants;  $\alpha$ -synuclein SAAs are foundational to these frameworks.
- Biomarker-based definitions of PD are expected to facilitate early detection of disease regardless of clinical symptoms, address disease heterogeneity and enable selection of populations for clinical trials to test potential disease-modifying or disease-preventing interventions.
- Major challenges remain for biomarker-based pre-clinical diagnosis, including biomarker validity, scalability and predictive value, ethical concerns and healthcare infrastructure requirements.

## Introduction

Since James Parkinson's seminal *Essay on the Shaking Palsy* of 1817, diagnosis of Parkinson disease (PD) has been a clinical exercise. According to Jean-Martin Charcot, the founding father of clinical neurology, who suggested the illness be named 'maladie de Parkinson', one did not even need particular skill to recognize it: "I have seen such patients everywhere on the streets of Rome, of Amsterdam, in Spain – it is always the same picture. They can be identified from afar; you do not need a medical history"<sup>1</sup>.

Current clinical criteria for diagnosis of PD are anchored on establishing the presence of parkinsonism (defined as bradykinesia together with classical resting tremor and/or rigidity identified by clinical neurological examination) and checking for defined supportive and exclusionary features<sup>2</sup>. Clinical and neuropathological validation studies of these criteria have shown that they have high specificity of up to 98%, enabling PD to be distinguished from other types of degenerative parkinsonism, but that their sensitivity is limited<sup>3,4</sup>. Furthermore, the sensitivity and specificity of these clinical criteria are lower at earlier stages of disease and with lower expertise of the diagnosing practitioner. A meta-analysis of 11 clinico-pathological studies found that the overall accuracy of the criteria was only 80.6% across various clinical scenarios<sup>5</sup>, and accuracy was still only 89% in a series in which the most recent clinical criteria were used<sup>4</sup>. The most common diagnostic errors involve mimics of PD, such as parkinsonian variants of multiple system atrophy (MSA) or progressive supranuclear palsy (PSP), which can be difficult to distinguish from PD in the early disease stages<sup>6</sup>.

In addition to their limited diagnostic accuracy, current clinical criteria for PD hinge on established motor features and are, therefore, inherently insensitive to the earliest stages of disease, when classical motor signs might be absent. Clinically manifest PD is now generally accepted to be preceded by a period of subtle motor signs and disease-related nonmotor symptoms, which form the basis of research criteria for prodromal PD<sup>7,8</sup> (Box 1). However, many of these signs and

symptoms have limited sensitivity, specificity and positive predictive value. Among the nonmotor manifestations of prodromal PD, isolated rapid eye movement sleep behaviour disorder (iRBD) has the highest predictive value – 73 to 90% of people with iRBD develop Lewy-body disorders, such as PD or dementia with Lewy bodies (DLB), within 10–14 years<sup>9,10</sup>. Neuropathological studies of people with a diagnosis of iRBD have shown the presence of extensive neurodegeneration and accumulation of  $\alpha$ -synuclein that meet pathological criteria for PD, DLB or MSA<sup>11</sup>.

The limitations of clinical diagnostic criteria for PD have negative consequences for clinical care and research. Diagnostic errors compromise clinical management, and failure to intervene at the earliest stages of disease is one reason for negative outcomes in previous trials of disease-modifying interventions. In this Review, we consider the potential of biomarkers to increase diagnostic accuracy and enable earlier diagnosis of PD. We also discuss proposals for biological disease definitions that are based on these biomarkers, highlighting their merits and limitations, and their implications for current PD research and future clinical management.

## Parkinson disease biomarkers — where we stand

Ideal PD biomarkers should enable differentiation between PD and other types of degenerative parkinsonism, identification of pathogenic subtypes, monitoring of disease progression and/or responses to treatment, and detection of disease-related dysfunction and pathology before the occurrence of classical motor signs. Although no single biomarker candidate currently meets all these requirements, major progress has been made in the field of molecular disease markers. Most notable is the development and validation of seed amplification assays (SAAs), which enable detection of very low levels of pathological  $\alpha$ -synuclein in the cerebrospinal fluid (CSF) and some other biofluids and tissue. Current work to develop biomarker-based diagnostic frameworks for PD focus on combinations of molecular assays for pathological  $\alpha$ -synuclein, neuroimaging evidence for neurodegeneration or neuronal dysfunction, and detection of PD-associated genetic variants<sup>12,13</sup>. The current status of each of these biomarker types is discussed below.

## Molecular biomarkers

Despite intensive research, particularly focused on  $\alpha$ -synuclein, few molecular biomarkers are available to aid diagnosis and care. However, the field of fluid-based and tissue-based biomarkers is changing rapidly, and several molecular disease markers have shown promise for research and clinical management in PD (Fig. 1). The most promising to date are  $\alpha$ -synuclein SAAs, which provide a biological marker of PD pathology with the potential to enable a biological definition of the disease.

## $\alpha$ -Synuclein seed amplification assays

$\alpha$ -Synuclein SAAs measure the ability of  $\alpha$ -synuclein in biofluids or tissue samples to induce self-aggregation and are a variation of the CSF diagnostic assay used for sporadic Creutzfeldt–Jakob disease, which exploits self-aggregation of the prion protein<sup>14</sup>. A biological sample is added to a buffer that contains monomeric  $\alpha$ -synuclein and the fluorescent dye thioflavin T. Pathological forms of  $\alpha$ -synuclein in the biological sample induce the formation of  $\beta$ -pleated sheet aggregates of  $\alpha$ -synuclein that bind to thioflavin T, resulting in a fluorescent signal. The main types of SAA are real-time quaking-induced conversion (RT-QuIC) and protein misfolding cyclic amplification – we refer to these collectively as SAAs.

## Box 1 | Parkinson disease stages and diagnostic challenges

Parkinson disease (PD) is a progressive neurodegenerative disorder characterized by  $\alpha$ -synuclein aggregation and dopaminergic neuron loss. PD evolves through three major stages — preclinical, prodromal and manifest — each with specific diagnostic challenges and opportunities.

### Preclinical PD

Preclinical PD is defined by the presence of disease-specific pathology that is clinically silent. Although genetic testing can identify asymptomatic carriers of PD-associated genetic variants, additional tests are needed to detect extant pathology.  $\alpha$ -Synuclein seed amplification assays and dopaminergic and other imaging modalities enable biological detection of PD-associated pathology, and these measures form the basis of biological diagnostic classification frameworks for PD. At this preclinical stage, environmental and lifestyle-related risk factors can be modified, but pharmacological intervention is not currently possible.

### Prodromal PD

Prodromal PD is defined by the presence of various nonmotor symptoms, such as loss of smell and constipation, that often develop before the onset of classic motor symptoms. Prodromal symptoms have been linked to an increased risk of classical PD in

population-based studies and can precede clinical diagnosis of PD by 10–20 years. Some of these symptoms, such as constipation and depression, are nonspecific, whereas others, such as rapid eye movement sleep behaviour disorder and hyposmia, are strong risk factors for the development of motor symptoms. The boundary between prodromal and early manifest PD is often unclear.

$\alpha$ -Synuclein seed amplification assays have strong diagnostic potential at this stage, particularly among people with rapid eye movement sleep behaviour disorder and/or hyposmia. People with prodromal PD are prime candidates for participation in future disease-modification trials.

