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Biomarkers of conversion to α-synucleinopathy in isolated rapid-eye-movement sleep behaviour disorder

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Contributions

All authors were involved in the drafting and editing of the manuscript, apart from AI and AJ, who were involved only during the editing stage. MGM coordinated the autonomic function section, EA coordinated the tissue biopsy section, DA coordinated the neuroimaging section, BFB and J-FG coordinated the cognition section, NJD coordinated the ophthalmic function section, RF coordinated the neurophysiology section, ZG-O coordinated the genetics section, MTH coordinated the olfaction section, JL coordinated the biofluids section, and JR coordinated the motor function section. MGM and WHO coordinated section editor submissions. RF, DA, J-FG, and BFB sourced the figures.

See *Online* for appendix

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Abstract

Patients with isolated rapid-eye-movement sleep behaviour disorder (RBD) are commonly regarded as being in the early stages of a progressive neurodegenerative disease involving α-synuclein pathology, such as Parkinson's disease, dementia with Lewy bodies, or multiple system atrophy. Abnormal α-synuclein deposition occurs early in the neurodegenerative process across the central and peripheral nervous systems and might precede the appearance of motor symptoms and cognitive decline by several decades. These findings provide the rationale to develop reliable biomarkers that can better predict conversion to clinically manifest α-synucleinopathies. In addition, biomarkers of disease progression will be essential to monitor treatment response once disease-modifying therapies become available, and biomarkers of disease subtype will be essential to enable prediction of which subtype of α-synucleinopathy patients with isolated RBD might develop.

Introduction

Rapid-eye-movement (REM) sleep behaviour disorder (RBD) has been established as one of the earliest and most specific prodromal signs of the α-synucleinopathies, including Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. Although not all patients with an α-synucleinopathy have RBD, several longitudinal studies have shown that more than 80% of patients with isolated RBD—ie, RBD not associated with a known neurological disorder—will be diagnosed with Parkinson's disease, dementia with Lewy bodies, or multiple system atrophy within their lifetimes.[1] For this reason, patients with isolated RBD will be an ideal population in which to use disease-modifying therapies when they become available. However, the delay from diagnosis of isolated RBD to phenoconversion (ie, conversion from isolated RBD to a diagnosis of Parkinson's disease, dementia with Lewy bodies, or multiple system atrophy) is variable, with the prodromal period lasting from years to decades, and RBD alone cannot predict α-synucleinopathy subtype. Identification of patients with isolated RBD who are most likely to phenoconvert within several years is crucial if participants are to reach endpoints within the time-frame of disease-modifying therapy trials, as is the identification of biomarkers that can monitor the neurodegenerative process and treatment outcomes.

The ideal biomarker must be highly sensitive and specific, reproducible, cost-effective, readily available, and able to serve as a therapy-responsive progression marker. The goal of this Review is to summarise the field of potential biomarkers of α-synucleinopathies in patients with isolated RBD with this ideal in mind. We focus on ten biomarker categories that have shown substantial promise, presented in the order in which we consider them to be easily obtainable and, therefore, available for use in potential international clinical trials. We have also categorised candidate biomarkers according to how they might be used (table

1). On the basis of current evidence, the potential usefulness of each biomarker will be highlighted, with specific focus on its role in future disease-modifying therapy trials.

Biomarker categories

Neurophysiology—REM sleep without atonia is the neurophysiological hallmark of RBD (figure 1) and is required for diagnosis. REM sleep without atonia is recorded during the mandatory diagnostic step of video-polysomnography, making it the most readily available diagnostic biomarker. The presence of REM sleep without atonia has been identified before dream-enacting behaviours, establishing isolated REM sleep without atonia as one of the earliest signs of neurodegeneration.[3,4] REM sleep without atonia might also offer the potential to predict phenotypic subtypes of evolving α-synucleinopathy, thus enhancing its diagnostic potential.[3] Several visual and automated methods for scoring REM sleep without atonia have shown largely convergent agreement, with acceptable sensitivity and specificity (both ranging 85–95%). Finally, REM sleep without atonia might prove to be a valuable prognostic biomarker of disease progression as it might increase over time in some individuals, with greater severity associated with accelerated phenoconversion.[5,6]

RBD seems to result from the breakdown of a broad neural network underlying REM sleep atonia, with an interaction between the brainstem and both rostral and caudal CNS structures.[7] This hypothesis has prompted the development of advanced EEG analysis that has shown potential as a diagnostic and prognostic biomarker. For example, lower cyclic alternating pattern rates on EEG have been associated with increased rates of phenoconversion in individuals with isolated RBD,[8] as has the time–frequency structure of resting wakeful EEG.[9] Further gains in diagnostic and prognostic value might be achieved by the use of artificial intelligence and machine learning-based methods, such as a recently developed random forest classifier that combines muscle atonia data with sleep architecture to accurately identify the presence of RBD.[10] Other more experimental approaches, including transcranial magnetic stimulation to probe early cortical dysfunction[11] and vestibular-evoked myogenic potentials assessing brainstem neurophysiology,[12] require additional investigation before being proposed as prognostic biomarkers.

Motor function—Given the prominence of parkinsonism in patients with isolated RBD who go on to be diagnosed with an α-synucleinopathy,[1] formalised motor assessments represent appealing and readily available biomarkers, although the specific protocol is yet to be optimised. Motor abnormalities in patients with isolated RBD emerge relatively late in the prodromal disease process and might indicate which patients are at most risk of phenoconversion in the near future. A longitudinal multicentre trial by the International RBD Study Group in 1280 patients with isolated RBD showed that quantitative motor tests are one of the most powerful predictive markers of future phenoconversion, with a hazard ratio (HR) of 3·16 (95% CI 1·86–5·37).[1] Performance on quantitative motor assessments and, in particular, an upper extremity alternating-tap test can become significantly different from that of healthy individuals 5–8 years before phenoconversion,[13] offering potential as both a prognostic and monitoring biomarker.

