Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

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

07 July 2024

Biomarkers of Conversion to Alpha-Synucleinopathy in Isolated REM Sleep Behaviour Disorder

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Abstract:

Patients with isolated rapid-eye-movement sleep behaviour disorder (iRBD) are commonly regarded as being in the early stages of a progressive neurodegenerative disease of α-synuclein pathology. Studies have demonstrated that abnormal α-synuclein deposition occurs early in the neurodegenerative process across the central and peripheral nervous system and may precede the appearance of motor symptoms and cognitive decline by several decades. This provides the rationale to develop reliable biomarkers that can better predict conversion to clinically manifest α-synucleinopathies including Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. In addition, biomarkers of disease progression will be essential to monitor treatment response once disease-modifying treatments become available, and biomarkers of disease subtype will better predict which subtype of α-synucleinopathy iRBD patients 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 (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA). While not all patients with an α-synucleinopathy have RBD, several longitudinal studies have demonstrated that >80% of patients with isolated RBD (iRBD)—or RBD without PD, DLB, or MSA —will be diagnosed with one of these conditions within their lifetimes.[1] For this reason, patients with

iRBD represent an ideal population in which to employ disease modifying therapies (DMT) when they become available. However, the delay from diagnosis of iRBD to phenoconversion (i.e, conversion from iRBD to a diagnosis of PD, DLB or MSA) is variable, with the prodromal period lasting years to decades, and RBD alone does not predict α-synucleinopathy subtype. Identification of iRBD patients more likely to phenoconvert within several years is critical for outcome measures within the time frame of DMT trials, as are biomarkers that can monitor the neurodegenerative process and treatment outcomes.

The ideal biomarker must be highly sensitive and specific, reproducible and cost-effective, readily available, and able to serve as a therapy-responsive progression marker. The goal of this review is to summarize the field of potential biomarkers with this ideal in mind. We will focus on ten biomarker categories that have demonstrated significant promise, presented in order of ease of obtainability for international recruitment strategies. We have also categorized candidate biomarkers according to how they might be utilised (table 1). Based on current evidence, the potential usefulness of each biomarker will be highlighted, with specific focus on its role in future DMT trials (table 2).

Search Strategy and Selection Criteria

This review is a collaboration by the biomarker working group of the International RBD Study Group (IRBDSG). Each section was coordinated by a section editor respected as an expert in the field, with two members (MGM, WHO) serving as coordinating editors. All members of the section teams independently performed a review of the relevant literature, in PUBMED, SCOPUS, Google Scholar, MEDLINE Ovid and Web of Science. The first literature search was conducted January 1st, 2019 and the final search was on January 4th, 2021. There were no language restrictions. Search terms for each section are listed in the supplementary material. The eligibility of each manuscript was assessed by all members of the section teams, under the leadership of the section editors. Studies meeting the following criteria were included in the review: iRBD confirmed by video-polysomnography (vPSG) according to standard diagnostic criteria, included human participants only, and published, with few exceptions (seminal work or landmark study), within the past three years. Case reports and case-series were excluded, and reviews were included only when containing aspects not covered in the original articles.

Biomarker Categories

1. Neurophysiology

REM sleep without atonia (RSWA) is the neurophysiological hallmark of RBD (figure 1) and is required for the diagnosis. RSWA is recorded during the mandatory diagnostic step of video polysomnography (vPSG), making it the most readily available diagnostic biomarker. The presence of RSWA has been identified prior to dream-enacting behaviors, establishing isolated RSWA as one of the earliest signs of neurodegeneration.[2,3] RSWA may also offer the potential to predict phenotypic subtypes of evolving α-synucleinopathy, thus enhancing its diagnostic potential.[2] Several visual and automated methods for scoring RSWA have all demonstrated largely convergent agreement, with acceptable sensitivity and specificity (both ranging from 85-95%). Finally, RSWA may prove to be a valuable prognostic biomarker of disease progression as it may increase over time in some individuals, with greater severity associated with accelerated phenoconversion.[4,5]

RBD seems to result from the breakdown of a broad network underlying REM sleep atonia, with an interaction between the brainstem and both rostral and caudal central nervous system (CNS) structures.[6] This has prompted advanced electroencephalography (EEG) analysis that has demonstrated potential as a diagnostic and prognostic biomarker. For example, lower cyclic alternating pattern (CAP) rates on EEG have been associated with increased rates of phenoconversion in those with iRBD,[7] as has the time-frequency structure of resting wakeful EEG.[8] Further gains in diagnostic and prognostic value may be achieved by the use of artificial intelligence and machine learning-based methods, such as a recent random forest classifier combining muscle atonia data with sleep architecture to accurately identify the presence of RBD.[9] Other more experimental approaches, including transcranial magnetic stimulation to probe early cortical dysfunction[10] and vestibular-evoked myogenic potentials assessing brainstem neurophysiology,[11] require additional investigation before being proposed as prognostic biomarkers.

2. Motor Function

Given the prominence of Parkinsonism in patients with iRBD who phenoconvert, formalised motor assessments represent appealing and readily available biomarkers, although the specific protocol is yet to be optimised. Motor abnormalities in patients with iRBD emerge relatively late in the prodromal disease process and may indicate those at more imminent risk of phenoconversion. A longitudinal multicentre trial of the IRBDSG in 1280 patients demonstrated that quantitative motor tests are one of the most powerful predictive markers of future phenoconversion in those with iRBD, with a hazard ratio (HR) of 3.16 (95% CI 1.86–5.37).[1] Quantitative motor assessments, and in particular an upper extremity alternating-tap test, can become abnormal five to eight years before phenoconversion,[12] offering potential as both a prognostic and monitoring biomarker.

Several cross-sectional studies using instrumental assessments have highlighted the use of gait,[13,14] speech,[15] saccadic eye movements,[16] rhythm,[17] and finger tapping[18] with sensitivity and specificity of up to 80% to identify the presence of iRBD. For example, changes in home-based spontaneous walking tasks, decreased gait speed, cadence and step variability have been reported in patients with iRBD, compared to age-matched controls,[13] while in laboratory assessments have revealed deficits in postural control and foot step asymmetry during dual-task walking in iRBD patients compared to controls, suggesting an overlap between motor and cognitive domains.[14] Acoustic speech analysis has indicated that levels of monopitch, longer duration of pauses and a decreased rate of follow-up speech segments may best discriminate between iRBD patients, PD patients, and controls,[15] likely reflecting both vocal cord hypokinesia and deficits in orolingual movement initiation. Poor spontaneous rhythm timing and perception has also been demonstrated, where performance in a small cohort of iRBD patients was similar to those with mild PD, [19] while in another cohort finger tapping amplitude and velocity decrement was impaired compared to controls, suggesting prodromal bradykinesia.[18] Finally, increased error rates for anti-saccadic but not pro-saccadic eye movements have been reported in iRBD patients compared with controls.[16] Despite the precision offered by these approaches, there is 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 shown to be highly effective in discriminating iRBD, PD and control populations, with a mean sensitivity of 85% and specificity of 92%,[17] highlighting the potential of future technology in prognostic and monitoring biomarkers for DMT trials.

3. Cognition

Cognitive decline is common in iRBD, thus cognitive testing represents another valuable and readily available biomarker. Mild cognitive impairment (MCI), an intermediate state between normal cognitive functioning and dementia, coexists in more than a third of patients with iRBD, and patients with iRBD and concomitant MCI are at higher risk of phenoconversion.[1,20] Indeed, both amnestic and non-amnestic MCI subtypes in patients with iRBD are predictive of the development of DLB or parkinsonism with cognitive impairment.[1,21-23]

Deficits in cognitive performance also affect iRBD patients who do not fulfill MCI criteria,[20] and cross-sectional studies have reported deficits in attention, executive function, memory, and visuospatial function.[20] The presence of pareidolias and deficits in prospective memory have also been identified.[24,25] Importantly, the pattern and severity of cognitive deficits in iRBD may predict phenoconversion subtype. Cognitive deterioration over time is more common in patients who will develop DLB, whereas stable cognitive performance over a period of six years is more predictive of those who will develop PD or remain disease-free.[23] In one longitudinal study, assessments of executive function, such as the Trail Making Test part B, demonstrated deficits in iRBD patients six years before diagnosis of DLB,[23] whereas verbal episodic and semantic memory, assessed by the Rey Auditory-Verbal Learning Test and semantic verbal fluency, respectively, were abnormal two to four years prior to diagnosis.[23] This predictive value may be heightened when combined with multi-modal imaging approaches, which have more recently elaborated structural and functional correlates of MCI in iRBD (see neuroimaging section).

Cognitive testing in iRBD may thus prove useful as a diagnostic biomarker, particularly to identify prodromal DLB, as well as a prognostic and monitoring biomarker. The psychometric properties of cognitive testing are well-established, widely available at low cost, and easily performed with administration times of 15-25 minutes.[23] The Montreal Cognitive Assessment (MOCA), a screening test that takes 10 minutes to administer, could be an alternative to identify iRBD patients at risk of DLB,[12] however further studies are needed to validate its psychometric properties in this population.