### Manifest PD

Manifest PD is defined by classical motor symptoms, but early diagnosis remains challenging owing to overlaps with atypical parkinsonian disorders, such as parkinsonism-predominant forms of progressive supranuclear palsy and multiple system atrophy. Ancillary tests, such as dopaminergic imaging, can support a diagnosis of PD but are not specific for PD.  $\alpha$ -Synuclein seed amplification assays can facilitate differential diagnosis by confirming the presence of  $\alpha$ -synuclein pathology, which helps to distinguish PD from non-synucleinopathies, even at early clinical stages. However, these assays are currently moderately invasive and not ready for wider clinical use.

The first  $\alpha$ -synuclein SAA to be published showed that the assay can detect  $\alpha$ -synuclein aggregation in CSF from people with PD with a sensitivity of 95% and a specificity of 100% when compared with CSF from healthy people and people with Alzheimer disease (AD) or tauopathies such as PSP and corticobasal degeneration<sup>15</sup>. Subsequently, the results of  $\alpha$ -synuclein SAAs have been replicated in many laboratories, and a pooled meta-analysis reported that SAAs enable people with synucleinopathies, such as PD or DLB, to be distinguished from people with non-synucleinopathies and healthy individuals with a sensitivity of 88% and a specificity of 95%<sup>16</sup>.

A large study from the Parkinson's Progression Marker Initiative (PPMI) has also shown that a positive  $\alpha$ -synuclein SAA had an average sensitivity of 88% for sporadic PD, ranging from 99% in people with hyposmia to 78% in people without impairment of olfaction<sup>17</sup>. As indicated above, sensitivity was lower (67%) in people with *LRRK2*-associated PD. In this study,  $\alpha$ -synuclein SAAs enabled people with PD to be distinguished from healthy individuals with a specificity of 96%. In accordance with earlier work in people with iRBD<sup>18</sup>,  $\alpha$ -synuclein SAAs were positive for 86% of PPMI participants with prodromal disease defined by rapid eye movement sleep behaviour disorder or hyposmia and a dopamine transporter deficit (detected with imaging) but an absence of PD motor symptoms<sup>17</sup>.

Use of the same  $\alpha$ -synuclein SAA protocol as that used in the PPMI cohort has indicated that the assay can also distinguish people with PD from people with MSA<sup>19,20</sup>. Differences in aggregation kinetics, including maximum fluorescence, in post-mortem brain and CSF samples enabled a promising separation between the two disorders<sup>19,20</sup>. In line with these findings, a study of multiple cohorts with clinically diagnosed MSA and PD showed that Lewy-body disorders are characterized by a high (type 1) fluorescence amplification pattern, whereas MSA is characterized by an intermediate (type 2) fluorescence amplification

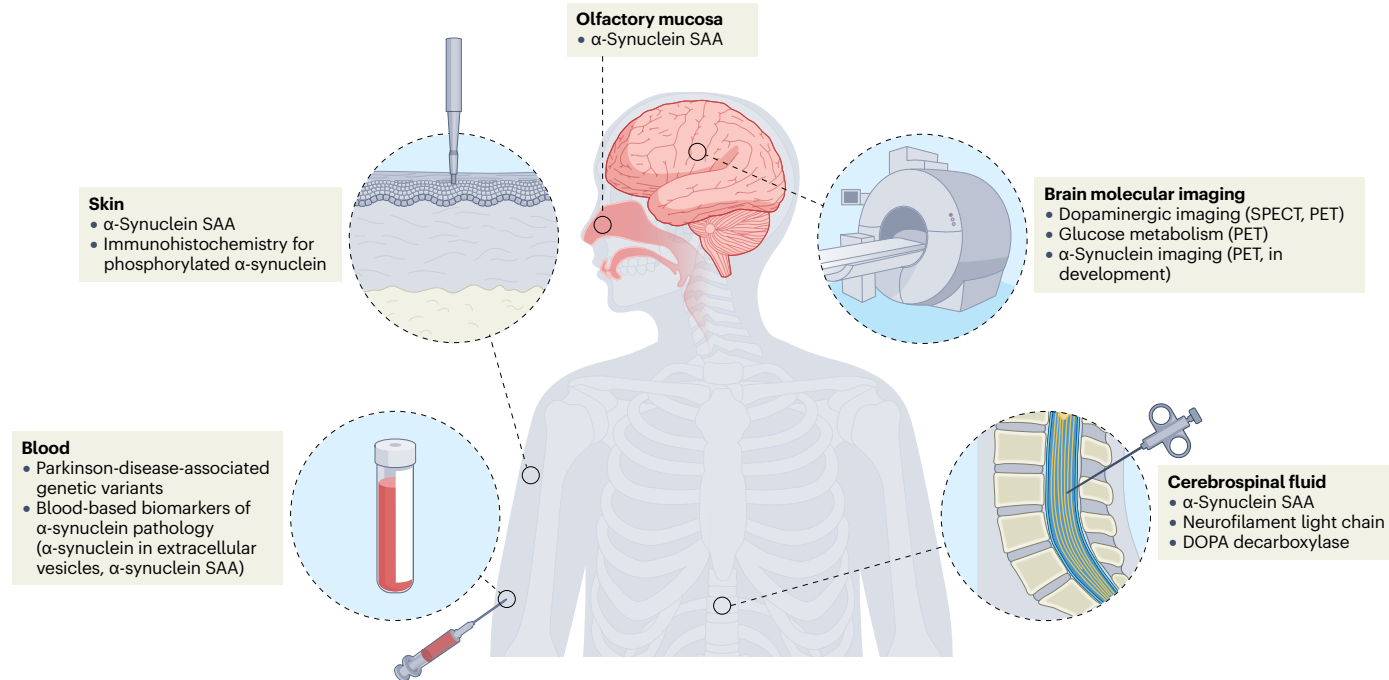
pattern, and that the type 2 fluorescence pattern had a sensitivity of 87% and a specificity of 77% for MSA<sup>21</sup>.

### $\alpha$ -Synuclein in peripheral tissues and fluids

The invasive nature of lumbar punctures limits the utility of CSF  $\alpha$ -synuclein SAAs. Consequently, assays that use peripheral tissue and/or fluids need to be developed and validated. In addition, mapping the presence and distribution of  $\alpha$ -synuclein across tissues and fluids by use of SAAs could provide insight into PD pathology, subtypes and disease progression.

Use of skin biopsy  $\alpha$ -synuclein SAAs has shown that their sensitivity and specificity are similar to those achieved with CSF  $\alpha$ -synuclein SAAs in manifest and prodromal PD<sup>22</sup>. A pooled meta-analysis demonstrated that use of skin biopsy  $\alpha$ -synuclein SAAs enabled people with PD to be distinguished from healthy individuals with a specificity of 91% and a sensitivity of 92%<sup>23</sup>. Similar specificities and sensitivities have been reported for the use of immunohistochemistry to detect  $\alpha$ -synuclein that is abnormally phosphorylated at the serine-129 residue (pSer129  $\alpha$ -synuclein) in skin biopsies<sup>24</sup>. One caveat with skin biopsy  $\alpha$ -synuclein SAAs is that the results are influenced by biopsy location — positivity in PD is highest in samples taken from the neck<sup>24</sup>.