Several cross-sectional studies using instrumental assessments have highlighted the use of gait,[14,15] speech,[16] saccadic eye movements,[17] rhythm,[18] and finger tapping,[19] with sensitivity and specificity of up to 80%, to identify the presence of isolated RBD. For example, changes in home-based spontaneous walking tasks and decreased gait speed, cadence, and step variability have been reported in patients with isolated RBD, compared with age-matched controls.[14] In addition, laboratory assessments have revealed deficits in postural control and foot step asymmetry during dual-task walking in patients with isolated RBD compared with controls, suggesting an overlap between motor and cognitive domains.[15] Acoustic speech analysis has indicated that levels of monopitch (reduced ability for intonation during speech), longer duration of pauses, and a decreased rate of follow-up speech segments might best discriminate between patients with isolated RBD, patients with Parkinson's disease, and controls,[16] probably reflecting both vocal cord hypokinesia and deficits in orolingual movement initiation. Poor spontaneous rhythm timing and perception has also been shown: performance in a small cohort of patients with isolated RBD was similar to that in individuals with mild Parkinson's disease,[20] whereas, in another cohort of patients with isolated RBD, finger tapping amplitude and velocity decrement were impaired when compared with controls, suggesting prodromal bradykinesia.[19] Finally, increased error rates for antisaccadic but not prosaccadic eye movements have been reported in patients with isolated RBD compared with controls.[17] Despite the precision offered by these approaches, there is also a recognised need to quickly and accurately assess motor function in the clinic and home environments. A combination of motor markers evaluated with a smartphone was highly effective in discriminating patients with isolated RBD, patients with Parkinson's disease, and controls, with a mean sensitivity of 85% and specificity of 92%,[18] highlighting the potential of future technology in prognostic and monitoring biomarkers for disease-modifying therapy trials.

Cognition—Cognitive decline is common in patients with isolated RBD, and so cognitive testing represents another valuable and readily available biomarker. Mild cognitive impairment is present in more than a third of patients with isolated RBD, and patients with isolated RBD and concomitant mild cognitive impairment are at higher risk of phenoconversion than patients with isolated RBD and normal cognitive functioning.[1,21] Indeed, both amnestic and non-amnestic mild cognitive impairment subtypes in patients with isolated RBD are predictive of the development of dementia with Lewy bodies or parkinsonism with cognitive impairment.[1,22–24]

Deficits in cognitive performance also affect patients with isolated RBD who do not fulfill criteria for mild cognitive impairment.[21] Cross-sectional studies have reported deficits in attention, executive function, memory, and visuospatial function in this population,[21] and pareidolias (the tendency to perceive a meaningful image in an ambiguous visual pattern) and deficits in prospective memory have also been identified.[25,26] Importantly, the pattern and severity of cognitive deficits in patients with isolated RBD might predict subtype of α-synucleinopathy. Cognitive deterioration over time is more common in patients who will develop dementia with Lewy bodies, whereas stable cognitive performance over a period of 6 years is more common in those who will develop Parkinson's disease or remain free of an α-synucleinopathy diagnosis.[24] In one longitudinal study, assessments of executive

function, such as the Trail Making Test Part B, showed deficits in patients with isolated RBD 6 years before diagnosis of dementia with Lewy bodies,[24] whereas verbal episodic memory, assessed by the Rey Auditory-Verbal Learning Test, and semantic memory, assessed by semantic verbal fluency, were abnormal 2–4 years before such a diagnosis.[24] This predictive value might be heightened when combined with multimodal imaging approaches, which have more recently been used to elaborate the structural and functional correlates of mild cognitive impairment in patients with isolated RBD (see Neuroimaging section).

Cognitive testing in patients with isolated RBD might thus prove useful as a diagnostic biomarker, particularly to identify prodromal dementia with Lewy bodies, and as a prognostic and monitoring biomarker. The psychometric properties of cognitive testing are well established, and the tests are widely available at low cost and easily done, with administration times of 15–25 min.[24] The Montreal Cognitive Assessment, a screening test that takes 10 min to administer, could be another option to identify patients with isolated RBD at risk of dementia with Lewy bodies;[13] however, further studies are needed to validate its psychometric properties in this population.

Olfaction—Hyposmia is recognised as one of the earliest prodromal signs of Parkinson's disease and is present in many patients with isolated RBD.[27,28] In a multicentre study of more than 600 patients with isolated RBD, hyposmia was present in 67%,[1] and back-extrapolation of disease (to estimate the time at which olfaction crossed normal control values) has identified evidence of hyposmia more than 20 years before phenoconversion.[13] The Sniffin' Sticks test (Burghardt, Wedel, Germany),[29] comprising multi-use felt-tip style pens, and the University of Pennsylvania Smell Identification Test (UPSIT),[30] which uses single-use scratch cards, are the most frequently used instruments to assess olfaction, with similar discrimination accuracy. A link between the severity of isolated RBD symptoms and olfactory deficit has been suggested through comparison of sleep clinic-ascertained patients with isolated RBD with those ascertained from a general population of older individuals;[31] olfactory function was worse in the clinic-ascertained compared to population-ascertained cohort, with clinic-ascertained patients with isolated RBD manifesting higher rates of dream-enactment behaviours during video-polysomngraphy than population-ascertained patients with isolated RBD, indicating worse RBD severity.

The HR attributed to hyposmia (2·62, 95% CI 1·67–4·12), based on pooled multicentre data from more than 600 individuals, exceeds that of all other non-motor markers.[1] Its use alongside an age cut-off of at least 55 years has been suggested to stratify individuals at risk of phenoconversion within several years,[32] although the absence of worsening olfactory deficit over time has led to caution over its use as an outcome measure in disease-modifying therapy trials.[33] In one study, hyposmia was closely correlated with progressive decline in visuospatial function and verbal memory in patients with isolated RBD followed over 2 years, suggesting that hyposmia might also predict future conversion to dementia with Lewy bodies.[34] Although hyposmia also correlates strongly with the presence of phosphorylated α-synuclein aggregates on skin biopsy,[35] it cannot distinguish underlying Parkinson's disease from dementia with Lewy bodies and has incomplete penetrance in the α-synucleinopathies.[35,36] As part of a two-tiered screening strategy aimed at identifying individuals at risk of incident Parkinson's disease, hyposmia combined with a dopamine

transporter deficit on ^{123}I-*N*-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane (^{123}I-FP-CIT) SPECT imaging predicted phenoconversion within a 4-year period with a sensitivity of 74%, specificity of 97%, positive predictive value of 67%, and negative predictive value of 97%.[37] The combination of hyposmia and abnormal dopamine transporter imaging in this cohort of 280 hyposmic and normosmic individuals was associated with a phenoconversion rate of 25%, compared with a rate of 2·5% using hyposmia alone, highlighting the potential of hyposmia as a combined biomarker.[37]

Finally, although olfactory discrimination is often reduced in patients with isolated RBD, partial recovery can occur due to neurogenesis in the subventricular zone. This zone contains neural progenitor cells that migrate via the rostral migratory stream to the olfactory bulb and differentiate into interneurons. Studies show variable impairment of olfactory bulb-related neurogenesis that is directly triggered by α-synuclein accumulation, in both post-mortem brain investigations from humans with Parkinson's disease and transgenic Parkinson's disease mouse models.[38] Therefore, variable neurogenesis might contribute to the substantial interindividual differences in olfaction observed in people with isolated RBD and intraindividual differences observed throughout ageing.