4. Olfaction

Hyposmia is recognised as one of the earliest prodromal signs of PD, and is present in many patients with iRBD.[26,27] In a multicentre study where olfaction was assessed in over 600 patients with iRBD, hyposmia was present in 67%,[1] and back-extrapolation of disease has identified evidence of hyposmia more than 20 years prior to phenoconversion.[12] The Sniffin' sticks test,[28] comprising multi-use felt-tip style pens, and the University of Pennsylvania Smell Identification Test (UPSIT),[29] utilising single-use scratch cards, are the most frequently used instruments to assess smell, with similar discrimination accuracy. A link between the extent of iRBD symptomatology and the degree of hyposmia has been suggested by lower olfactory test scores in individuals with iRBD detected on population screening (with a lower frequency of dream enactment behaviours) compared to those presenting clinically.[30]

The HR attributed to hyposmia (2.62, 95% CI 1.67–4.12), based on pooled multicentre data from over 600 individuals, exceeds that of all other non-motor markers.[1] Its use alongside an age cut-off of ≥ 55 has been suggested to stratify individuals at risk of imminent conversion,[31] though the lack of worsening olfactory deficit over time has led to caution over its use as an outcome measure in DMT trials.[32] In one study, hyposmia was closely correlated with progressive decline in visuospatial function and verbal memory in iRBD patients followed over two years, suggesting that hyposmia might also predict future conversion to DLB.[33] While hyposmia also correlates highly with phosphorylated α-synuclein (p-syn) aggregates on skin biopsy,[34] an inability to distinguish underlying PD from DLB and incomplete penetrance of hyposmia in the α-synucleinopathies, based on UPSIT-40 scores, has been described.[34,35] As part of a two-tiered screening strategy aimed at identifying individuals at risk of incident PD, hyposmia combined with dopamine transporter (DaT) deficit on ^{123}I-FP-CIT-SPECT imaging predicted phenoconversion within a four-year period with a sensitivity, specificity, positive predictive value and negative predictive value of 74%, 97%, 67% and 97%, respectively.[36] The combination of hyposmia and abnormal DaT imaging in this cohort was associated with a phenoconversion rate of 25%, compared to a rate of 2.5% using hyposmia alone, highlighting hyposmia's potential as a combined biomarker.[36]

Finally, while olfactory function is often abnormal iRBD patients, partial recovery can occur, due to neurogenesis in the subventricular zone (SVZ). The SVZ contains neural progenitor cells that migrate via the rostral migratory stream to the olfactory bulb (OB) and differentiate into interneurons. Studies show variably impaired OB-related neurogenesis that is directly triggered by α-synuclein accumulation, in both human PD postmortem brain and transgenic PD models.[37] Therefore, variable neurogenesis may contribute to the significant inter and intra-individual differences in olfaction observed in RBD and ageing.

5. Ophthalmic Function

Despite its ease of use, colour discrimination (CD) has rarely been systematically evaluated in iRBD,[1,12,38] and only by the Farnsworth-Munsell 100-Hue test. In a study of 154 iRBD patients, olfactory dysfunction was first to develop, followed by impaired CD.[12] In another study of 62 iRBD patients followed over five years, 13/21 (74%) of iRBD patients with impaired CD phenoconverted to PD, compared to 12/40 (30%) of those with normal vision.[38] In a multicentre study of 1,280 iRBD patients, in which 1/5 underwent colour testing,[1] HR for phenoconversion to PD or DLB was 1.69 (1.01–2.78).[1] In a monocentric study of 154 iRBD patients, impaired CD began 12.8 years before phenoconversion to PD or DLB,[12] suggesting that CD holds potential as both a diagnostic and prognostic biomarker. The mechanism of heightened phenoconversion risk in those with impaired CD remains unclear.

While data are less robust, optical coherence tomography (OCT) also holds potential as a diagnostic and prognostic biomarker, as parafoveal ganglion cell complex thinning in the retina has been found to correlate with olfactory loss and striatal DaT reduction in iRBD.[39] In PD, such thinning has been correlated with nigral dopaminergic loss and visual impairment.[40] Furthermore, iRBD patients seem to demonstrate a reduction of the retinal nerve fibre layer,[41] however longitudinal studies utilising OCT in iRBD will have to confirm these findings.[42] Although the retinal contribution to colour perception is well established, OCT findings have yet to be correlated with CD in iRBD. Interestingly, Lewy type pathology in the retinal ganglion cell complex layers has also been found in incidental Lewy body disease involving the brainstem.[43] While the mechanism of CD impairment at the retinal level remains uncertain, these findings suggest parallel initiation of the neurodegenerative process at anatomically distinct sites. Future

studies are needed to rule out methodological inconsistencies[44] and to determine if these retinal changes are unique to the α-synucleinopathies.[45]

6. Autonomic Function

Autonomic impairment is common in iRBD, occurs early in the disease process,[12] and has been demonstrated in studies utilizing both questionnaires and objective measures of autonomic function with heart rate variability (HRV), cardiac metaiodobenzylguanidine (MIBG) scintigraphy, and autonomic reflex testing. Autonomic symptoms in iRBD encompass adrenergic and cardiovagal deficits, sexual and urinary dysfunction, and constipation.[1,46,47] The severity of autonomic impairment is mild to moderate in most iRBD patients, intermediate between controls and those with PD.[46]

Several questionnaire-based studies have revealed that iRBD patients report significantly more autonomic symptoms than controls, with greatest impairment in cardiovascular, gastrointestinal and urinary domains.[1,46,47] In a prospective study of 1,280 iRBD patients, constipation and erectile dysfunction were associated with greatest risk of phenoconversion.[1] The severity of autonomic symptoms has also been associated with putaminal DaT abnormalities and an accelerated rate of phenoconversion in iRBD patients, highlighting potential as both a diagnostic and prognostic biomarker.[27,47,48]

While some HRV studies have demonstrated impairment in low frequency spectra on vPSG, suggestive of cardiac sympathetic impairment, other studies have demonstrated impairment in high frequency spectra, suggestive of parasympathetic impairment.[49] MIBG scintigraphy studies have shown that iRBD patients have markedly reduced MIBG uptake ratios compared to controls, suggestive of post-ganglionic sympathetic impairment,[50] a finding more commonly seen in PD than MSA, allowing for potential diagnostic distinction between prodromal phenotypes. While HRV and MIBG abnormalities are seen early in iRBD, no longitudinal data have demonstrated association with phenoconversion rates, making these biomarkers more appealing for diagnostic use.

Autonomic reflex testing has demonstrated consistent impairment across cardiovagal, sympathetic adrenergic, and sudomotor domains, with greatest impairment in measures of sympathetic adrenergic function,[51-53] which may worsen with disease progression. In addition, more severe cardiovagal dysfunction has been associated with phenoconversion to DLB rather than PD.[48] While validated as the most quantitative and comprehensive method of assessing autonomic function, autonomic reflex testing requires a specialized autonomic laboratory with beat-to-beat blood pressure recording, thus limiting access, in contrast to less-expensive, but less precise questionnaires.

Though available literature has demonstrated that autonomic impairment may serve as a diagnostic marker in iRBD, data on the prognostic value of such impairment are limited, and longitudinal studies are necessary to determine whether autonomic impairment in iRBD can help predict the development of α-synucleinopathy subtypes. Finally, the large intraindividual variability of autonomic symptoms in iRBD patents may pose challenges in accurately phenotyping those at risk of more imminent phenoconversion.

7. Biofluids

Given the neuroanatomical proximity, biomarkers obtained from CSFs represent appealing candidates for molecular characterization of the α-synucleinopathies. The use of Real-Time Quaking-Induced Conversion (RT-QuIC) has emerged as an ultrasensitive technique to identify pathological CSF α-synuclein in PD and DLB with a high degree of sensitivity and specificity. Among the few studies analysing CSF biomarkers in iRBD patients, RT-QuIC can detect pathogenic species of α-synuclein with a sensitivity of 90-100% and specificity of 90-98%,[54] with a positive result suggesting greater risk of phenoconversion,[55] highlighting potential as both a diagnostic and prognostic biomarker. Furthermore, nasal swabs with RT-QuIC on olfactory mucosa has been reported as a potential diagnostic marker. The technique is less invasive than lumbar puncture and has good specificity (90%) but moderate sensitivity (44.45%), though sensitivity is enhanced in those iRBD patients with hyposmia.[56]

Biomarkers obtained directly from blood represent an attractive candidate due to relatively low cost and ease of obtainability, however results have been thus far suboptimal. While α-synuclein

in plasma neuronal exosomes may aid in early diagnosis of PD, no significant differences in exosomal α-synuclein concentrations were found in those with iRBD in one longitudinal study.[57] Another cross-sectional study demonstrated that neuronal exosome α-synuclein concentrations were elevated in iRBD patients when compared to controls and those with MSA, but no different when compared to those with PD, suggesting a potential role in predicting subtype, based on the fact that α-syn in MSA accumulates primarily in oligodendrocytes.[58] In addition, serum neurofilament light chain (NfL), a neuronal cytoskeletal protein released upon neuronal damage, might mark the conversion iRBD to clinically manifest PD.[59] Techniques such as proteomics analysis of serum samples, which have identified several significantly altered proteins, have provided further insight into the protein signature profile and molecular pathways involved in the pathogenesis of iRBD,[60,61] however confirmatory studies are needed.