In addition to skin biopsy  $\alpha$ -synuclein SAAs, an immunoprecipitation-based  $\alpha$ -synuclein SAA has been used to detect pathogenic  $\alpha$ -synuclein in the serum<sup>25</sup>. In two cohorts, this assay enabled people with PD to be distinguished from those with MSA and healthy individuals with an overall accuracy of 86% or 95%. Similarly, use of isolated LICAM-positive extracellular vesicles from the plasma of people with PD led to positive  $\alpha$ -synuclein SAAs in all samples tested<sup>26</sup>. However, the results of these two blood-based assays require confirmation from other laboratories.



**Fig. 1 | Detection of biomarkers of Parkinson disease at different sites.** Brain biomarkers are detected with positron emission tomography (PET) or magnetic resonance imaging (MRI).  $\alpha$ -Synuclein is common to all sites. DOPA,

3,4-dihydroxyphenylalanine; SAA, seed amplification assay; SPECT, single-photon emission computed tomography.

## Limitations and development of $\alpha$ -synuclein seed amplification assays

Although  $\alpha$ -synuclein SAAs provide a biological anchor for diagnosis of PD, much remains to be discovered (reviewed in detail elsewhere<sup>27</sup>). One major caveat with current CSF  $\alpha$ -synuclein SAA protocols is that they might have limited sensitivity for detection of  $\alpha$ -synuclein neuropathology that is restricted to the amygdala or lower brainstem<sup>28,29</sup>. Comparative studies of CSF SAA positivity rates and brain Lewy-body load have shown that antemortem CSF  $\alpha$ -synuclein SAAs have close to 100% sensitivity when Lewy-body disease pathology is in the neocortex or limbic system, but less than 50% when  $\alpha$ -synuclein pathology is predominantly in the amygdala<sup>29</sup>. Whether this difference relates to the burden of Lewy-body pathology or whether it also relates to distinct strains of  $\alpha$ -synuclein with different SAA reactivity remains unclear.

Another limitation of current  $\alpha$ -synuclein SAAs is that the result is a dichotomous positive or negative, and development of quantitative assays is a priority. Kinetic parameters such as lag time, maximum fluorescence and time to 50% fluorescence could be calculated to provide quantitative measures. These parameters could reflect structurally distinct  $\alpha$ -synuclein aggregates that underlie the clinical heterogeneity of PD, but studies are needed to test this hypothesis. In line with this idea, one study showed that distinct biophysical, kinetic and seeding properties of  $\alpha$ -synuclein fibrils that were amplified from the CSF of people with PD by recursive SAA were associated with different clinical features<sup>30</sup>.

A further difficulty with the use of CSF  $\alpha$ -synuclein SAAs as a diagnostic anchor for PD is that positivity has been reported in a proportion of people with tauopathies, such as PSP and corticobasal degeneration, the clinical features of which overlap with PD. In a cohort of people in

the UK, CSF  $\alpha$ -synuclein SAAs were positive for 96% of 66 people with PD, but SAAs were also positive for 15% of 52 people with PSP<sup>31</sup>. Another study reported  $\alpha$ -synuclein SAA positivity in 10% of people with PSP and 30% of people with a corticobasal syndrome<sup>32</sup>. Furthermore, estimates suggest that CSF  $\alpha$ -synuclein SAAs are positive in 20–40% of people with AD<sup>33–35</sup>. These findings are likely to reflect the frequent occurrence of multiple co-pathologies in the brains of people with neurodegenerative diseases<sup>36</sup>, making it challenging to rely on a single proteinopathy marker for diagnostic classification. In addition, CSF  $\alpha$ -synuclein SAAs are positive in 4–15% of healthy individuals<sup>17,18,34</sup>, and the positive predictive value of these assays in the general population remains unknown.

To enable implementation of  $\alpha$ -synuclein SAAs in routine clinical settings worldwide, harmonization of existing SAA protocols is needed. Reproducibility and standardization need to exceed the levels reported in research settings to date, because specificities obtained with protocols from different laboratories differ considerably.

## Beyond $\alpha$ -synuclein

CSF  $\alpha$ -synuclein SAAs have been used in combination with measurement of neurofilament light chain (NfL) levels to distinguish MSA from Lewy-body disorders, as NfL levels are consistently higher in MSA than in PD or in healthy individuals<sup>37</sup>. In one study of pure autonomic failure – a synucleinopathy that can progress to either PD or MSA – baseline CSF levels of NfL were elevated only in people who later developed MSA, enabling these individuals to be distinguished from people who went on to develop PD or DLB and people who did not progress to any of these diseases<sup>38</sup>. These findings suggest that CSF levels of NfL can be used to predict phenoconversion of prodromal synucleinopathies into one

or other phenotype. An advantage of NfL is that it can be measured in peripheral blood, and evidence suggests that measurement of plasma levels of NfL can enable PD to be differentiated from MSA and other atypical Parkinsonian disorders<sup>39</sup>.

In most people with PD, CSF levels of the AD biomarkers amyloid- $\beta_{42}$  (A $\beta_{42}$ ) and total or phosphorylated tau (p-tau) are normal<sup>40</sup>. However, low CSF levels of A $\beta_{42}$  predict worsening of motor and cognitive performance in PD<sup>41</sup>, and robust pathological evidence demonstrates that multiple co-pathologies, including amyloid- $\beta$ , tau and TDP43 pathology, can occur in the brains of people with PD<sup>42</sup>.

In the past three years, several studies have demonstrated that biofluid levels of dopa-decarboxylase (DDC) are increased in PD<sup>43–46</sup> and that increased CSF levels of DDC correlate with lower levels of dopamine transporters (detected with imaging) in people with PD<sup>47</sup>. CSF analysis has shown that DDC is upregulated in treatment-naive people with PD and even in people with rapid eye movement sleep behaviour disorder<sup>48,49</sup>. Furthermore, increased CSF levels of DDC in early PD predict later cognitive decline<sup>50</sup>. One caveat is that CSF and plasma levels of DDC are further increased in people with PD who are receiving L-DOPA and a DDC inhibitor<sup>43,48,49</sup>. Currently, it is unclear whether increased levels of DDC reflect strong monoaminergic neurodegeneration or upregulation to compensate for low dopamine levels.

Besides NfL, no blood biomarkers have been established for clinical assessment of PD or atypical parkinsonism. However, several studies suggest that plasma biomarkers could be developed in the near future. Large-scale proteomics studies have identified blood panels of proteins that can enable people with PD to be distinguished from healthy individuals<sup>51,52</sup>. Use of a machine learning model to analyse blood samples from 99 people with recently diagnosed PD, 72 people with iRBD and 36 healthy donors identified a panel of eight plasma proteins that correctly identified all people with PD. In a subset of 54 people with iRBD followed prospectively, the panel classified 79% as PD up to seven years prior to their phenoconversion to clinical PD<sup>52</sup>. Several of the markers in these blood panels regulate inflammatory processes that have a role in PD pathogenesis. Upregulation of inflammatory markers, such as IL17, in the CSF has also been found in people with PD<sup>43</sup>, highlighting inflammation as a contributing factor in PD.