Ophthalmic function—Despite its ease of use, colour discrimination has rarely been systematically evaluated in people with isolated RBD,[1,13,39] and the only test to have been evaluated is the Farnsworth-Munsell 100-Hue test. In a study of 154 patients with isolated RBD, olfactory dysfunction was first to develop, followed by impaired colour discrimination.[13] In another study of 62 patients with isolated RBD followed over 5 years, 13 (74%) of 21 patients with isolated RBD and impaired colour discrimination converted to Parkinson's disease, compared with 12 (30%) of 40 patients with isolated RBD and unimpaired colour discrimination.[39] In a multicentre study of 1280 patients with isolated RBD, in which a fifth underwent colour testing,[1] the HR for phenoconversion to Parkinson's disease or dementia with Lewy bodies was 1·69 (95% CI 1·01–2·78).[1] In a monocentric study of 154 patients with isolated RBD, impaired colour discrimination began an estimated 12·8 years before phenoconversion to Parkinson's disease or dementia with Lewy bodies,[13] suggesting that colour discrimination holds potential as both a diagnostic and prognostic biomarker. The mechanism of heightened phenoconversion risk in those with impaired colour discrimination remains unclear.

Although the data are less robust than those for discrimination, optical coherence tomography also holds potential as a diagnostic and prognostic biomarker, as thinning of the parafoveal ganglion cell complex in the retina has been found to correlate with olfactory loss and striatal dopamine transporter reduction in patients with isolated RBD.[40] For Parkinson's disease, such thinning has been correlated with nigral dopaminergic loss and visual impairment[41] Furthermore, patients with isolated RBD seem to show a reduction of the retinal nerve fibre layer.[42] However, longitudinal studies using optical coherence tomography in patients with isolated RBD are needed to confirm these findings.[43] Although the retinal contribution to colour perception is well established, optical coherence tomography findings have yet to be correlated with colour discrimination in patients with isolated RBD. Interestingly, Lewy-type pathology in the retinal ganglion cell complex layers has also been found in incidental Lewy body disease involving the brainstem.[44] Although

the mechanism of colour discrimination impairment in the retina remains uncertain, these findings suggest parallel initiation of the neurodegenerative process at anatomically distinct sites. Future studies are needed to rule out methodological inconsistencies[45] between different optical coherence tomography studies and to establish whether these retinal changes are unique to α-synucleinopathies.[46]

Autonomic function—Autonomic impairment is common in patients with isolated RBD, occurs early in the disease process,[13] and has been shown in studies using both questionnaires and objective measures of autonomic function, such as heart rate variability, cardiac metaiodobenzylguanidine scintigraphy, and autonomic reflex testing. Autonomic symptoms in patients with isolated RBD encompass adrenergic and cardiovagal deficits, sexual and urinary dysfunction, and constipation.[1,47,48] The severity of autonomic impairment is mild to moderate in most patients with isolated RBD, at an intermediate level between healthy individuals and people with Parkinson's disease.[47]

Several questionnaire-based studies have revealed that patients with isolated RBD report significantly more autonomic symptoms than controls do, with the greatest impairment reported in cardiovascular, gastrointestinal, and urinary domains.[1,47,48] In a prospective study of 1280 patients with isolated RBD, constipation and erectile dysfunction were associated with the greatest risk of phenoconversion.[1] The severity of autonomic symptoms has also been associated with putaminal dopamine transporter abnormalities and an accelerated rate of phenoconversion in patients with isolated RBD, highlighting potential as both a diagnostic and prognostic biomarker.[28,48,49]

Although some studies on heart rate variability have shown impairment in low-frequency spectra on video-polysomnography, suggestive of cardiac sympathetic impairment, other studies suggest impairment in high-frequency spectra, suggestive of parasympathetic impairment.[50] Metaiodobenzylguanidine scintigraphy studies have shown that patients with isolated RBD have markedly reduced uptake ratios compared with controls, suggestive of postganglionic sympathetic impairment,[51] a finding more commonly seen in patients with Parkinson's disease than in those with multiple system atrophy, allowing for a potential diagnostic distinction between prodromal phenotypes. Although heart rate variability and metaiodobenzylguanidine abnormalities are seen early in the isolated RBD disease course, there are no longitudinal data showing an association with phenoconversion rates, making these biomarkers more appealing for diagnostic than for prognostic use.

Autonomic reflex testing has shown consistent impairment across cardiovagal, sympathetic adrenergic, and sudomotor domains in patients with isolated RBD, with the greatest impairment in measures of sympathetic adrenergic function,[52–54] which might worsen with disease progression. In addition, more severe cardiovagal dysfunction is more strongly associated with phenoconversion to dementia with Lewy bodies than Parkinson's disease.[49] Although validated as the most quantitative and comprehensive method of assessing autonomic function, autonomic reflex testing requires a specialised autonomic laboratory with beat-to-beat blood pressure recording, thus limiting access, in contrast to less expensive but less precise questionnaires.

Although the available literature shows that autonomic impairment can serve as a diagnostic marker in isolated RBD, data on the prognostic value of such impairment are scarce, and longitudinal studies are necessary to establish whether autonomic impairment in patients with isolated RBD can help predict the development of α-synucleinopathy subtypes. Finally, the large intraindividual variability of autonomic symptoms in patients with isolated RBD might pose challenges in accurately phenotyping those at risk of more imminent phenoconversion.

Biofluids—Given the neuroanatomical proximity of CSF to the brain, biomarkers obtained from CSF represent appealing candidates for molecular characterisation of α-synucleinopathies. Real-time quaking-induced conversion (RT-QuIC) has emerged as an ultrasensitive technique to identify pathological α-synuclein in the CSF of patients with Parkinson's disease and patients with dementia with Lewy bodies, with a high degree of sensitivity and specificity. From the few studies analysing CSF biomarkers in patients with isolated RBD, RT-QuIC can detect pathogenic species of α-synuclein with a sensitivity of 90–100% and specificity of 90–98%,[55] with a positive result suggesting an increased risk of phenoconversion,[56] highlighting the potential of RT-QuIC to detect pathogenic α-synuclein in CSF as both a diagnostic and prognostic biomarker. Furthermore, use of RT-QuIC with swabs from olfactory mucosa to detect pathogenic α-synuclein has been reported as a potential diagnostic marker. The technique is less invasive than lumbar puncture and has good specificity (90%) but moderate sensitivity (44%), although the sensitivity increases to 73% in patients with isolated RBD who have hyposmia.[57]

Biomarkers obtained directly from blood represent an attractive candidate due to the relatively low cost and ease of obtainability; however, results have been suboptimal. α-Synuclein in plasma neuronal exosomes might aid in the early diagnosis of Parkinson's disease, but no significant differences in exosomal α-synuclein concentrations were found in individuals with isolated RBD compared with healthy controls in one longitudinal study.[58] Another cross-sectional study showed that neuronal exosome α-synuclein concentrations were elevated in individuals with isolated RBD when compared with controls and individuals with multiple system atrophy, but no different when compared with people with Parkinson's disease; this finding suggests a potential role in predicting subtype, based on the fact that α-synuclein in patients with multiple system atrophy accumulates primarily in oligodendrocytes.[59] In addition, serum neurofilament light chain, a neuronal cytoskeletal protein released upon neuronal damage, might mark the conversion of isolated RBD to clinically manifest Parkinson's disease.[60] Techniques such as proteomics analysis of serum samples, which have identified several proteins at significantly altered expression levels, have provided further insight into the protein signature profile and molecular pathways involved in the pathogenesis of isolated RBD,[61,62] but confirmatory studies are needed.