Alterations in circulating microRNAs have been demonstrated in several neurodegenerative diseases including iRBD. One study demonstrated that miR-19b was significantly downregulated in iRBD patients who phenoconverted, but not in those who remained disease free, indicating that dysregulation of miR-19b might contribute to phenoconversion and offer potential as a prognostic biomarker.[62] With respect to the functional bioenergetics in peripheral blood cells, one study revealed decreased antioxidant superoxide dismutase and increased glycolysis in iRBD patients using peripheral blood mononuclear cells.[63] The potential of other samples such as saliva, tears, and microbiome analysis is yet to be explored in iRBD, and longitudinal studies are required to determine whether such biosamples can be used to assess phenoconversion risk.[64]

8. Neuroimaging

Evidence of nigro-striatal dopaminergic impairment, usually measured as basal ganglia DaT availability, has consistently been found in iRBD on both PET and SPECT imaging (figure 2a), with ^{123}I-FP-CIT-SPECT currently the most studied and available DaT-SPECT imaging modality. Abnormal DaT imaging appears to signal an increased risk of phenoconversion,[1,65,66] especially when combined with cognitive and autonomic impairment.[67,68] Furthermore, nigrostriatal DaT abnormalities seem to correlate with changes in brain glucose metabolism as assessed by ^{18}F-fluoro-deoxyglucose (^{18}F-FDG)-PET .[69] The ^{18}F-FDG-PET-derived RBD-/PD-

related pattern (RBDRP; figure 2b)[70] appears to be a prodromal progression marker, having demonstrated potential to both assess progression and predict α-synucleinopathy subtype.[71]

While radionuclide studies with DaT-SPECT and FDG-PET are widely available and their costs for clinical trial purposes are acceptable, dependence on ionizing radiation may limit their utility. MRI, therefore, remains an attractive alternative. MRI techniques have demonstrated abnormalities in the substantia nigra related to the degree of dopaminergic dysfunction (figure 2c)[72] and gray matter changes in the motor cortico-subcortical loop that correlate with motor abnormalities.[73] Findings on MRI have also been correlated with cognitive impairment in RBD patients with MCI, 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 iRBD patients.[74]

Other MRI approaches include deformation-based morphometry, which has identified a brain signature, combining cortical and subcortical deformation and subarachnoid and ventricular expansion, that predicts the development of dementia in iRBD patients.[75] MRI has also shown promising results in identifying patients at risk of developing MSA.[76] Whole-brain resting-state functional MRI (fMRI) has demonstrated correlations between reduced performance in a processing speed task and disrupted connectivity in the associative areas of the parieto-temporal lobes,[77] as well as an association between verbal learning and left thalamo-fusiform connectivity.[23] In addition, fMRI has demonstrated a disrupted posterior brain network associated with iRBD-related cognitive impairment.[77] Accordingly, cholinergic denervation on ^{11}C-Donepezil-PET, known to be related to cognitive impairment, was found in iRBD patients, particularly in the temporal, occipital, cingulate and dorsolateral prefrontal cortex.[78]

Despite best efforts, imaging biomarkers that delineate neuropathological spread of α-synuclein are lacking in iRBD.[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 as a way to select participants for DMT trials in prodromal PD. ^{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,[71] however confirmatory

studies are required. While several MRI techniques offer potential as diagnostic and prognostic markers, longitudinal data are needed before recommending this technique for DMT trials.

9. Tissue Biopsy

pSyn deposits in the substantia nigra are a neuropathological hallmark of PD, however autopsy studies have also demonstrated pSyn in peripheral structures such as the autonomic nerves, enteric mucosa and salivary glands in PD and DLB patients.[79,80] One of the first tissues to be analysed in both PD and iRBD was colonic tissue, however limited data demonstrated a positivity rate of only 24% in 4/17 iRBD patients.[81] Transcutaneous core needle biopsy of the submandibular gland with ultrasound guidance has demonstrated high sensitivity and specificity in major salivary gland tissue in one study,[82] however adequate biopsy material was obtained in only 9/21 (43%) of patients. Biopsy of minor salivary glands in the inner side of the lower lip obtains adequate tissue in all cases however is less sensitive, with only 31/62 (50%) of iRBD patients demonstrating pSyn positivity.[83]

More recently, skin biopsy has emerged as a promising, less invasive technique (supplementary figure).[80] This technique is easier to perform than colon or salivary gland biopsies, is well-tolerated, relatively inexpensive, and can be performed in any outpatient setting under aseptic technique, although dual-immunofluorescence analysis does require operator experience. One study utilising biopsies of multiple unilateral sites (C7 paraspinal area, T10 paraspinal area, proximal and distal leg) demonstrated pSyn positivity in 10/18 (56%) of iRBD patients, 20/25 (80%) early PD patients, and 0/20 controls.[34] The likelihood of pSyn positivity was greater in those with olfactory dysfunction, whereas the relation with reduced DaT-SPECT ligand density was less robust, indicating that skin biopsy positivity can be found already in iRBD patients with a normal DaT-SPECT, at least two years before nigrostriatal decline. A second study independently confirmed this finding using bilateral biopsies at C8 and the distal leg demonstrated pSyn positivity in 9/12 (75%) of iRBD patients and 0/55 of controls.[84] A third study of unilateral biopsies at C8 and the distal leg demonstrated pSyn deposits in 26/30 (87%) of iRBD and 0/17 patients with RBD secondary to type 1 narcolepsy, confirming the specificity of this technique.[85] A more recent study of a single biopsy from a single C8 cervical paravertebral site utilising an automated immunohistochemical assay demonstrated α-synuclein

(non-phosphorylated, non-pathological variant) in 23/28 (82.1%) of iRBD patients.[86] These studies have demonstrated a combined specificity of 100% and a sensitivity of 58%-87%. In addition, the analysis technique has been shown to have excellent inter-observer reliability in two independent experienced laboratories.[87]

Peripheral tissue biopsy, especially skin biopsy, thus demonstrates great promise as an in-vivo diagnostic biomarker in iRBD. While some evidence also suggests promise in the ability to differentiate PD and DLB from MSA,[88] this remains to be validated in those with iRBD, and it remains unclear if the severity of pSyn deposition confers greater risk of phenoconversion. Longitudinal studies are thus pivotal to better understand its full potential as not only a diagnostic but also a prognostic biomarker.

10. Genetic Markers

Recent studies suggest that the genetic background of iRBD does not fully overlap with those of PD, DLB and MSA. Genetic variants in *LRRK2* [89] and *MAPT*,[90] which are associated with PD, show no association with iRBD. The *APOE*-ε4 haplotype, which is strongly associated with DLB, is also not associated with iRBD.[91] However, *GBA* variants, which are associated with PD, DLB, and arguably MSA, are also associated with iRBD.[92] Two coding variants in *TMEM175* affect the risk for PD, yet only one of them has been confirmed in iRBD.[93] These genetic studies demonstrate that iRBD has a distinct genetic background, and we cannot assume that genetic variants that are relevant for risk or progression in PD or DLB are also relevant for phenoconversion in iRBD.

GBA variants are found in approximately 10% of iRBD patients and are associated with probable RBD (pRBD) in PD.[92] A multi-center study of 1,061 iRBD patients showed that 52% of *GBA* variant carriers had phenoconverted, compared to 35% of non-carriers, despite similar disease duration.[94] Furthermore, this study demonstrated that individuals with severe *GBA* variants, defined as variants that cause the severe types of Gaucher disease (types 2 and 3), may be at higher risk of more rapid conversion, compared to individuals with mild or absent *GBA* variants.[94] Fine-mapping of the *SNCA* locus in iRBD, pRBD, PD and DLB has also demonstrated differential genetic background between some of the different traits, and potential

effect of some *SNCA* variants on rate of conversion in iRBD.[95] In PD, the main effect on risk is driven by variants in the 3' region of the gene, while in iRBD and DLB, different and independent variants at the 5' *SNCA* region are associated with risk, and specific 5' *SNCA* variants may also affect the rate of conversion.[95] While 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 iRBD.

Combined biomarkers

No single biomarker that fulfills the ideals of precision and accuracy, availability and cost-effectiveness. Some biomarkers may appear early and change very slowly over time in those with iRBD, such as hyposmia and CD, while others may appear closer to phenoconversion, such as motor impairment, cognitive impairment, presynaptic dopaminergic imaging and FDG-PET. Still others may hold value for the exclusion of atypical Parkinsonism syndromes—cognitive testing and neuroimaging to help exclude DLB, for example, or autonomic testing and skin biopsy to help exclude MSA. Ideally, diagnostic biomarkers will be used to identify subtype of future α-syncucleinopathy, while a combination of prognostic biomarkers will inform proximity to phenoconversion, and monitoring biomarkers will aid in tracking therapy response, acknowledging that different α-synucleinopathy subtypes will evolve differently (figure 3).[96]

How will a combination of biomarkers be used in future DMT trials? Thus far, most studies in iRBD 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 iRBD patients by members of the IRBDSG, where the presence of mild motor impairment and of hyposmia increased the observed annual conversion rate from 6.3% in all RBD to 15.7%, providing a basis for calculating a realistic sample size for a DMT trial.[1] This combined biomarker approach requires significant investment, rigorous standardization across multiple sites for sample collection,

storage and assays, and data harmonization followed by replication and confirmation, before it can inform clinical trials and change medical practice.

Conclusions and Future Directions

DMT trials are currently ongoing in PD. The next challenge will be to focus these therapies on iRBD, to slow or even prevent the full manifestation of disease. It will be important to enrich target populations with biomarkers of short-term conversion (e.g., abnormal DaT-SPECT)[96] and be able to monitor disease progression with serial measurements (e.g., motor function, cognition, DaT-SPECT). The PPMI 2.0 prodromal cohort starting in 2020 as well as other key initiatives including the North American Prodromal Synucleinopathy (NAPS) cohort[110] and the IRBDSG must work together in this wider initiative to deliver meaningful change for patients. Future research will focus on longitudinal outcome data of multiple biomarkers across multiple centers worldwide.