Other possible blood-based biomarkers of PD that have been identified include a marker of mitochondrial DNA damage<sup>53</sup>, which is a characteristic of PD, and the specific microRNA miR-4438, which is enriched in plasma extracellular vesicles from people with synucleopathies, including PD<sup>54</sup>. Further work is needed to determine the potential diagnostic potential of these markers.

## Imaging biomarkers

Molecular imaging using dopaminergic tracers and magnetic resonance have become part of the routine clinical work-up for parkinsonism. These markers increase diagnostic accuracy and enable PD to be distinguished from non-PD conditions with overlapping clinical features<sup>55,56</sup>. However, imaging biomarkers have limitations in relation to their specificity for PD (Table 1).

## Radiotracer imaging

Molecular imaging markers of presynaptic dopaminergic function include a variety of positron emission tomography (PET) and single-photon emission computed tomography (SPECT) radioligands that bind to the dopamine transporter, L-aromatic amino acid decarboxylase or the vesicular monoamine transporter type 2 (VMAT2), all of which are sensitive to a reduction in nigro-striatal dopaminergic nerve terminals<sup>57</sup>. However, these markers lack specificity for PD and cannot differentiate between PD and other forms of degenerative parkinsonism, such as MSA or PSP. In addition, reductions in signal do not consistently correlate with nigral neuronal loss<sup>58</sup>.

Dopamine transporter imaging is sensitive to early presynaptic dopaminergic dysfunction in individuals who exhibit non-motor signs of prodromal PD<sup>59</sup>. In the Parkinson Associated Risk Syndrome (PARS) study, dopamine transporter imaging with SPECT (DaT-SPECT) in 185 people with hyposmia and no clinical signs of PD showed reduced striatal tracer binding in 51 (27%) of people at baseline. Among people with tracer binding levels below 65% of the age-expected mean ( $n = 21$ ), 67% developed clinical PD over a mean follow-up period of six years<sup>60</sup>. In a study of 100 PARS participants for whom baseline CSF samples were available, 34 of 71 (48%) people with hyposmia had positive CSF  $\alpha$ -synuclein SAAs compared with just one of 25 (4%) participants without hyposmia. Of the 34 people with hyposmia and a positive  $\alpha$ -synuclein SAA, 12 (35%) also had a dopamine transporter deficit, and seven of these 12 people developed PD over four years<sup>61</sup>. These observations suggest that biomarker evidence of  $\alpha$ -synuclein pathology can precede dopaminergic dysfunction, and that the combination of both is associated with clinical conversion. Similarly, studies of people with iRBD have consistently found correlations between reduced dopamine transporter binding at baseline and conversion to PD or DLB<sup>62–64</sup>.

Noradrenergic cardiac imaging with either <sup>123</sup>I-metaiodo-benzylguanidine (MIBG) SPECT or <sup>18</sup>F-dopamine PET can detect the loss of post-ganglionic sympathetic innervation, which is present in a majority of people with PD and can also be found in individuals

**Table 1 | Imaging markers of neurodegeneration associated with Parkinson disease**

Imaging modality	Changes detected	Sensitivity for PD	Specificity for PD	Abnormal in prodromal PD	Changes with PD progression
Dopaminergic SPECT/PET	Striatal dopaminergic denervation	High	Low	+	+
FDG-PET	PD-related metabolic pattern	High	High	+	+
MIBG-SPECT	Cardiac sympathetic denervation	Moderate to high	Moderate	+	(+)
Neuromelanin MRI	Substantia nigra neuronal loss	Moderate to high	Low	(+)	+
Iron-sensitive MRI (R2*, QSM, DNH)	Substantia nigra pathology	Moderate to high	Low	Unknown	(+)
Diffusion tensor imaging	Substantia nigra structural changes	Moderate to high	Moderate	(+)	(+)

+ indicates consistent findings in multiple studies, (+) indicates positive findings in some studies but insufficient evidence. DNH, dorsal nigral hyperintensity; FDG, <sup>18</sup>F-fluorodeoxyglucose; MIBG, metaiodo-benzylguanidine; MRI, magnetic resonance imaging; PD, Parkinson disease; PET, positron emission tomography; QSM, quantitative susceptibility mapping; SPECT, single-photon emission computed tomography.

who are at risk of PD, such as people with iRBD or hyposmia<sup>65</sup>. Prospective studies have indicated that an increased risk of subsequent clinical Lewy-body disorders (PD or DLB) is associated with reduced noradrenergic cardiac tracer binding<sup>66</sup>.

Metabolic resting state imaging with FDG–PET and spatial covariance analysis has revealed PD-specific regional changes (referred to as PD-related pattern) that are characterized by hypermetabolism in the thalamus, globus pallidus and primary motor cortex, associated with relative metabolic reductions in the lateral premotor cortex and posterior parietal cortex<sup>67</sup>. Such changes can already be detected in prodromal disease stages, as exemplified by studies in people with iRBD<sup>68,69</sup>, and this type of analysis also enables differentiation between different forms of degenerative parkinsonism.

## Magnetic resonance imaging

The main role of structural magnetic resonance imaging (MRI) in the diagnostic work-up of PD is to rule out pathologies that are either inconsistent with PD or to indicate co-morbidities. However, newer MRI techniques, including neuromelanin-sensitive and iron-sensitive sequences and diffusion tensor imaging, have the potential to detect changes that are directly related to PD pathology<sup>70</sup>.

On high-field fast spin-echo MRI sequences, nuclei that contain neuromelanin appear as high-signal-intensity areas relative to the surrounding brain tissue<sup>71,72</sup>. Neuromelanin-MRI has consistently demonstrated that the signal is reduced in the substantia nigra and locus coeruleus, where neuromelanin is usually higher in people with PD than in healthy people. Reductions in neuromelanin signal intensity and volume in the substantia nigra and locus coeruleus have also been detected in people with iRBD and people with pure autonomic failure who met the Movement Disorder Society (MDS) criteria for ‘probable prodromal PD’, indicating that this imaging marker is also sensitive to prodromal disease<sup>73–76</sup>.

Iron-sensitive MRI techniques, such as T2-weighted imaging or quantitative susceptibility mapping enable quantification of regional brain iron levels; in particular, quantitative susceptibility mapping can

be used to distinguish between people with PD and healthy controls with high sensitivity and specificity<sup>77–80</sup>. Iron-sensitive high-field MRI can identify an ovoid-shaped area of signal hyperintensity, known as the dorsolateral nigral hyperintensity, within the hypointense substantia nigra<sup>81</sup>, and several studies have shown that unilateral or bilateral loss of this signal enables people with PD to be distinguished from healthy controls with a specificity of >90%<sup>82,83</sup>. Loss of dorsolateral nigral hyperintensity has also been described in iRBD, suggesting that this marker is sensitive to early neurodegeneration<sup>84</sup>.

Neurodegenerative changes in PD also affect diffusion of water molecules along fibre tracts, which can be detected using diffusion tensor imaging. Diffusion tensor imaging studies of people with early PD or iRBD have revealed increases in free water and reduced fractional anisotropy (a measure of tissue integrity) in the substantia nigra, and these changes seem to increase with greater disease duration<sup>85,86</sup>.