Alterations in circulating microRNAs have been shown in several neurodegenerative diseases including isolated RBD. One study showed that miR-19b was significantly downregulated in patients with isolated RBD who phenoconverted, but not in those who remained disease-free after 4·7 (±2·6) years of follow-up, indicating that dysregulation of miR-19b might contribute to phenoconversion and offer potential as a prognostic biomarker.[63] One study revealed decreased antioxidant superoxide dismutase and increased

glycolysis in patients with isolated RBD using peripheral blood mononuclear cells.[64] The potential of other samples, such as those from saliva, tears, and the microbiome, is yet to be explored in patients with isolated RBD, and longitudinal studies are required to establish whether such biosamples can be used to assess phenoconversion risk.[65]

Neuroimaging—Evidence of nigrostriatal dopaminergic impairment, usually measured as availability of dopamine transporters in the basal ganglia, has consistently been found in patients with isolated RBD on both PET and SPECT imaging (figure 2A), with ^{123}I-FP-CIT SPECT currently the most studied and readily available dopamine transporter SPECT imaging modality. Abnormal dopamine transporter imaging appears to signal an increased risk of phenoconversion,[1,69,70] especially when combined with cognitive and autonomic impairment.[66,71] Furthermore, nigrostriatal dopamine transporter abnormalities seem to correlate with changes in brain glucose metabolism as assessed by ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET.[72] The ^{18}F-FDG PET-derived isolated RBD-related (figure 2B) and Parkinson's disease-related pattern[67] appears to be a prodromal progression marker, having shown potential both to assess progression and to predict α-synucleinopathy subtype.[73]

Although radionuclide studies with dopamine transporter SPECT and ^{18}F-FDG-PET imaging are widely available and their costs for clinical trial purposes are acceptable, dependence on ionising radiation might restrict their utility. Therefore, MRI remains an attractive alternative. MRI techniques have shown abnormalities in the substantia nigra related to the degree of dopaminergic dysfunction (figure 2C)[74] and grey matter changes in the motor cortico-subcortical loop that correlate with motor abnormalities.[75] Findings on MRI have also been correlated with cognitive impairment in patients with RBD and mild cognitive impairment, with cortical thinning in the left anterior temporal cortex best differentiating patients from controls (figure 2D). In addition, associations have been noted between reduced attention and executive function and thinning of the frontal cortex, between reduced verbal learning and thinning of the left temporal cortex, and between visuospatial function and thinning of the fronto-temporo-occipital cortex in patients with isolated RBD.[68]

Other MRI approaches include deformation-based morphometry, which has been used to identify a brain signature by combining cortical and subcortical deformation and subarachnoid and ventricular expansion, that predicts the development of dementia with Lewy bodies in patients with isolated RBD.[76] MRI has also shown promising results in identifying patients at risk of developing multiple system atrophy.[77] Whole-brain resting-state functional MRI has shown correlations between reduced performance in a processing speed task and disrupted connectivity in the associative areas of the parieto-temporal lobes,[78] as well as an association between verbal learning and left thalamo-fusiform connectivity.[24] In addition, functional MRI has shown a disrupted posterior brain network associated with isolated RBD-related cognitive impairment.[78] Accordingly, cholinergic denervation on ^{11}C-donepezil PET, known to be related to cognitive impairment, was found in patients with isolated RBD, particularly in the temporal, occipital, cingulate, and dorsolateral prefrontal cortex.[79]

Despite best efforts, imaging biomarkers that delineate neuropathological spread of α-synuclein are lacking for patients with isolated RBD. ^{123}I-FP-CIT SPECT remains the

most reliable prognostic marker of phenoconversion in this context and is increasingly being considered as an enrichment tool to select individuals with prodromal Parkinson's disease for partidpation in disease-modifying therapy trials. ^{18}F-FDG PET has shown diagnostic promise in detecting disease-specific patterns with the potential to predict α-synucleinopathy subtype, in addition to potential as a prognostic progression marker,[73] but confirmatory studies are required. Although several MRI techniques offer potential as diagnostic and prognostic markers, longitudinal data are needed before recommending this technique for disease-modifying therapy trials.

Tissue biopsy—Phosphorylated α-synuclein deposits in the substantia nigra are a neuropathological hallmark of Parkinson's disease; however, autopsy studies have also shown phosphorylated α-synuclein in peripheral structures, such as the autonomic nerves, enteric mucosa, and salivary glands, in patients with Parkinson's disease or dementia with Lewy bodies.[80,81] One of the first tissues to be analysed in both people with Parkinson's disease and people with isolated RBD was colonic tissue, with only one study showing a positivity rate of only 24% (4 of 17 patients with isolated RBD).[82] Transcutaneous core needle biopsy of the submandibular gland with ultrasound guidance showed high sensitivity and specificity in major salivary gland tissue in one study,[83] but adequate biopsy material was obtained in only 9 (43%) of 21 patients. Biopsy of minor salivary glands in the inner side of the lower lip obtains adequate tissue in all cases but is less sensitive, with only 31 (50%) of 62 patients with isolated RBD showing phosphorylated α-synuclein positivity.[84]

More recently, skin biopsy has emerged as a promising and less invasive technique (appendix p 3).[81] This technique is well tolerated, relatively inexpensive, and easier to do than colon or salivary gland biopsies, and can be done in any outpatient setting under aseptic technique, although dual-immunofluorescence analysis does require operator experience. One study using biopsies of multiple unilateral sites (C7 paraspinal area, T10 paraspinal area, and proximal and distal leg) showed phosphorylated α-synuclein positivity in 10 (56%) of 18 patients with isolated RBD, 20 (80%) of 25 patients with early Parkinson's disease, and 0 of 20 controls.[35] The likelihood of phosphorylated α-synuclein positivity was greater in those with olfactory dysfunction, whereas the relationship with reduced dopamine transporter SPECT ligand density was less robust, indicating that skin biopsy positivity can be found in patients with isolated RBD with a normal dopamine transporter SPECT, at least 2 years before nigrostriatal decline. A second study independently confirmed this finding using bilateral biopsies at C8 and the distal leg, showing phosphorylated α-synuclein positivity in 9 (75%) of 12 patients with isolated RBD and 0 of 55 controls.[85] A third study of unilateral biopsies at C8 and the distal leg showed phosphorylated α-synuclein deposits in 26 (87%) of 30 patients with isolated RBD and 0 of 17 patients with RBD secondary to type 1 narcolepsy, confirming the specificity of this technique.[86] A more recent study of a single biopsy from a C8 cervical paravertebral site using an automated immunohistochemical assay showed phosphorylated α-synuclein in 23 (82%) of 28 patients with isolated RBD.[87] These studies have shown a combined specificity of 100% and a sensitivity of 58–87%. In addition, the analysis technique has been shown to have excellent interobserver reliability in two independent experienced laboratories.[88]