Dr. Lee has nothing to disclose.

Dr. Leenders has nothing to disclose.

Dr. Lewis has nothing to disclose.

Dr. Liguori has nothing to disclose.

Dr. Liu has nothing to disclose.

Dr. Lo reports grants from Oxford NIHR Biomedical Research Centre, outside the submitted work.

Dr. Ehgoetz Martens has nothing to disclose.

Dr. Nepozitek has nothing to disclose.

Dr. Plazzi reports personal fees from UCB, personal fees from Bioprojet, personal fees from Jazz Pharma, personal fees from Idorsia, outside the submitted work; .

Dr. Provini reports personal fees from Vanda Pharmaceutical, personal fees from Sanofi, personal fees from Zambon, personal fees from Fidia, personal fees from Bial, personal fees from Eisai Japan, personal fees from Italfarmaco, outside the submitted work.

Monica Puligheddu has nothing to disclose.

Dr. Rolinski reports grants from National Institute for Health Research (NIHR), during the conduct of the study; grants from Britannia Pharmaceuticals Ltd., non-financial support from IXICO , grants from Eli Lilly , outside the submitted work.

Dr. Rusz has nothing to disclose.

Dr. Stefani has nothing to disclose.

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References:

1.   Postuma RB, Iranzo A, Hu M, et al. Risk and predictors of dementia and parkinsonism in idiopathic REM sleep behaviour disorder: a multicentre study. *Brain* 2019; **142**(3): 744-59.
2.   Ferri R, Arico D, Cosentino FII, Lanuzza B, Chiaro G, Manconi M. REM sleep without atonia with REM sleep-related motor events: broadening the spectrum of REM sleep behavior disorder. *Sleep* 2018; **41**(12).
3.   Hogl B, Stefani A, Videnovic A. Idiopathic REM sleep behaviour disorder and neurodegeneration - an update. *Nat Rev Neurol* 2018; **14**(1): 40-55.
4.   Nepozitek J, Dostalova S, Dusek P, et al. Simultaneous tonic and phasic REM sleep without atonia best predicts early phenoconversion to neurodegenerative disease in idiopathic REM sleep behavior disorder. *Sleep* 2019; **42**(9).
5.   McCarter SJ, Sandness DJ, McCarter AR, et al. REM sleep muscle activity in idiopathic REM sleep behavior disorder predicts phenoconversion. *Neurology* 2019; **93**(12): e1171-e9.
6.   Peever J, Luppi PH, Montplaisir J. Breakdown in REM sleep circuitry underlies REM sleep behavior disorder. *Trends Neurosci* 2014; **37**(5): 279-88.
7.   Melpignano A, Parrino L, Santamaria J, et al. Isolated rapid eye movement sleep behavior disorder and cyclic alternating pattern: is sleep microstructure a predictive parameter of neurodegeneration? *Sleep* 2019; **42**(10).
8.   Ruffini G, Ibanez D, Castellano M, et al. Deep Learning With EEG Spectrograms in Rapid Eye Movement Behavior Disorder. *Front Neurol* 2019; **10**: 806.
9.   Cooray N, Andreotti F, Lo C, Symmonds M, Hu MTM, De Vos M. Detection of REM sleep behaviour disorder by automated polysomnography analysis. *Clin Neurophysiol* 2019; **130**(4): 505-14.
10.   Lanza G, Arico D, Lanuzza B, et al. Facilitatory/inhibitory intracortical imbalance in REM sleep behavior disorder: early electrophysiological marker of neurodegeneration? *Sleep* 2020; **43**(3).
11.   Puligheddu M, Figorilli M, Serra A, et al. REM Sleep without atonia correlates with abnormal vestibular-evoked myogenic potentials in isolated REM sleep behavior disorder. *Sleep* 2019; **42**(9).

12.   Fereshtehnejad SM, Yao C, Pelletier A, Montplaisir JY, Gagnon JF, Postuma RB. Evolution of prodromal Parkinson's disease and dementia with Lewy bodies: a prospective study. *Brain* 2019; **142**(7): 2051-67.

13.   Del Din S, Yarnall AJ, Barber TR, et al. Continuous Real-World Gait Monitoring in Idiopathic REM Sleep Behavior Disorder. *J Parkinsons Dis* 2020; **10**(1): 283-99.

14.   Ehgoetz Martens KA, Matar E, Hall JM, et al. Subtle gait and balance impairments occur in idiopathic rapid eye movement sleep behavior disorder. *Mov Disord* 2019; **34**(9): 1374-80.

15.   Rusz J, Hlavnicka J, Tykalova T, et al. Smartphone Allows Capture of Speech Abnormalities Associated With High Risk of Developing Parkinson's Disease. *IEEE Trans Neural Syst Rehabil Eng* 2018; **26**(8): 1495-507.

16.   Hanuska J, Rusz J, Bezdicek O, et al. Eye movements in idiopathic rapid eye movement sleep behaviour disorder: High antisaccade error rate reflects prefrontal cortex dysfunction. *J Sleep Res* 2019; **28**(5): e12742.

17.   Arora S, Baig F, Lo C, et al. Smartphone motor testing to distinguish idiopathic REM sleep behavior disorder, controls, and PD. *Neurology* 2018; **91**(16): e1528-e38.

18.   Krupicka R, Kryze P, Netukova S, et al. Instrumental analysis of finger tapping reveals a novel early biomarker of parkinsonism in idiopathic rapid eye movement sleep behaviour disorder. *Sleep Med* 2020; **75**: 45-9.

19.   Cochen De Cock V, de Verbizier D, Picot MC, et al. Rhythm disturbances as a potential early marker of Parkinson's disease in idiopathic REM sleep behavior disorder. *Ann Clin Transl Neurol* 2020; **7**(3): 280-7.

20.   Ferini-Strambi L, Fasiello E, Sforza M, Salsone M, Galbiati A. Neuropsychological, electrophysiological, and neuroimaging biomarkers for REM behavior disorder. *Expert Rev Neurother* 2019; **19**(11): 1069-87.

21.   Terzaghi M, Toscano G, Casoni F, et al. Assessment of cognitive profile as a prodromal marker of the evolution of rapid eye movement sleep behavior disorder. *Sleep* 2019; **42**(8).

22.   Genier Marchand D, Montplaisir J, Postuma RB, Rahayel S, Gagnon JF. Detecting the Cognitive Prodrome of Dementia with Lewy Bodies: A Prospective Study of REM Sleep Behavior Disorder. *Sleep* 2017; **40**(1).

23. Genier Marchand D, Postuma RB, Escudier F, et al. How does dementia with Lewy bodies start? prodromal cognitive changes in REM sleep behavior disorder. *Ann Neurol* 2018; **83**(5): 1016-26.

24. Honeycutt L, Gagnon JF, Pelletier A, De Roy J, Montplaisir JY, Postuma RB. Pareidolias and cognition in isolated REM sleep behavior disorder. *Parkinsonism Relat Disord* 2020; **75**: 76-9.

25. Bezdicek O, Nikolai T, Nepozitek J, et al. Prospective memory impairment in idiopathic REM sleep behavior disorder. *Clin Neuropsychol* 2018; **32**(5): 1019-37.

26. Barber TR, Lawton M, Rolinski M, et al. Prodromal Parkinsonism and Neurodegenerative Risk Stratification in REM Sleep Behavior Disorder. *Sleep* 2017; **40**(8).

27. Dusek P, Ibarburu V, Bezdicek O, et al. Relations of non-motor symptoms and dopamine transporter binding in REM sleep behavior disorder. *Sci Rep* 2019; **9**(1): 15463.

28. Hummel T, Sekinger B, Wolf SR, Pauli E, Kobal G. 'Sniffin' sticks': olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. *Chem Senses* 1997; **22**(1): 39-52.

29. Doty RL, Shaman P, Applebaum SL, Giberson R, Siksorski L, Rosenberg L. Smell identification ability: changes with age. *Science* 1984; **226**(4681): 1441-3.

30. Sasai-Sakuma T, Takeuchi N, Asai Y, Inoue Y, Inoue Y. Prevalence and clinical characteristics of REM sleep behavior disorder in Japanese elderly people. *Sleep* 2020; **43**(8).

31. Postuma RB, Gagnon JF, Bertrand JA, Genier Marchand D, Montplaisir JY. Parkinson risk in idiopathic REM sleep behavior disorder: preparing for neuroprotective trials. *Neurology* 2015; **84**(11): 1104-13.

32. Iranzo A, Serradell M, Vilaseca I, et al. Longitudinal assessment of olfactory function in idiopathic REM sleep behavior disorder. *Parkinsonism Relat Disord* 2013; **19**(6): 600-4.

33. Shin C, Lee JY, Kim YK, et al. Cognitive decline in association with hyposmia in idiopathic rapid eye movement sleep behavior disorder: a prospective 2-year follow-up study. *Eur J Neurol* 2019; **26**(11): 1417-20.

34. Doppler K, Jentschke HM, Schulmeyer L, et al. Dermal phospho-alpha-synuclein deposits confirm REM sleep behaviour disorder as prodromal Parkinson's disease. *Acta Neuropathol* 2017; **133**(4): 535-45.