## Genetic markers

Our understanding of the genetic basis of PD has progressed rapidly over the past three decades (Table 2). Identification of triplications, duplications and point mutations in *SNCA*, which encodes  $\alpha$ -synuclein, that cause dominantly inherited PD led to the discovery that  $\alpha$ -synuclein has a key role in the formation of Lewy bodies<sup>87,88</sup>. In turn, this understanding has led to the central role of  $\alpha$ -synuclein in the development of diagnostic markers of PD<sup>17</sup>. Identification of recessive variants in the mitochondrial genes *PRKN*, *PINK1* and *PARK7* (also known as *DJI*) also provided an understanding that mitochondrial dysfunction is an important component of PD pathogenesis<sup>89–91</sup>.

Over the past 20 years, the era of genome-wide association studies (GWAS) and growing case–control sample sizes has substantially increased the number of loci implicated in sporadic PD from tens to hundreds<sup>92</sup>. The latest European GWAS, which included 63,000 cases and 1.7 million controls, identified 134 risk loci for PD<sup>93</sup>. Reassuringly, many of the genes associated with monogenic disease are in loci that have also been identified by GWAS, highlighting that specific pathways

**Table 2 | Characteristics of Parkinson disease caused by or associated with specific genes**

Gene	Median age of onset (years)	Clinical features	Inheritance, penetrance	Pathology	$\alpha$ -Synuclein seed amplification assay
<i>SNCA</i>	46	Rapid progression, dementia, autonomic dysfunction	Autosomal dominant, fully penetrant	Lewy bodies and $\alpha$ -synuclein aggregates, nigral degeneration	Positive
<i>LRRK2</i>	56	Typical PD, often mild, variable progression	Autosomal dominant, variable penetrance	Variable, including Lewy bodies, tau, TDP43 or pure nigral degeneration	2 of 3 positive, 1 of 3 negative
<i>VPS35</i>	52	Typical PD	Autosomal dominant, highly penetrant	Unknown	Unknown
<i>CHCHD2</i>	48–55	Tremor-dominant PD, psychiatric symptoms	Autosomal dominant, variable penetrance	Unknown	Unknown
<i>PRKN</i>	31	Early-onset PD, slow progression, dystonia, good L-DOPA response, dementia rare	Autosomal recessive, fully penetrant	Usually no Lewy bodies (~80%), nigral degeneration	Mostly negative
<i>PINK1</i>	32	Early-onset PD, mild progression, psychiatric symptoms, good L-DOPA response, dementia rare	Autosomal recessive, fully penetrant	Limited data, with Lewy bodies in one person, none in another	Unknown
<i>PARK7</i>	27	Early-onset PD, similar to <i>PINK1</i> and <i>PRKN</i> , dementia rare	Autosomal recessive, fully penetrant	Limited data, Lewy bodies in one person	Unknown
<i>RAB32</i>	54	Typical PD	Autosomal dominant, reduced penetrance	Limited data, no Lewy bodies in one person	Unknown
<i>GBA1</i>	51	Typical PD, earlier onset, dementia, faster progression	Risk gene (variant dependent)	Lewy bodies and $\alpha$ -synuclein aggregates, nigral degeneration	Positive

Information from the [MDSGene](#) website and ref. 131. PD, Parkinson disease; TDP43, TAR DNA-binding protein 43.

**Table 3 | Comparison of proposed frameworks for biological definition and classification of Parkinson disease**

Element of framework	Framework		
	NSD-ISS	SynNeurGe	
Scope	Biological definition of neurodegenerative diseases with predominant neuronal $\alpha$ -synuclein pathology	Biological classification of Parkinson disease	
Biomarker anchors <sup>a</sup>	$\alpha$ -Synuclein pathology	Positive CSF $\alpha$ -synuclein seed amplification assay	Positive CSF or skin biopsy $\alpha$ -synuclein seed amplification assay; evidence for $\alpha$ -synuclein pathology in skin biopsies (immunohistochemistry)
	Imaging evidence of neurodegeneration	Dopaminergic dysfunction detected with dopamine transporter imaging	Dopaminergic dysfunction detected with dopamine transporter imaging; cardiac sympathetic denervation detected with MIBG-SPECT; PD-related pattern detected with FDG-PET
	Genetic variants	Fully penetrant <i>SNCA</i> variants only (included as an anchor for stage 0)	Fully penetrant variants of <i>SNCA</i> or biallelic variants of <i>PRKN</i> , <i>PINK1</i> or <i>PARK7</i>
Clinical symptoms	Not considered to be defining features of disease; biomarker anchors define transitions from stage 2 to stages 3–6	Not considered to be defining features of disease	
Disease staging	An integral part of the framework; seven stages based on transition from asymptomatic to symptomatic with increasing functional impact	Not part of framework	

<sup>a</sup>Biomarkers are complementary rather than hierarchical. CSF, cerebrospinal fluid; FDG, <sup>18</sup>F-fluorodeoxyglucose; MIBG, metaiodo-benzylguanidine; NSD-ISS, Neuronal Synuclein Disease Integrated Staging System; PD, Parkinson disease; PET, positron emission tomography; SPECT, single-photon emission computed tomography.

involved in familial forms of PD are also important in sporadic disease. Variants in *LRRK2* have been identified as the most common cause of dominantly inherited PD, and variants in *GBA1* have been identified as the most common genetic risk factor for PD, although penetrance is age-dependent for both<sup>94,95</sup>. Genetic discovery has continued apace, with *VPSI3C* and *RAB32* variants most recently identified as causes of monogenic PD<sup>96,97</sup>. Consequently, our understanding of PD has shifted dramatically – in the 1980s, the disease was considered unlikely to have a genetic component, whereas we now know that monogenic causes account for up to 10% of cases (population-dependent), and variants identified in GWAS account for another 16–22% of heritable PD risk<sup>90</sup>.

The role of PD genetics in routine clinical practice has also grown substantially in high-income countries. In many of these countries, genetic testing is no longer reserved for people with an age at onset less than 40 years or multiple family members with PD, but is increasingly used for characterization, stratification and prognostication of PD<sup>98</sup>. In addition, pathways that are affected by specific PD-associated mutations are now being targeted in ongoing disease-modification trials in people with PD who carry *GBA1* or *LRRK2* mutations<sup>99</sup>.

Genetic characterization of PD is also part of proposed biological classification schemes for the disease; for example, fully penetrant *SNCA* mutations can be classified as stage 0 of neuronal synuclein disease in the Neuronal Synuclein Disease Integrated Staging System (NSD-ISS), and PD-associated genetic variants can be used as one of three classes of biological diagnostic anchors in the SynNeurGe classification<sup>12,13</sup> (see section ‘Proposed biomarker-based diagnostic frameworks’ and Table 3).

Some genetic forms of PD, such as those associated with *PRKN* or *LRRK2* mutations, pose a challenge to definitions of PD that require a positive  $\alpha$ -synuclein SAA status, given that people with the same mutation and identical clinical phenotypes can test positive or negative with SAAs<sup>17,100</sup>. In a post-mortem study of people with PD with *LRRK2* mutations, use of CSF  $\alpha$ -synuclein SAAs discriminated *LRRK2* PD with and without Lewy-body-type pathology with high sensitivity and specificity<sup>101</sup>. Furthermore, preliminary data suggest that any

association between PD polygenic risk score and CSF  $\alpha$ -synuclein SAA status is limited in people with dementia and in healthy people<sup>102</sup>.