Peripheral tissue biopsy, especially skin biopsy, thus shows great promise as an in-vivo diagnostic biomarker for isolated RBD. Although some evidence also suggests promise in the ability to differentiate Parkinson's disease and dementia with Lewy bodies from multiple system atrophy,[89] this ability remains to be validated in individuals with isolated RBD, and it remains unclear whether the severity of phosphorylated α-synuclein deposition confers increased risk of phenoconversion. Longitudinal studies are needed to better understand its full potential as not only a diagnostic but also a prognostic biomarker.

Genetic markers—Recent studies suggest that the genetic background of isolated RBD does not fully overlap with those of Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. Genetic variants in the LRRK2[90] and *MART* genes,[91] which are associated with Parkinson's disease, show no association with isolated RBD. The *APOE* ε4 haplotype, which is strongly associated with dementia with Lewy bodies, is also not associated with isolated RBD.[92] However, *GBA* variants that are associated with Parkinson's disease, dementia with Lewy bodies, and, arguably, multiple system atrophy, are also associated with isolated RBD.[93] Two coding variants in *TMEM175* affect the risk of Parkinson's disease, yet only one of them has been found in patients with isolated RBD.[94] These genetic studies show that isolated RBD has a distinct genetic background, and we cannot assume that genetic variants that are relevant for the risk of, or for progression in, Parkinson's disease or dementia with Lewy bodies are also relevant for phenoconversion in isolated RBD.

GBA variants are found in approximately 10% of patients with isolated RBD and are associated with probable RBD in Parkinson's disease.[93] A cross-sectional multicentre study of 1061 patients with isolated RBD showed that 52% of *GBA* variant carriers phenoconverted, compared with 35% of non-carriers, despite similar disease duration.[95] Furthermore, this study showed that individuals with severe *GBA* variants, defined as variants that cause the severe types of Gaucher disease (types 2 and 3), might be at increased risk of more rapid phenoconversion, compared with individuals with mild or absent *GBA* variants.[95] Fine-mapping of the *SNCA* locus in isolated RBD, probable RBD, Parkinson's disease, and dementia with Lewy bodies has also shown differences in genetic background between people with these different diagnoses and in the potential effects of some *SNCA* variants on rate of conversion in isolated RBD.[96] In Parkinson's disease, the main effect on risk is driven by variants in the 3′ region of the gene, whereas, in isolated RBD and dementia with Lewy bodies, different and independent variants at the 5′ *SNCA* region are associated with risk, and specific 5′ *SNCA* variants might also affect the rate of phenoconversion.[96] Although these results are all preliminary and require confirmation in larger cohorts, they provide a proof of concept for the use of genetic variants as prognostic biomarkers to assess phenoconversion risk. Additional analyses, including polygenic risk scores from genome-wide association studies, as well as burden analyses of rare genetic variants, will also be needed to increase our ability to use genetic signatures as biomarkers in isolated RBD.

Combined biomarkers

No single biomarker for phenoconversion to α-synucleinopathies in people with isolated RBD fulfils the ideals of precision, accuracy, availability, and cost-effectiveness (table 2). Some biomarkers might appear early and change very slowly over time in individuals with isolated RBD, such as hyposmia and colour discrimination, whereas others might appear closer to phenoconversion, such as motor impairment, cognitive impairment, and reduced presynaptic dopaminergic uptake on ^{18}F-FDG PET imaging. Still others might hold value for the exclusion of atypical parkinsonism syndromes: cognitive testing and neuroimaging to help exclude dementia with Lewy bodies, for example, or autonomic testing and skin biopsy to help exclude multiple system atrophy. Ideally, diagnostic biomarkers will be used to identify the subtype of future α-syncucleinopathy, whereas a combination of prognostic biomarkers will inform proximity to phenoconversion, and monitoring biomarkers will aid in tracking therapy response, taking into consideration that different α-synucleinopathy subtypes will evolve differently (figure 3).[109]

How will a combination of biomarkers be used in future disease-modifying therapy trials? Thus far, most studies in patients with isolated RBD have evaluated single or very small groups of biomarkers in isolation. However, combined biomarkers that span multiple modalities hold the greatest promise. The power of combining multiple biomarkers was illustrated in a collaborative study of 1280 patients with isolated RBD by members of the International RBD Study Group, in which the presence of mild motor impairment and hyposmia increased the observed annual phenoconversion rate from 6·3% in all patients with RBD to 15·7%, providing a basis for calculating a realistic sample size for a disease-modifying therapy trial.[1] This combined biomarker approach requires substantial investment, rigorous standardisation across multiple sites for sample collection, storage, and assays, and data harmonisation followed by replication and confirmation, before it can inform clinical trials and change medical practice.

Conclusions and future directions

Disease-modifying therapy trials are currently ongoing in patients with Parkinson's disease. The next challenge will be to test these therapies in people with isolated RBD, to slow or even prevent the full manifestation of disease. It will be important to enrich target populations with biomarkers of short-term conversion (eg, abnormal dopamine transporter SPECT)[109] and be able to monitor disease progression with serial measurements (eg, motor function, cognition, dopamine transporter SPECT). The Parkinson's Progression Markers Initiative 2.0 prodromal cohort (NCT04477785), which started in 2020, as well as other key initiatives such as the North American Prodromal Synucleinopathy cohort (NCT03623672) and the International RBD Study Group, must work together towards this broader goal of slowing or preventing phenoconversion in the α-synucleinopathies. Future research will focus on longitudinal outcome data of multiple biomarkers across multiple centres worldwide.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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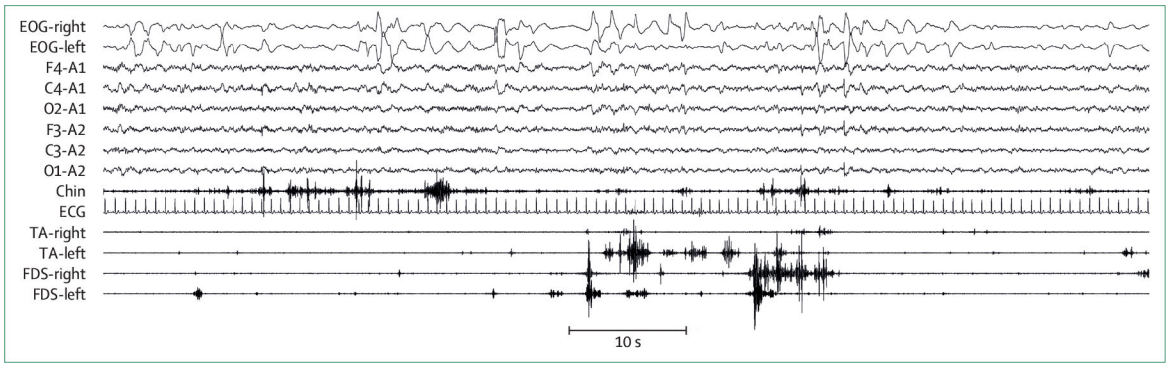