35.   Iranzo A, Marrero-González P, Serradell M, Gaig C, Santamaria J, Vilaseca I. Significance of hyposmia in isolated REM sleep behavior disorder. *J Neurol*. 2021; **268**(3):963-966.

36.   Jennings D, Siderowf A, Stern M, et al. Conversion to Parkinson Disease in the PARS Hyposmic and Dopamine Transporter-Deficit Prodromal Cohort. *JAMA Neurol* 2017; **74**(8): 933-40.

37.   Horgusluoglu E, Nudelman K, Nho K, Saykin AJ. Adult neurogenesis and neurodegenerative diseases: A systems biology perspective. *Am J Med Genet B Neuropsychiatr Genet* 2017; **174**(1): 93-112.

38.   Postuma RB, Gagnon JF, Vendette M, Desjardins C, Montplaisir JY. Olfaction and color vision identify impending neurodegeneration in rapid eye movement sleep behavior disorder. *Ann Neurol* 2011; **69**(5): 811-8.

39.   Lee JY, Ahn J, Oh S, et al. Retina thickness as a marker of neurodegeneration in prodromal lewy body disease. *Mov Disord* 2020; **35**(2): 349-54.

40.   Ahn J, Lee JY, Kim TW, et al. Retinal thinning associates with nigral dopaminergic loss in de novo Parkinson disease. *Neurology* 2018; **91**(11): e1003-e12.

41.   Yang ZJ, Wei J, Mao CJ, et al. Retinal nerve fiber layer thinning: a window into rapid eye movement sleep behavior disorders in Parkinson's disease. *Sleep Breath* 2016; **20**(4): 1285-92.

42.   Lee JY, Ahn J, Yoon EJ, Oh S, Kim YK, Jeon B. Macular ganglion-cell-complex layer thinning and optic nerve integrity in drug-naive Parkinson's disease. *J Neural Transm (Vienna)* 2019; **126**(12): 1695-9.

43.   Mailankody P, Lenka A, Pal PK. The role of Optical Coherence Tomography in Parkinsonism: A critical review. *J Neurol Sci* 2019; **403**: 67-74.

44.   Cheung CY, Chan VTT, Mok VC, Chen C, Wong TY. Potential retinal biomarkers for dementia: what is new? *Curr Opin Neurol* 2019; **32**(1): 82-91.

45.   Ortuno-Lizaran I, Beach TG, Serrano GE, Walker DG, Adler CH, Cuenca N. Phosphorylated alpha-synuclein in the retina is a biomarker of Parkinson's disease pathology severity. *Mov Disord* 2018; **33**(8): 1315-24.

46.   Ferini-Strambi L, Oertel W, Dauvilliers Y, et al. Autonomic symptoms in idiopathic REM behavior disorder: a multicentre case-control study. *J Neurol* 2014; **261**(6): 1112-8.

47.   Li Y, Kang W, Yang Q, et al. Predictive markers for early conversion of iRBD to neurodegenerative synucleinopathy diseases. *Neurology* 2017; **88**(16): 1493-500.

48.	McCarter SJ, Gehrking TL, St Louis EK, et al. Autonomic dysfunction and phenoconversion in idiopathic REM sleep behavior disorder. *Clin Auton Res* 2020; **30**(3): 207-13.

49.	Zitser J, During EH, Chiaro G, Miglis MG. Autonomic impairment as a potential biomarker in idiopathic REM-sleep-behavior disorder. *Auton Neurosci* 2019; **220**: 102553.

50.	Knudsen K, Fedorova TD, Hansen AK, et al. In-vivo staging of pathology in REM sleep behaviour disorder: a multimodality imaging case-control study. *Lancet Neurol* 2018; **17**(7): 618-28.

51.	Lee H, Cho YW, Kim HA. The Severity and Pattern of Autonomic Dysfunction in Idiopathic Rapid Eye Movement Sleep Behavior Disorder. *Mov Disord* 2015; **30**(13): 1843-8.

52.	Rocchi C, Placidi F, Liguori C, et al. Daytime autonomic activity in idiopathic rapid eye movement sleep behavior disorder: a preliminary study. *Sleep Med* 2018; **52**: 163-7.

53.	Zitser J, Muppidi S, Sinn DI, Jaradeh S, Miglis MG. Quantitative sudomotor abnormalities in clinically isolated rapid eye movement sleep behavior disorder. *Auton Neurosci* 2020; **224**: 102645.

54.	Rossi M, Candelise N, Baiardi S, et al. Ultrasensitive RT-QuIC assay with high sensitivity and specificity for Lewy body-associated synucleinopathies. *Acta Neuropathol* 2020; **140**(1): 49-62.

55.	Iranzo A FG, Chumbala A, Serradell M, Gelpi E, Vilaseca I, Sanchez-Valle R, Gaig C, Santamaria J, Tolosa E, Riha RL, Green AJE. Detection of α-synuclein in CSF by RT-QuIC in patients with isolated rapid-eye-movement sleep behaviour disorder: a longitudinal observational study. *The Lancet Neurology* 2021; **20**(3):203-212.

56.	Stefani A IA, Holzknecht E, Perra D, Bongianni M, Gaig C, Heim B, Serradell M, Sacchetto L, Garrido A, Capaldi S, Sanchez-Gomez MPC, Mariotto S, Ferrari S, Fiorini M, Schmutzhard J, Cocchiara P, Vilaseca I, Brozzetti L, Monaco S, Marti MJ, Seppi K, Tolosa E, Santamaria J, Hogl B, Poewe W, Zanusso G. Alpha-synuclein seeds in olfactory mucosa of patients with isolated rapideye-movement sleep behaviour disorder. *Brain* 2021;**144**(4):1118-1126.

57.	Niu M, Li Y, Li G, et al. A longitudinal study on alpha-synuclein in plasma neuronal exosomes as a biomarker for Parkinson's disease development and progression. *Eur J Neurol* 2020; **27**(6): 967-74.

58.   Jiang C, Hopfner F, Katsikoudi A, et al. Serum neuronal exosomes predict and differentiate Parkinson's disease from atypical parkinsonism. *J Neurol Neurosurg Psychiatry* 2020; **91**(7): 720-9.

59.   Wilke C, Dos Santos MCT, Schulte C, et al. Intraindividual Neurofilament Dynamics in Serum Mark the Conversion to Sporadic Parkinson's Disease. *Mov Disord* 2020; **35**(7): 1233-8.

60.   Mondello S, Kobeissy F, Mechref Y, et al. Novel biomarker signatures for idiopathic REM sleep behavior disorder: A proteomic and system biology approach. *Neurology* 2018; **91**(18): e1710-e5.

61.   Dong X, Mondello S, Kobeissy F, Talih F, Ferri R, Mechref Y. LC-MS/MS glycomics of idiopathic rapid eye movement sleep behavior disorder. *Electrophoresis* 2018; **39**(24): 3096-103.

62.   Fernandez-Santiago R, Iranzo A, Gaig C, et al. MicroRNA association with synucleinopathy conversion in rapid eye movement behavior disorder. *Ann Neurol* 2015; **77**(5): 895-901.

63.   Smith AM, Depp C, Ryan BJ, et al. Mitochondrial dysfunction and increased glycolysis in prodromal and early Parkinson's blood cells. *Mov Disord* 2018; **33**(10): 1580-90.

64.   Heintz-Buschart A, Pandey U, Wicke T, et al. The nasal and gut microbiome in Parkinson's disease and idiopathic rapid eye movement sleep behavior disorder. *Mov Disord* 2018; **33**(1): 88-98.

65.   Iranzo A, Valldeoriola F, Lomena F, et al. Serial dopamine transporter imaging of nigrostriatal function in patients with idiopathic rapid-eye-movement sleep behaviour disorder: a prospective study. *Lancet Neurol* 2011; **10**(9): 797-805.

66.   Iranzo A, Santamaria J, Valldeoriola F, et al. Dopamine transporter imaging deficit predicts early transition to synucleinopathy in idiopathic rapid eye movement sleep behavior disorder. *Ann Neurol* 2017; **82**(3): 419-28.

67.   Arnaldi D, Chincarini A, De Carli F, et al. The fate of patients with REM sleep behavior disorder and mild cognitive impairment. *Sleep Med* 2021; **79**: 205-10.

68.   Arnaldi D, Chincarini A, Hu MT, et al. Dopaminergic imaging and clinical predictors for phenoconversion of REM sleep behaviour disorder. *Brain* 2021; **144**(1): 278-87.

69. Huang Z, Jiang C, Li L, et al. Correlations between dopaminergic dysfunction and abnormal metabolic network activity in REM sleep behavior disorder. *J Cereb Blood Flow Metab* 2020; **40**(3): 552-62.

70. Meles SK, Renken RJ, Janzen A, et al. The Metabolic Pattern of Idiopathic REM Sleep Behavior Disorder Reflects Early-Stage Parkinson Disease. *J Nucl Med* 2018; **59**(9): 1437-44.

71. Kogan RV, Janzen A, Meles SK, et al. Four-Year Follow-up of [(18) F]Fluorodeoxyglucose Positron Emission Tomography-Based Parkinson's Disease-Related Pattern Expression in 20 Patients with Isolated Rapid Eye Movement Sleep Behavior Disorder Shows Prodromal Progression. *Mov Disord* 2021; **36**(1): 230-5.