## Proposed biomarker-based diagnostic frameworks

In principle, biomarker-based, biological diagnostic schemes could address major unmet needs in the diagnosis and treatment of neurodegenerative diseases, including PD. Such schemes could improve diagnostic accuracy, identification of pathogenetic subtypes, and early and pre-symptomatic diagnosis, leading to new opportunities for disease-modifying and pathway-specific symptomatic therapies. Two diagnostic frameworks have been proposed for PD that are based on combinations of complementary molecular, imaging and genetic markers (Table 3). The SynNeurGe criteria<sup>13</sup> form a framework for biological classification of PD, and the NSD-ISS<sup>12</sup> is a framework for biological definition and staging of PD.

In both frameworks, demonstration of pathological  $\alpha$ -synuclein seeding activity in the CSF with an SAA is a core diagnostic anchor, and pathological findings in dopaminergic imaging are used as an indicator of neurodegeneration. The SynNeurGe criteria also take into account a broad spectrum of PD-associated genetic variants, regardless of their association with  $\alpha$ -synuclein pathology but classified by their degree of penetrance, whereas in the NSD-ISS system, only fully penetrant *SNCA* mutations are considered to be disease-defining genetic variants and individuals with pathogenic variants that are associated with PD risk but no evidence of neuronal  $\alpha$ -synuclein pathology are categorized as having low or high genetic risk but are not assigned a stage<sup>12</sup>. This difference reflects a fundamental distinction between the two systems – the SynNeurGe classification is designed to encompass the full spectrum of what is currently recognized clinically as PD, whereas the NSD-ISS focuses strictly on the part of that spectrum in which  $\alpha$ -synuclein pathology is present, under the umbrella term of ‘neuronal synuclein disease’, and does not include conditions in which  $\alpha$ -synuclein pathology is not present, which excludes, for example, a proportion of symptomatic and presymptomatic carriers of *PRKN* or *LRRK2* mutations. Another difference between the systems is that

people who are asymptomatic but have a positive CSF  $\alpha$ -synuclein SAA would be classified in the NSD-ISS as being in stage 1 of neuronal synuclein disease<sup>12</sup> (stage 1a if dopamine transporter imaging is normal and stage 1b if both are pathological) but would be classified as having ‘Parkinson’s type synucleinopathy’ in the SynNeurGe classification<sup>13</sup>.

Both proposals indicate that future refinements are likely to incorporate additional biomarkers, which will be important given that co-pathologies are often present in pathologically defined synucleinopathies – the most common are AD-related pathological changes, neurovascular pathology and limbic predominant TDP43 encephalopathy<sup>11,42</sup>. These co-pathologies can be associated with specific phenotypical features and can drive disease. For example, neuropathological studies in animals and humans have shown that pathological tau isoforms and tau pathology facilitate  $\alpha$ -synuclein aggregation and propagation<sup>103,104</sup>. In DLB, coexistence of AD pathology, which is common, is associated with faster progression of clinical symptoms<sup>40,105,106</sup>.

## Opportunities and challenges in research and practice

The move towards disease definitions that are based on biomarkers regardless of the presence or absence of clinical symptoms has far-reaching implications, initially for research but also for future clinical practice. The proposed biological frameworks are intended to facilitate research in two inter-related areas: prospective studies to define the risk of clinically manifest disease in people who are asymptomatic but biomarker-positive, and implementation of disease prevention trials in biologically defined participants (Table 4).

### Implications for prediction and prognosis

Population-based prospective studies to evaluate the predictive validity of biomarker-based diagnosis in people without symptoms are lacking, but predictive validity has already been demonstrated for some imaging markers, such as DaT-SPECT changes in people with iRBD and/or prodromal PD (see section ‘Radiotracer imaging’). In addition, several studies have shown that CSF  $\alpha$ -synuclein SAAs are positive in 58–86% of individuals with features of prodromal PD, such as iRBD or hyposmia,

and the highest rates of positive SAAs are in cohorts that are enriched on the basis of reduced striatal tracer uptake in DaT-SPECT<sup>17,61,106</sup>.

The findings of prospective cohort studies highlight the fact that the relationships between biomarkers and subsequent disease development and progression remain to be fully defined. In one prospective study of 96 people in the PPMI cohort who were classified as having prodromal PD owing to the presence of hyposmia and/or iRBD and mild reductions in striatal tracer uptake on DaT-SPECT, 56% had a positive CSF  $\alpha$ -synuclein SAA, and 39% of these developed PD or DLB during a median follow-up period of five years<sup>107</sup>. By contrast, only 5% of people with a negative SAA developed PD or DLB during follow-up. In a large study of 1,182 participants in the BioFINDER cohort without cognitive and neurological impairment, 8% had a positive CSF  $\alpha$ -synuclein SAA, among whom 23% developed clinically defined PD or DLB during four years of follow-up<sup>108</sup>. These results provide the first evidence that  $\alpha$ -synuclein SAA positivity in healthy people has predictive value, but further large, long-term, prospective studies are clearly needed to understand the meaning of biological disease markers at the population level and in specific risk groups.

### Implications for disease-modification trials

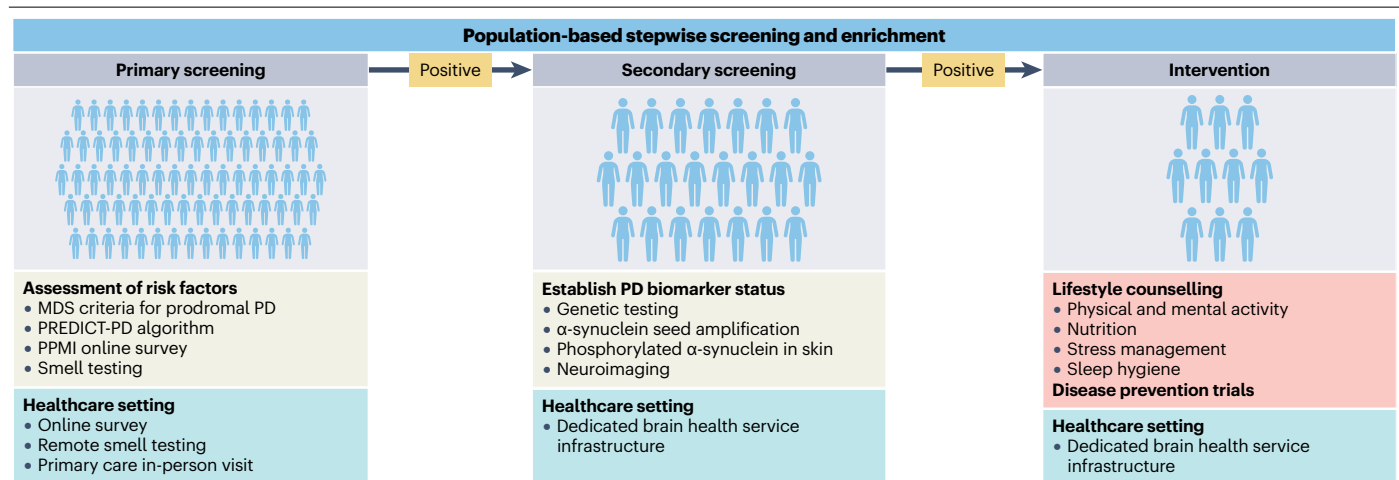
The plethora of failed disease-modification trials in PD in the past have been attributed to various factors, including the type of intervention, clinical trial design, the choice of primary outcome measures, trial duration and, importantly, inclusion of populations with disease that is either too advanced or too heterogeneous.