Figure 1: REM sleep recorded in a patient with RBD

The sleep pattern shows excessive chin muscle tone and excessive phasic EMG twitch activity over the chin, TA, and FDS muscles. A1=left mastoid reference. A2=right mastoid reference. C3=left central. C4=right central. ECG=electrocardiogram. EMG=electromyogram. EOG=electro-oculogram. FDS=flexor digitorum superficialis. F3=left frontal. F4=right frontal. O1= left occipital. O2=right occipital. TA=tibialis anterior.

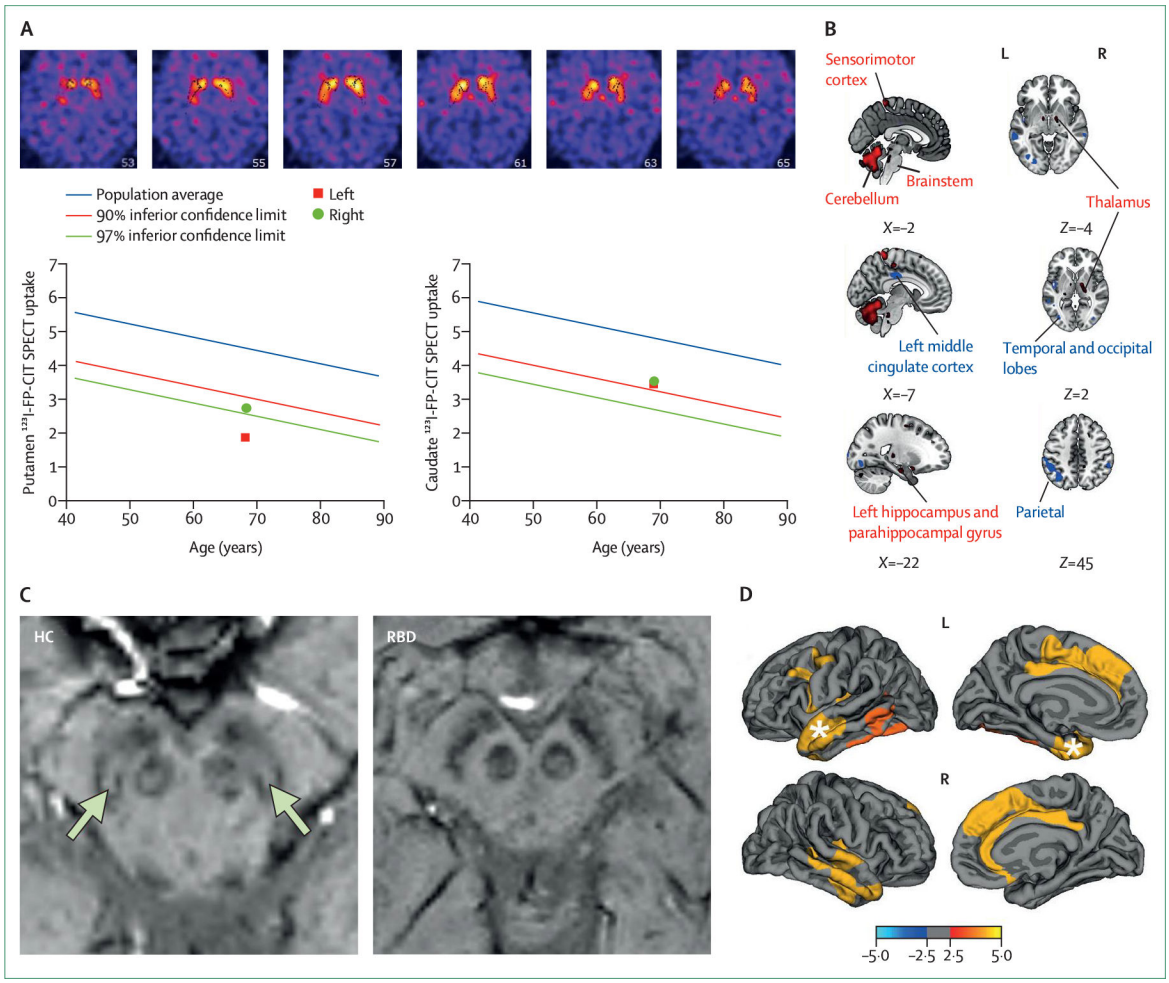

Figure 2: Functional and structural brain imaging findings in patients with isolated RBD

(A) Example of an ^{123}I-FP-CIT SPECT scan in a patient with isolated RBD, showing reduced uptake (yellow and orange) in the left putamen and, to a lesser extent, the right putamen. Scans are shown at different levels of the brain (denoted by the numbers in the bottom right corners). The population average data in the graphs have been obtained by analysis of individuals without isolated RBD from the European Normal Control database of DaTSCAN, using the basal ganglia matching tool.[66] Red and green lines show two different confidence limits for putamen and caudate ^{123}I-FP-CIT SPECT uptake. Red squares represent left putamen and caudate nuclei, and green circles represent right putamen and caudate nuclei of the patients whose scans are shown above. (B) Stable voxels (90% CI not straddling zero after bootstrap resampling) of ^{18}F-FDG PET-derived brain glucose isolated RBD-related pattern are visualised by overlaying them on a T1 MRI template. The arrows are pointing to all brain areas with stable voxels. Red indicates positive voxel weights (relative hypermetabolism) and blue indicates negative voxel weights (relative hypometabolism). Coordinates in axial (Z) and sagittal (X) planes are in Montreal Neurologic Institute standard space. Panel adapted from Meles et al.[67] (C) Examples of susceptibility-weighted imaging taken at the level of the substantia nigra in a healthy control and a patient with isolated RBD. Image HC reveals the presence

of a bilateral dorsal nigral hyperdensity (green arrows), corresponding to nigrosome 1. The dorsal nigral hyperdensity is lost bilaterally in the patient with isolated RBD. (D) Areas of cortical thinning in patients with isolated RBD and mild cognitive impairment compared with individuals without isolated RBD or cognitive impairment, corrected for family-wise error at $p<0{\cdot}05$, with age, sex, and education added as covariates. The colour bar represents the logarithmic scale of p values (−log10), with red-to-yellow areas representing significant thinning in patients with mild cognitive impairment and isolated RBD versus controls. The white asterisks represent the cluster of thinning (left anterior temporal lobe, including entorhinal cortex, insula, and inferior and middle frontal cortex) that best discriminated between patients with isolated RBD and mild cognitive impairment versus healthy controls (AUC 0·91 [95% CI 0·825–0·996]). Panel adapted from Rahayel et al.[68] AUC=area under the curve. FDG=fluorodeoxyglucose. ^{123}I-FP-CIT=^{123}I-N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane. HC=healthy control. RBD=rapid-eye-movement sleep behaviour disorder. L=left. R=right