72. Barber TR, Griffanti L, Bradley KM, et al. Nigrosome 1 imaging in REM sleep behavior disorder and its association with dopaminergic decline. *Ann Clin Transl Neurol* 2020; **7**(1): 26-35.

73. Rahayel S, Postuma RB, Montplaisir J, et al. Abnormal Gray Matter Shape, Thickness, and Volume in the Motor Cortico-Subcortical Loop in Idiopathic Rapid Eye Movement Sleep Behavior Disorder: Association with Clinical and Motor Features. *Cereb Cortex* 2018; **28**(2): 658-71.

74. Rahayel S, Postuma RB, Montplaisir J, et al. Cortical and subcortical gray matter bases of cognitive deficits in REM sleep behavior disorder. *Neurology* 2018; **90**(20): e1759-e70.

75. Rahayel S, Postuma RB, Montplaisir J, et al. A Prodromal Brain-Clinical Pattern of Cognition in Synucleinopathies. *Ann Neurol* 2021; **89**(2): 341-57.

76. Munoz-Lopetegi A, Berenguer J, Iranzo A, et al. Magnetic resonance imaging abnormalities as a marker of multiple system atrophy in isolated rapid eye movement sleep behavior disorder. *Sleep* 2021; **44**(1).

77. Campabadal A, Abos A, Segura B, et al. Disruption of posterior brain functional connectivity and its relation to cognitive impairment in idiopathic REM sleep behavior disorder. *Neuroimage Clin* 2020; **25**: 102138.

78. Gersel Stokholm M, Iranzo A, Ostergaard K, et al. Cholinergic denervation in patients with idiopathic rapid eye movement sleep behaviour disorder. *Eur J Neurol* 2020; **27**(4): 644-52.

79. Beach TG, Adler CH, Sue LI, et al. Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. *Acta Neuropathol* 2010; **119**(6): 689-702.

80.	Zitser J, Gibbons C, Miglis MG. The role of tissue biopsy as a biomarker in REM sleep behavior disorder. *Sleep Med Rev* 2020; **51**: 101283.

81.	Sprenger FS, Stefanova N, Gelpi E, et al. Enteric nervous system alpha-synuclein immunoreactivity in idiopathic REM sleep behavior disorder. *Neurology* 2015; **85**(20): 1761-8.

82.	Vilas D, Iranzo A, Tolosa E, et al. Assessment of alpha-synuclein in submandibular glands of patients with idiopathic rapid-eye-movement sleep behaviour disorder: a case-control study. *Lancet Neurol* 2016; **15**(7): 708-18.

83.	Iranzo A, Borrego S, Vilaseca I, et al. alpha-Synuclein aggregates in labial salivary glands of idiopathic rapid eye movement sleep behavior disorder. *Sleep* 2018; **41**(8).

84.	Antelmi E, Donadio V, Incensi A, Plazzi G, Liguori R. Skin nerve phosphorylated alpha-synuclein deposits in idiopathic REM sleep behavior disorder. *Neurology* 2017; **88**(22): 2128-31.

85.	Antelmi E, Pizza F, Donadio V, et al. Biomarkers for REM sleep behavior disorder in idiopathic and narcoleptic patients. *Ann Clin Transl Neurol* 2019; **6**(9): 1872-6.

86.	Al-Qassabi A, Tsao TS, Racolta A, et al. Immunohistochemical Detection of Synuclein Pathology in Skin in Idiopathic Rapid Eye Movement Sleep Behavior Disorder and Parkinsonism. *Mov Disord* 2021; **36**(4):895-904.

87.	Donadio V, Doppler K, Incensi A, et al. Abnormal alpha-synuclein deposits in skin nerves: intra- and inter-laboratory reproducibility. *Eur J Neurol* 2019; **26**(10): 1245-51.

88.	Donadio V, Incensi A, Rizzo G, et al. Skin Biopsy May Help to Distinguish Multiple System Atrophy-Parkinsonism from Parkinson's Disease With Orthostatic Hypotension. *Mov Disord* 2020; **35**(9): 1649-57.

89.	Ouled Amar Bencheikh B, Ruskey JA, Arnulf I, et al. LRRK2 protective haplotype and full sequencing study in REM sleep behavior disorder. *Parkinsonism Relat Disord* 2018; **52**: 98-101.

90.	Li J, Ruskey JA, Arnulf I, et al. Full sequencing and haplotype analysis of MAPT in Parkinson's disease and rapid eye movement sleep behavior disorder. *Mov Disord* 2018; **33**(6): 1016-20.

91.	Gan-Or Z, Montplaisir JY, Ross JP, et al. The dementia-associated APOE epsilon4 allele is not associated with rapid eye movement sleep behavior disorder. *Neurobiol Aging* 2017; **49**: 218 e13- e15.

92.	Gan-Or Z, Mirelman A, Postuma RB, et al. GBA mutations are associated with Rapid Eye Movement Sleep Behavior Disorder. *Ann Clin Transl Neurol* 2015; **2**(9): 941-5.

93. Krohn L, Ozturk TN, Vanderperre B, et al. Genetic, Structural, and Functional Evidence Link TMEM175 to Synucleinopathies. *Ann Neurol* 2020; **87**(1): 139-53.

94. Krohn L, Ruskey JA, Rudakou U, et al. GBA variants in REM sleep behavior disorder: A multicenter study. *Neurology* 2020; **95**(8): e1008-e16.

95. Krohn L, Wu RYJ, Heilbron K, et al. Fine-Mapping of SNCA in Rapid Eye Movement Sleep Behavior Disorder and Overt Synucleinopathies. *Ann Neurol* 2020; **87**(4): 584-98.

96. Arnaldi D, Fama F, Girtler N, et al. Rapid eye movement sleep behavior disorder: A proof-of-concept neuroprotection study for prodromal synucleinopathies. *Eur J Neurol* 2021; **28**(4): 1210-7.

97. Califf RM. Biomarker definitions and their applications. *Exp Biol Med (Maywood)* 2018; **243**(3): 213-21.

98. Ferri R, Gagnon JF, Postuma RB, Rundo F, Montplaisir JY. Comparison between an automatic and a visual scoring method of the chin muscle tone during rapid eye movement sleep. *Sleep Med* 2014; **15**(6): 661-5.

99. Ferri R, Fulda S, Manconi M, et al. Night-to-night variability of periodic leg movements during sleep in restless legs syndrome and periodic limb movement disorder: comparison between the periodicity index and the PLMS index. *Sleep Med* 2013; **14**(3): 293-6.

100. Figorilli M, Ferri R, Zibetti M, et al. Comparison Between Automatic and Visual Scorings of REM Sleep Without Atonia for the Diagnosis of REM Sleep Behavior Disorder in Parkinson Disease. *Sleep* 2017; **40**(2).

101. Figorilli M, Marques AR, Meloni M, et al. Diagnosing REM sleep behavior disorder in Parkinson's disease without a gold standard: a latent-class model study. *Sleep* 2020; **43**(7).

102. Frauscher B, Gabelia D, Biermayr M, et al. Validation of an integrated software for the detection of rapid eye movement sleep behavior disorder. *Sleep* 2014; **37**(10): 1663-71.

103. Frauscher B, Iranzo A, Gaig C, et al. Normative EMG values during REM sleep for the diagnosis of REM sleep behavior disorder. *Sleep* 2012; **35**(6): 835-47.

104. Khalil A, Wright MA, Walker MC, Eriksson SH. Loss of rapid eye movement sleep atonia in patients with REM sleep behavioral disorder, narcolepsy, and isolated loss of REM atonia. *J Clin Sleep Med* 2013; **9**(10): 1039-48.

105. McCarter SJ, St Louis EK, Duwell EJ, et al. Diagnostic thresholds for quantitative REM sleep phasic burst duration, phasic and tonic muscle activity, and REM atonia index in REM

sleep behavior disorder with and without comorbid obstructive sleep apnea. *Sleep* 2014; **37**(10): 1649-62.

106.   McCarter SJ, St Louis EK, Sandness DJ, et al. Diagnostic REM sleep muscle activity thresholds in patients with idiopathic REM sleep behavior disorder with and without obstructive sleep apnea. *Sleep Med* 2017; **33**: 23-9.

107.   Campabadal A, Segura B, Junque C, et al. Comparing the accuracy and neuroanatomical correlates of the UPSIT-40 and the Sniffin' Sticks test in REM sleep behavior disorder. *Parkinsonism Relat Disord* 2019; **65**: 197-202.

108.   Lawton M, Hu MT, Baig F, et al. Equating scores of the University of Pennsylvania Smell Identification Test and Sniffin' Sticks test in patients with Parkinson's disease. *Parkinsonism Relat Disord* 2016; **33**: 96-101.

109.   Pyatigorskaya N, Gaurav R, Arnaldi D, et al. Magnetic Resonance Imaging Biomarkers to Assess Substantia Nigra Damage in Idiopathic Rapid Eye Movement Sleep Behavior Disorder. *Sleep* 2017; **40**(11).

110. https://clinicaltrials.gov/ct2/show/NCT03623672; Accessed 5/18/21.

Table 1. Biomarker Categories and Definitions[97]

Biomarker Subtype	Definition	Application to iRBD
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 (e.g., PD, DLB, MSA)
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 and/or disease severity.
Monitoring/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/neuroprotection and demonstrate efficacy of DMTs.
Combined	Composite and multidimensional, through combination of multiple biomarkers, and as such better reflecting biological systems than single biomarkers**.**	To refine and enhance the diagnostic, prognostic and monitoring capabilities single biomarkers in iRBD.