To date, disease-modification trials in PD have included only people with early but clinically established disease, in whom neuronal pathology might already have advanced too far for a given intervention to have a measurable and clinically meaningful effect. The ability to detect the underlying pathology before the symptomatic threshold is reached could facilitate a paradigm shift whereby people who are positive for disease biomarkers, with or without prodromal symptoms, are enrolled in disease-prevention trials (Table 4). Pivotal efficacy trials in such populations with an end point of phenoconversion to clinically defined disease currently seem unrealistic, because the low rate of conversion would necessitate long trial durations (2–5 years)

**Table 4 | Design features of disease modification trials at different stages of Parkinson disease**

Trial feature	Preclinical PD		Prodromal PD		Early clinical PD	
Target population	People at risk, defined biologically		People who are positive for biomarkers and have hyposmia and/or RBD		People with clinically defined early PD, untreated or on stable treatment	
Intervention	Lifestyle modification, repurposed safe drugs		Lifestyle modification, low-to-moderate risk (repurposed) drugs		Low-to-moderate risk (repurposed) drugs, novel pathway-specific drugs	
Endpoints	Phase II	Biomarker	Phase II	Biomarker and clinical (cognitive or motor) change	Phase IIb/III	Motor and biomarker progression
	Phase III	Clinical change, including phenoconversion	Phase III	Clinical change, including phenoconversion		
Sample size <sup>a</sup>	Phase II	>200	Phase II	160	Phase IIb/III	200–500
	Phase III	10,000 (owing to low presumed effect size)	Phase III	570–1,860		
Duration <sup>a</sup>	Phase II	12–24 months	Phase II	12–24 months	Phase IIb/III	12–24 months
	Phase III	3–5 years	Phase III	2–5 years		

<sup>a</sup>Estimates partly based on ref. 108. PD, Parkinson disease; RBD, rapid eye movement sleep behaviour disorder.



**Fig. 2 | Proposed design of population-based stepwise risk screening programmes for Parkinson disease.** Identification of people at high risk of Parkinson disease (PD) in the general population requires a primary screening step to assess for the presence of risk factors. People whose primary screening is positive could then proceed to secondary screening to assess the status of several

PD biomarkers. People whose secondary screening is positive could then receive interventions such as lifestyle-modification recommendations or enrolment in clinical trials. MDS, Movement Disorders Society; PPMI, Parkinson's Progression Markers Initiative.

and large sample sizes (several thousand participants). However, phase II trial platforms with biomarker outcomes in populations that are enriched for biomarker positivity and prodromal symptoms, such as hyposmia and iRBD, are feasible and currently in development<sup>109,110</sup>. Biomarker-based stratification could also enable pathogenetically homogeneous subgroups and subgroups with co-pathology to be defined.

When testing disease-modifying interventions in people without symptoms, the intervention must be safer than in trials in people with disease to maintain acceptable risk–benefit ratios. For this reason, re-purposed drugs with established safety or non-pharmacological interventions, such as exercise or other lifestyle modifications, should be prioritized. Examples of this approach include the ongoing trial of idebenone<sup>111</sup> and the SLOW-Speed trial of titrated exercise via a smartphone app in people with prodromal symptoms<sup>112</sup>.

## Future directions

### Biomarkers to measure disease progression

One obstacle in past trials of disease-modifying interventions in PD has been a lack of reliable biomarkers of disease progression that are more sensitive to changes than clinical outcomes. Molecular dopaminergic imaging and novel MRI approaches, including iron-sensitive MRI sequences, neuromelanin and free-water imaging, are all sensitive to changes, but the progression rates of these markers in early and prodromal PD are yet to be fully defined.

$\alpha$ -Synuclein PET imaging could have the high sensitivity and specificity needed to detect the earliest signs of pathology in PD, similar to the role of amyloid and tau PET in AD. No  $\alpha$ -synuclein PET tracer that can specifically label pathological  $\alpha$ -synuclein aggregates has yet been validated in PD, but proof-of-concept studies in small numbers of participants with MSA or PD have produced encouraging results<sup>113,114</sup>.

Studies are also in progress to determine whether measures of progression could be developed from  $\alpha$ -synuclein SAAs<sup>115</sup>. To date,  $\alpha$ -synuclein SAAs have mainly been used as a binary measure, but their underlying principles theoretically allow for the development of a

quantitative readout. A correlation has been found between the burden of Lewy-body pathology in brain tissue and kinetic parameters in CSF  $\alpha$ -synuclein SAAs<sup>29</sup>, and a quantitative measure of  $\alpha$ -synuclein seeds in the CSF correlated with clinical severity in a group of seven people with PD<sup>116</sup>, but further work is needed to confirm these findings. Less invasive approaches could include quantification of  $\alpha$ -synuclein in skin biopsy samples by immunohistochemistry<sup>24</sup>.

### Biomarkers and future PD early detection programmes

Biomarker characterization of people who are considered to be at high risk of PD could identify people with prodromal PD for inclusion in clinical trials. Current evidence suggests that a primary remote screen with a smell test that is adapted to the cultural specifics of a given population will identify up to 27% of people with hyposmia<sup>117</sup>, up to 50% of whom could be positive for  $\alpha$ -synuclein in the CSF<sup>61</sup>. These data suggest the possibility of two-stage PD risk screening programmes that involve remote smell testing as a low-cost, non-invasive primary step that is followed by a  $\alpha$ -synuclein biomarker testing (Fig. 2). The first step seems feasible now, but  $\alpha$ -synuclein SSAs are not ready to be implemented in this way at a population level globally owing to issues with reliability, reproducibility and scalability to the level necessary for cost-effective screening. In this context, CSF sampling would be too invasive, but blood-based assays could eventually meet the requirements for reliability, availability and cost.

Other tools for remote assessment of PD risk could also be used as an initial step in screening programmes (Fig. 2). Risk factor assessment methods that have been tested for their predictive validity include the MDS criteria for prodromal PD<sup>7</sup> and the PREDICT-PD algorithm<sup>118</sup>. The PREDICT-PD algorithm has been designed for remote administration as an online survey, as has the PPMI prodromal survey<sup>117</sup>. People with increased risk on the basis of such remote assessments could be invited for secondary screening with in-person and biomarker assessments. People with positive tests for PD biomarkers, such as DaT-SPECT or CSF  $\alpha$ -synuclein SAA, could then be candidates for research interventions

## Box 2 | From research to real-world benefits

Progress in biomarker research — particularly  $\alpha$ -synuclein seed amplification assays — has fuelled efforts to enable more accurate and earlier diagnosis of Parkinson disease (PD) than is possible at present, and biological, biomarker-based diagnostic frameworks for PD have been developed. Redefining PD on the basis of specific biomarker profiles has enormous potential to lead to real-world benefits. These benefits include more accurate diagnosis of PD and differentiation of PD from mimics, such as multiple system atrophy, progressive supranuclear palsy or non-degenerative parkinsonism. Crucially, biomarker-based criteria would also enable diagnosis independently of the presence of clinical symptoms, thereby enabling detection of disease at a very early, even preclinical, stage. Such early detection raises the possibility of disease-prevention trials in people who are at high risk of developing PD. In addition, biomarker signatures could help to distinguish between different forms of PD and guide precision medicine approaches. Ultimately, once the current frameworks have matured, biomarkers will become an essential element for population-based risk screening for PD, which is necessary for the prevention of PD to become a reality.