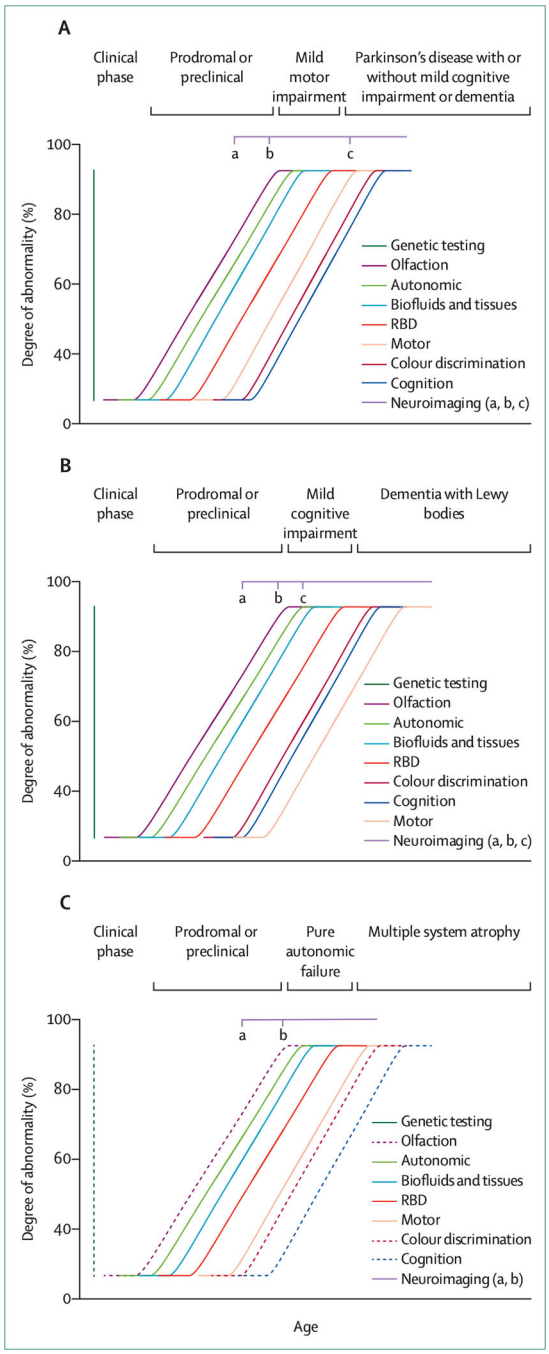

Figure 3: Hypothetical timeline of isolated RBD and associated clinical manifestations in relation to evolving α-synucleinopathies

The hypothetical timelines are for Parkinson's disease (A), dementia with Lewy bodies (B), and multiple system atrophy (C). In Parkinson's disease and dementia with Lewy bodies, changes in smell and autonomic functioning typically precede RBD, followed by other features; parkinsonism precedes cognitive changes in evolving Parkinson's disease, whereas cognitive changes precede parkinsonism in evolving dementia with Lewy bodies. In multiple system atrophy, autonomic dysfunction manifests around the time of isolated RBD, followed by elements of parkinsonism or cerebellar dysfunction, or both, in many

individuals. Changes in smell and cognition are minimal or absent in multiple system atrophy, and genetic variants associated with multiple system atrophy are still being studied (represented by dashed lines). For the neuroimaging timeline, brainstem alterations (a) occur first, followed by nigrostriatal dopaminergic alterations (b), and then other subcortical and cortical alterations (c). RBD=rapid-eye-movement sleep behaviour disorder.

Table 1:

Biomarker categories and definitions

	Definition	Application to isolated RBD
Diagnostic	To detect or confirm presence of a disease or condition of interest or to identify individuals with a subtype of the disease	To confirm an underlying α-synucleinopathy; to distinguish subtype of α-synucleinopathy (ie, Parkinson's disease, dementia with Lewy bodies, multiple system atrophy)
Prognostic	To identify likelihood of a clinical event, disease recurrence, or progression in patients who have the disease or medical condition of interest	To predict rate of phenoconversion; to predict disease severity
Monitoring or therapy-responsive *	To monitor progression of disease or show that a biological response has occurred in an individual who has been exposed to a medical product or an environmental agent	To monitor the progression of neurodegeneration; to detect the eventual effect of drug treatment (extent of neuroprotection); to establish efficacy of disease-modifying therapies
Combined	Composite and multidimensional, through combination of multiple biomarkers, and, as such, better reflects biological systems than single biomarkers	To refine and enhance the diagnostic, prognostic, and monitoring capabilities of single biomarkers in isolated RBD

Definitions in the second column adapted from Califf.[2] RBD=rapid-eye-movement sleep behaviour disorder.

* For simplicity, we have included biomarkers that hold promise as therapy-responsive markers in the monitoring category, as data are currently limited to longitudinal observational studies; when disease-modifying therapy trials are carried out, these monitoring biomarkers might also be considered as therapy-responsive biomarkers.