[*] 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 DMT trials are eventually employed, these monitoring biomarkers may also be considered as therapy responsive biomarkers.

iRBD = isolated REM sleep behavior disorder, PD = Parkinson's disease, DLB = Lewy body dementia, MSA = multiple system atrophy, DMT = disease modifying therapy.

Table 2. Summary of Biomarker Candidates in iRBD

Biomarker	Subtype	Availability*	Cost**	Sensitivity/specificity	Remarks
Neurophysiology					
RSWA quantified by visual or automated methods (e.g., SINBAR method, REM atonia index)	Diagnostic, prognostic, monitoring	+++	+	Diagnostic: 85-95%/85-95%[98-106] Prognostic: 78-89%/61-70%[4]	Robust data supporting both visual and automatic methods, with comparable results despite differences in methodology Only one study
CAP rate	Diagnostic, prognostic	++	++	N/A	Only one study available,[7] special analyses of EEG required, limited availability
Biomarker obtained through artificial intelligence, machine learning and deep neural network-based methods	Diagnostic, prognostic, combined	+	+++	Diagnostic: 91-98%/93-94% Prognostic: AUC: 78%[8,9]	Limited number of studies[8,9]
Motor Function					
Upper extremity alternate-tap test	Diagnostic, prognostic, monitoring, combined	+++	+	Year 0: 100%/83%[23] Year -1: 92%/86% Year -2: 88%/89% Year -3: 91%/86%	Easy to perform Year 0 = phenoconversion to PD or DLB Years -1, -2, -3 = years prior to phenoconversion to PD or DLB
Speech abnormalities quantified by means of acoustic analysis	Prognostic, monitoring	+++	+	67%/71%[15]	Easy to perform, only cross-sectional validation studies
Gait dysfunction by instrumental analysis	Prognostic, monitoring	++	+++	N/A	Limited to few specialized centers, cross-sectional studies only
Wearables devices/smartphones	Prognostic, monitoring	+++	+	92%/90%[17]	Cross-sectional validation studies only
Cognition					
Trail Making Test Part B	Diagnostic, prognostic, monitoring, combined	+++	+	Year 0: 100%/83%[23] Year -1: 92%/86% Year -2: 88%/89% Year -3: 91%/86%	Only one longitudinal study, early identification of prodromal DLB. Year 0 = phenoconversion to DLB Years -1, -2, -3 = years prior to phenoconversion
Semantic verbal fluency	Monitoring, diagnostic, prognostic, combined	+++	+	Year 0: 91%/97%[23] Year -1: 91%/91% Year -2: 80%/91% Year -3: 90%/74%	Only one longitudinal study, cognitive change over time for prodromal DLB Year 0 = phenoconversion to DLB Years -1, -2, -3 = years prior to phenoconversion
Rey Auditory-Verbal Learning Test (immediate recall)	Diagnostic, prognostic, monitoring, combined	+++	+	Year 0: 92%/89%[23] Year -1: 100%/89% Year -2: 100%/75% Year -3: 82%/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 (e.g., Sniffin' Sticks, UPSIT)	Diagnostic, prognostic, combined	+++	+	Sensitivity 86-91% Specificity 76-88%[107]	Easily performed with conversion data between Sniffin and UPSIT available[108]
Ophthalmic Function					
Farnsworth-Munsell 100-Hue test	Diagnostic, prognostic	++	+	N/A	Easily performed, limited data
Optical coherence tomography (structural imaging of the parafoveal avascular zone)	Diagnostic, prognostic	+	+++	N/A	Highly promising for investigating other pathways at risk for early degeneration
Autonomic Function					
Autonomic questionnaires	Diagnostic, prognostic, monitoring, combined	+++	+	N/A	Easily performed, can be easily repeated over time
Hear rate variability analysis	Diagnostic	+++	+	N/A	Easily obtained from baseline vPSG, however sensitive to artifact

MIBG	Diagnostic	++	++	N/A	May help distinguish PD/DLB from MSA[50]
Cardiovascular reflex testing	Diagnostic, prognostic, monitoring, combined	+	++	N/A	Limited to few specialized centers, may help distinguish PD/DLB from MS[48]
Biofluids					
CSF RT-QuIC	Diagnostic, prognostic, monitoring	+	++	100%/98%[54]	Somewhat invasive
Nasal swabs (olfactory mucosa RT-QuIC)	Diagnostic	++	++	44.4%/90%[56]	Somewhat invasive, ENT specialist needed for sampling
Serum neuronal exosomal α-synuclein	Diagnostic	+	+++	95%/93%[58]	Most appealing sensitivity and specificy
Neuroimaging					
^{123}I-FP-SPECT (DaT-SPECT)	Diagnostic, prognostic, monitoring, combined	++	++	29.3%/100%[68]	Limited diagnostic value in differentiating iRBD 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	++	++	52.4%/100%[70,71]	Fair diagnostic value in differentiating iRBD from controls, high diagnostic potential in predicting α-synucleinopathy subtype but requires independent validation, possible prognostic value to be demonstrated in large series, useful for monitoring disease progression, possibly responsive to therapy
MRI – nigrosome MRI – SN neuromelanin MRI – cortical thinning MRI – DBM	Diagnostic, prognostic, combined	++	++	MRI nigrosome: 27.5-77%/97-92.3%[72] MRI SN neuromelanin: 90%-94%[109]	Good diagnostic potential in differentiating iRBD from controls (nigrosome, SN neuromelanin) as well as RBD subtype (i.e., RBD w/MCI), cortical thinning, possible prognostic value for DLB (DBM), all markers require independent study confirmation
Tissue Biopsy					
Colon Biopsy	Diagnostic	+	++	24%/100%[81]	Invasive, poor sensitivity
Major salivary glands	Diagnostic	+	++	89%/100%[82]	Invasive, surgeon needed for sampling, high sensitivity if glandular tissue obtained
Minor salivary glands	Diagnostic	++	++	50%/97%[83]	Invasive, surgeon needed for sampling, poor sensitivity
Skin Biopsy	Diagnostic, prognostic, monitoring, combined	++	++	58%-87%/100%[34,85,86]	Easy to perform, minimally invasive, but analysis requires expertise, may help distinguish PD/DLB from MSA[50]
Genetic Testing					
GBA variants	Prognostic	++	++	N/A	May help predict the rate of phenoconversion[94]
SNCA 5' variants	Prognostic	++	++	N/A	May help predict the rate of phenoconversion[95]

* + limited availability, ++ moderate availability., +++ wide availability; ** + low cost, ++ moderate cost, +++ high cost

RSWA = REM sleep without atonia, SINBAR = Sleep Innsbruck Barcelona group, CAP = cyclic alternating pattern, AUC = area under the curve, EEG = electroencephalography, DLB = dementia with Lewy bodies, UPSIT = University of Pennsylvania smell identification test, MIBG = metaiodobenzylguanidine, vPSG = video polysomnography, ^{123}I-FP = [123]Ioflupane, DaT = dopamine transporter, ^{18}F-FDG = ^{18}F-fluoro-deoxyglucose, SN =

substantia nigra, MCI = mild cognitive impairment, DBM = deformation-based morphometry, RT-QuIC = real-time quaking-induced conversion, ENT = ear nose and throat, GBA = glucocerebrosidase, SNCA = alpha-synuclein gene

Figure 1. REM sleep recorded in a patient with RBD showing excessive chin muscle tone and excessive phasic EMG twitch activity over the chin, tibialis anterior, and flexor digitorum superficialis muscles. EMG = electromyogram, EOG = electrooculogram, ECG = electrocardiogram, TA = tibialis anterior muscle, FDS = flexor digitorum superficialis muscle.

Source: Raffaele Ferri, OASI research institute.

Figure 2. Functional and structural brain imaging findings in iRBD patients

A) Example of an 123I-FP-SPECT scan in an iRBD patient, showing reduced uptake in the left putamen and to a lesser extent the right putamen. The data in the graph have been obtained by analysis of normal subjects without iRBD from the ENC-DAT database using the Basal Ganglia matching tool (see Arnaldi et al.[67] for details). These areas show two different confidence levels (90% in dotted red line and 97% in dashed green line) respectively for putamen and caudate 123I-FP-SPECT uptake. Red squares represent left putamen and caudate nuclei. Green circles represent right putamen and caudate nuclei.

B) Stable voxels (90% CI not straddling zero after bootstrap resampling) of 18F-FDG-PET derived brain glucose iRBD-related pattern (RBDRP) are visualized by overlaying them on T1 MRI template. [Red indicates positive voxel weights (relative hypermetabolism), and blue indicates negative voxel weights (relative hypometabolism). L=left, R=right. Coordinates in axial (Z) and sagittal (X) planes are in Montreal Neurologic Institute standard space. Figure adapted from Meles S et al.[70]

C) Examples of susceptibility-weighted imaging taken at the level of the substantia nigra, in a healthy control (HC) and a patient with iRBD (RBD, see Barber et al.[72] for details). Image HC reveals the presence of a bilateral dorsal nigral hyperdensity (DNH, marked with red arrows), corresponding to nigrosome 1. The DNH is lost bilaterally in the patient with RBD.