Before this potential can be realized in real-world clinical practice, more research is needed to define the accuracy of biomarker signatures for risk prediction among healthy people and for the prognosis and monitoring of treatment response in people with symptoms. Concerted efforts will be required to harmonize protocols for  $\alpha$ -synuclein SAAs and other biomarkers to ensure that they are reproducible between laboratories and to broaden access to them. The emergence of scalable blood-based tests could ultimately provide the accessibility and feasibility needed to translate current scientific advances into meaningful benefits for people living with PD.

such as lifestyle modification or inclusion in a disease-modification trial (Fig. 2).

However, critical issues need to be resolved before PD risk screening could become a healthcare reality. Challenges include defining target populations and age thresholds for initial screening, establishing predictive values of positive tests, implementing an appropriate infrastructure for testing and counselling, and the availability of interventions that meaningfully reduce disease risk.

Before these challenges are met, screening and current biological definitions of disease carry a serious risk of overdiagnosis — that is, diagnosis of preclinical disease on the basis of markers that would not have progressed to clinically manifest disease in the individual's remaining lifetime<sup>119</sup>. Such false-positives can lead to unnecessary further diagnostic testing and healthcare costs, and can create anxiety and affect quality of life, employment and insurance options for the individual. This risk is illustrated by the fact that CSF  $\alpha$ -synuclein SSAs are positive in 5–12% of healthy people<sup>105</sup>. If all such healthy individuals are reclassified as having a PD synucleinopathy or stage 1 of neuronal synuclein disease in line with the SyNeurGe and NSD-ISS frameworks, the prevalence of PD would increase dramatically, posing a major challenge to the existing healthcare infrastructure. Nonetheless, studies of people with PD or iRBD or who are asymptomatic indicate that a high proportion of people would like to know their risk status if

there were a reliable predictive test, even in the absence of preventive therapies<sup>120–122</sup>.

### Digital technology and artificial intelligence

Artificial intelligence (AI) and the ability to analyse large volumes of data are revolutionizing all areas of medicine — and PD is no exception. These technologies offer powerful tools to improve diagnostic accuracy, personalize treatments and accelerate research into new therapies. For example, deep learning algorithms can analyse MRI and other neuroimaging scans to detect subtle brain changes that indicate early onset of disease even before clinical symptoms appear, and can assist in distinguishing PD from other forms of parkinsonism<sup>123</sup>. AI approaches are also expected to facilitate analysis of large fluid or tissue biomarker sets generated by use of omics approaches.

AI can also process information (including medical history, non-motor symptoms and genetic variables) from large patient databases to detect combinations of factors that suggest a high risk of developing PD. Similarly, AI can be used to extract PD-specific patterns from data collected with digital sensors that record a wide range of movement features, including dexterity, handwriting, voice parameters, eye movements, facial expression, nocturnal mobility, tremor and gait patterns, enabling early diagnosis and detection of disease progression<sup>124–126</sup>.

Beyond digital biomarker extraction, AI-based methods can also harmonize and integrate multimodal datasets, including fluid biomarkers, neuroimaging and omics-derived measures. By mitigating inter-cohort variability and generating unified latent representations, these approaches facilitate the identification of composite biomarker signatures. Emerging evidence indicates that multimodal AI frameworks that combine CSF or blood biomarkers, MRI and/or PET features and digital measures improve diagnostic and prognostic performance compared with single-modality approaches<sup>127,128</sup>.

Although these advances are at an early stage, ongoing research and technological improvements are expected to increase their accuracy and impact considerably<sup>129,130</sup>. An example of this research is the **AI-PROGNOSIS** project in Europe, the focus of which is to improve early detection, diagnosis and personalized management of PD by the use of AI and digital biomarkers.

### Conclusions

The characterization of  $\alpha$ -synuclein SAAs as sensitive and specific tools to detect  $\alpha$ -synuclein pathology in clinically or neuropathologically established synucleinopathies has opened up a new era in the diagnosis of PD, with considerable implications for clinical practice (Box 2). The use of  $\alpha$ -synuclein SAAs improves diagnostic accuracy for clinically manifest PD and DLB and can help to differentiate PD from MSA, PSP and other mimics. Most importantly,  $\alpha$ -synuclein SAAs also have the potential to enable diagnosis of prodromal and preclinical disease stages, and are a core component of proposals for the biological definition and classification of Lewy-body diseases. In addition, advances in imaging, genetic and other molecular biomarkers are also improving diagnostic accuracy in PD, enabling diagnosis at pre-symptomatic disease stages and facilitating identification of pathogenetic subtypes. Top of the agenda for current PD research is addressing disease heterogeneity and the development of precision medicine approaches, which are becoming a reality in ongoing clinical trials in genetically defined subtypes of PD.

Should disease-modifying therapies be developed for PD, the pressure to accurately diagnose manifest and premotor PD and to

accurately monitor disease progression will greatly increase. Trial platforms and trials for intervention in prodromal PD are already being planned and developed on the basis of the latest biomarker findings. However, important ethical issues need to be addressed when diagnosing PD in people with only nonmotor symptoms or no symptoms, particularly while no proven preventive treatments or strategies exist. People who have positive biomarker tests and those involved in prediagnostic research must be fully informed of the potential benefits and risks of any preventive strategies they might receive.

Published online: 13 February 2026

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## Author contributions

E.T. and W.P. conceived the scope and content of the article and oversaw content development. All authors researched data for the article, wrote the article, contributed substantially to discussion of the content and reviewed and edited the manuscript before submission.

## Competing interests

E.T. reports honoraria for consultancy from Prevail/Lilly and has received funding for research from the Michael J Fox Foundation. Unrelated to this work, W.P. has received personal lecture fees and/or consultancy fees from Alterity, AbbVie, AC Immune, BIAL, Boehringer, Britannia, Lilly, Lundbeck, Merck, Merz Pharma, Mitsubishi-Tanabe, Neuroderm, Novo Nordisk, Roche, Sanofi, Sumitomo, Takeda, Teva, UCB and Zambon, and has received grant support from the Michael J Fox Foundation and the European Union programmes FP7 and Horizon, and has received royalties from Cambridge University Press, Oxford University Press, Thieme and Wiley Blackwell. A.J.N. is supported by the Michael J Fox Foundation and reports honoraria from Teva and travel and meeting expenses from the International Movement Disorders Society. P.S. has received honoraria for lectures or courses from Abbvie, Britannia, Lundbeck, Merz Therapeutics and Zambon, owns stock in Bioarctic and Irlab, and has received major research grants from Aligning Science Across Parkinson's, the Knut and Alice Wallenberg Foundation, the Michael J Fox Foundation and the Swedish Research Council. M.J.M. has received grant support from the Michael J Fox Foundation and honoraria for consultancy from Bial. P.M. declares no competing interests.

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**Peer review information** *Nature Reviews Neurology* thanks the anonymous reviewers for their contribution to the peer review of this work.

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