Table 2:

Summary of biomarker candidates in isolated RBD

	Subtype	Availability	Cost	Sensitivity and specificity	Remarks
Neurophysiology					
RSWA quantified by visual or automated methods (eg, SINBAR, rapid-eye-movement atonia index)	Diagnostic, prognostic, monitoring	High	Low	Diagnostic: 85–95% and 85–95%;[97–105] prognostic: 78–89% and 61–70%[5]	Robust data supporting both visual and automatic methods, with similar results despite differences in methods; few studies
Cyclic alternating pattern rate	Diagnostic, prognostic	Moderate	Moderate	NA	Only one study;[8] special analyses of EEG required
Biomarkers obtained through artificial intelligence, machine learning, and deep neural network-based methods	Diagnostic, prognostic, combined	Low	High	Diagnostic: 91–98% and 93–94%; prognostic: AUC 78%[9,10]	Few studies[9,10]
Motor function					
Upper extremity alternate-tap test	Diagnostic, prognostic, monitoring, combined	High	Low	Year 0:100% and 83%;[24] year –1: 92% and 86%; year –2: 88% and 89%; year –3: 91% and 86%	Easy to do; year 0=phenoconversion to PD or DLB; years –1, –2, –3=years before phenoconversion
Speech abnormalities quantified by means of acoustic analysis	Prognostic, monitoring	High	Low	67% and 71%[16]	Easy to do; only cross-sectional validation studies
Gait dysfunction by instrumental analysis	Prognostic, monitoring	Moderate	High	NA	Limited to few specialised centres; cross-sectional studies only
Wearable devices and smartphones	Prognostic, monitoring	High	Low	92% and 90%[18]	Cross-sectional validation studies only
Cognition					
Trail Making Test Part B	Diagnostic, prognostic, monitoring, combined	High	Low	Year 0: 100% and 83%;[24] year –1: 92% and 86%; year –2: 88% and 89%; year –3: 91% and 86%	Only one longitudinal study; early identification of prodromal DLB; year 0=phenoconversion to DLB; years –1, –2, –3=years before phenoconversion
Semantic verbal fluency	Monitoring, diagnostic, prognostic, combined	High	Low	Year 0: 91% and 97%;[24] year –1: 91% and 91%; year –2: 80% and 91%; year –3: 90% and 74%	Only one longitudinal study; cognitive change over time for prodromal DLB; year 0=phenoconversion to DLB; years –1, –2, –3=years before phenoconversion
Rey Auditory-Verbal Learning Test (immediate recall)	Diagnostic, prognostic, monitoring, combined	High	Low	Year 0: 92% and 89 %;[24] year –1: 100% and 89%; year –2: 100% and 75%; year –3: 82% and 89%	Only one longitudinal study; cognitive change over time for prodromal DLB; year 0=phenoconversion to DLB; years –1, –2, –3=years prior to phenoconversion
Olfaction					
Odour identification testing (eg. Sniffin' Sticks, UPSIT)	Diagnostic, prognostic, combined	High	Low	86–91% and 76–88%[106]	Easily done with conversion data between Sniffin and UPSIT available[107]

	Subtype	Availability	Cost	Sensitivity and specificity	Remarks
Ophthalmic function					
Farnsworth-Munsell 100-Hue test	Diagnostic, prognostic	Moderate	Low	NA	Easily done; limited data
Optical coherence tomography (structural imaging of the parafoveal avascular zone)	Diagnostic, prognostic	Low	Moderate	NA	Highly promising for investigating other pathways at risk of early degeneration
Autonomic function					
Autonomic questionnaires	Diagnostic, prognostic, monitoring, combined	High	Low	NA	Easily done and can be easily repeated over time
Heart rate variability analysis	Diagnostic	High	Low	NA	Easily obtained from baseline vPSG; sensitive to artifact
Metaiodobenzylguanidine	Diagnostic	Moderate	Moderate	NA	Might help distinguish PD and DLB from MSA[51]
Cardiovascular reflex testing	Diagnostic, prognostic, monitoring, combined	Low	Moderate	NA	Limited to few specialised centres; might help distinguish PD and DLB from MSA[49]
Biofluids					
CSF RT-QuIC	Diagnostic, prognostic, monitoring	Low	Moderate	100% and 98%[55]	Somewhat invasive
Nasal swabs (olfactory mucosa) RT-QuIC	Diagnostic	Moderate	Moderate	44·4% and 90%[57]	Minimally invasive, ENT specialist needed for sampling
Serum neuronal exosomal α-synuclein	Diagnostic	Low	High	95% and 93%[59]	Most appealing serum marker sensitivity and specificity
Neuroimaging					
^{123}I-FP SPECT (dopamine transporter SPECT)	Diagnostic, prognostic, monitoring, combined	Moderate	Moderate	29·3% and 100%[71]	Low diagnostic value in differentiating patients with isolated RBD from controls; high prognostic value in identifying future phenoconverters; low prognostic value in identifying phenoconversion subtype; responsive to dopamine-oriented therapy
^{18}F-FDG PET	Diagnostic, monitoring, combined	Moderate	Moderate	52·4% and 100%[67,73]	Moderate diagnostic value in differentiating patients with isolated RBD from controls; high diagnostic potential in predicting α-synucleinopathy subtype but requires independent validation; possible prognostic value has yet to be shown in large series; useful for monitoring disease progression; possibly responsive to therapy
MRI for nigrosome, MRI for substantia nigra neuromelanin, MRI for cortical thinning, and MRI for DBM	Diagnostic, prognostic, combined	Moderate	Moderate	MRI nigrosome: 27·5–77% and 97–92·3%;[74] MRI substantia nigra neuromelanin: 90% and 94%[108]	Good diagnostic potential in differentiating patients with isolated RBD from controls (nigrosome, substantia nigra neuromelanin) as well as RBD subtype (ie, RBD with MCI or cortical thinning); possible prognostic value for DLB (DBM); all markers require independent study confirmation
Tissue biopsy					
Colon biopsy	Diagnostic	Low	Moderate	24% and 100%[82]	Invasive; poor sensitivity

	Subtype	Availability	Cost	Sensitivity and specificity	Remarks
Major salivary glands	Diagnostic	Low	Moderate	89% and 100%[83]	Invasive, surgeon needed for sampling; high sensitivity if glandular tissue obtained
Minor salivary glands	Diagnostic	Moderate	Moderate	50% and 97%[84]	Invasive, surgeon needed for sampling; poor sensitivity
Skin biopsy	Diagnostic, prognostic, monitoring, combined	Moderate	Moderate	58%–87% and 100%[35,86,87]	Easy to do, minimally invasive, but analysis requires expertise; might help distinguish PD and DLB from MSA[51]
Genetic testing					
GBA variants	Prognostic	Moderate	Moderate	NA	Might help predict the rate of phenoconversion[95]
SNCA 5′ variants	Prognostic	Moderate	Moderate	NA	Might help predict the rate of phenoconversion[96]

RSWA=rapid-eye-movement sleep without atonia. SINBAR=Sleep Innsbruck Barcelona group. AUC=area under the curve. PD=Parkinson's disease. DLB=dementia with Lewy bodies. UPSIT=University of Pennsylvania smell identification test. vPSG=video polysomnography. MSA= multiple system atrophy. RT-QuIC=real-time quaking-induced conversion. ENT=ear nose and throat. ^{123}I-FP=^{123}I-ioflupane. ^{18}F-FDG=^{18}F-fluorodeoxyglucose. MCI=mild cognitive impairment. DBM=deformation-based morphometry. RBD=rapid-eye-movement sleep behaviour disorder.