D) Areas of cortical thinning in iRBD patients with mild cognitive impairment (MCI) compared to controls with normal cognition and without iRBD, corrected for family-wise error at p<0.05 with age, sex, and education added as covariates. The color bar represents the logarithmic scale of p values (-log10), with red-to-yellow areas representing significant thinning in patients with MCI versus controls. The white asterisks represent the cluster of thinning (left anterior temporal lobe including entorhinal cortex, insula, and inferior/middle frontal cortex) that best discriminated between patients with MCI versus controls without MCI or iRBD [AUC: 0.91 (0.825–0.996)]. Figure adapted from Rahayel S et al.[74]
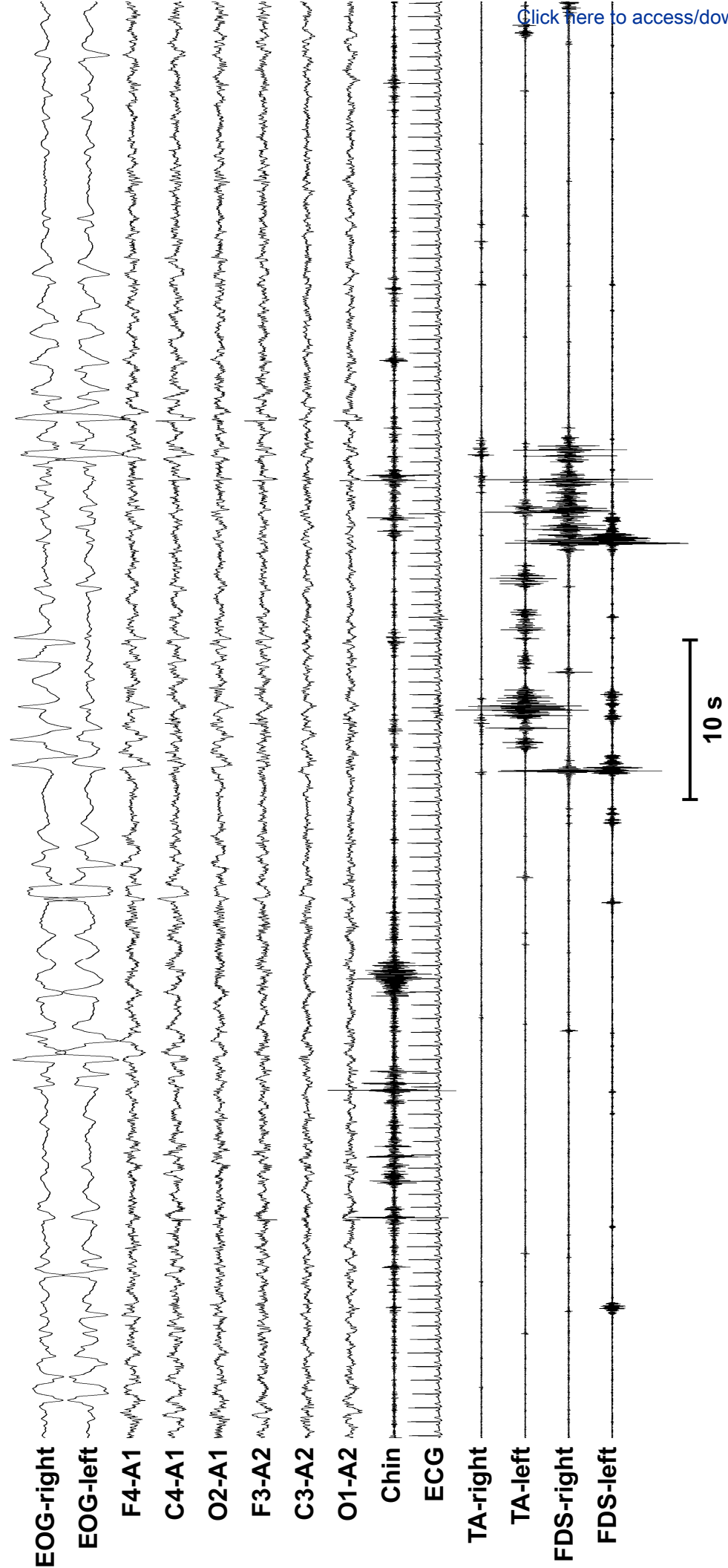
Source: Dario Arnaldi, University of Genoa.

Figure 3. Hypothetical Timeline of iRBD and Associated Clinical Manifestations in Relation to Evolving PD, DLB and MSA.

The hypothetical timelines for PD (A), DLB (B) and MSA (C) are depicted as shown. In PD and DLB, changes in smell and autonomic functioning typically precede RBD, followed by other features, with parkinsonism preceding cognitive changes occurring in evolving PD whereas cognitive changes occur prior to parkinsonism in evolving DLB. In MSA, autonomic dysfunction manifested occur around the time of iRBD, followed by elements of parkinsonism and/or cerebellar dysfunction in many. Changes in smell and cognition are minimal or absent in MSA, and genetic variants associated with MSA are still being studied (these curves are represented by dashed lines as they are hypothesized or inadequately studied). Neuroimaging: a=brainstem alterations, b=nigrostriatal dopaminergic alterations, c=other subcortical and cortical alterations). DLB=dementia with Lewy bodies, MMI=mild motor impairment, MCI=mild cognitive impairment, MSA=multiple system atrophy, PAF=pure autonomic failure, PD=Parkinson's disease, PDD=Parkinson's disease with dementia.
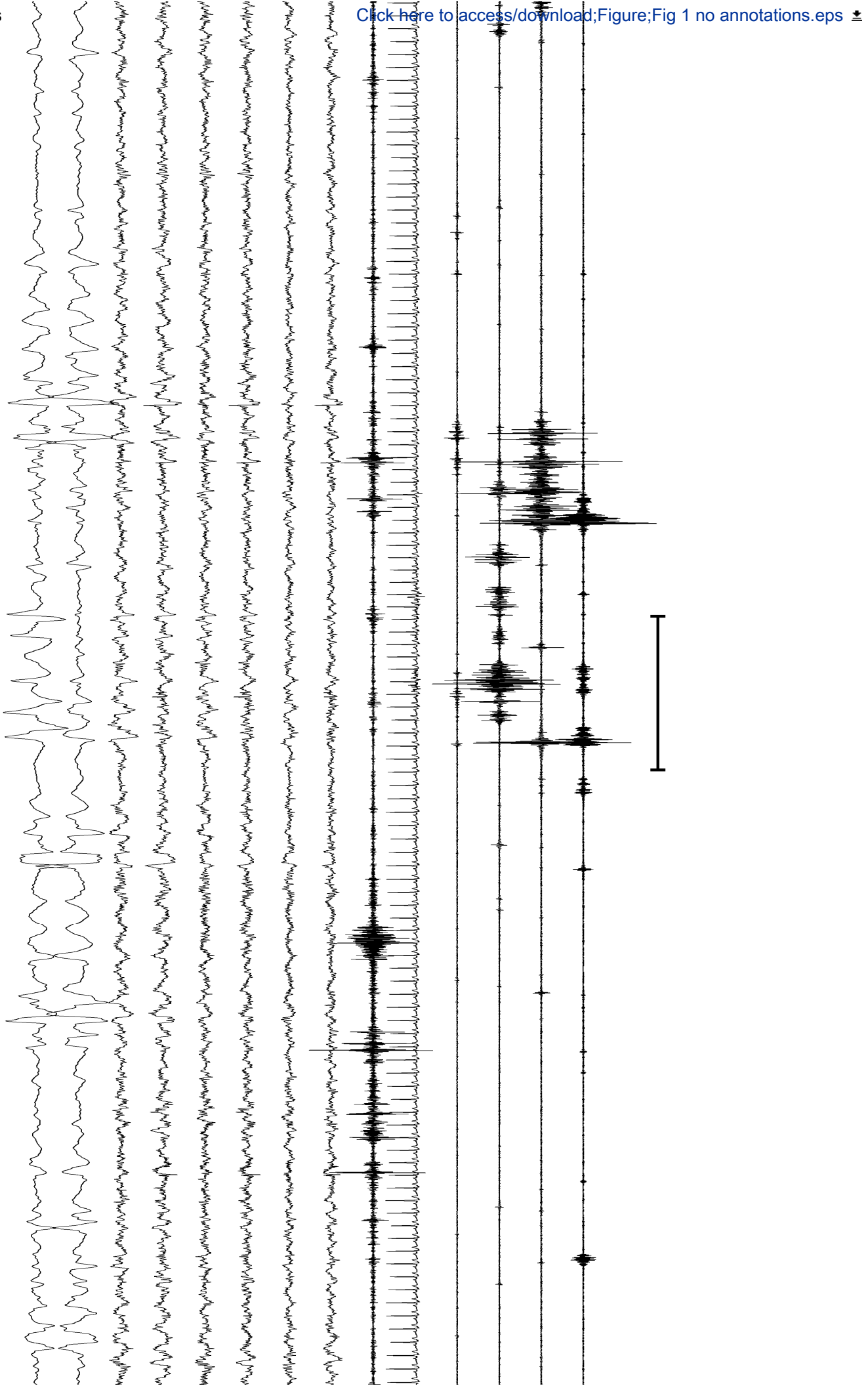
Source: Jean-Francois Gagnon, Centre intégré universitaire de santé et de services sociaux du Nord-de-l'Île-de-Montréal, and Bradley F. Boeve, Mayo Clinic.
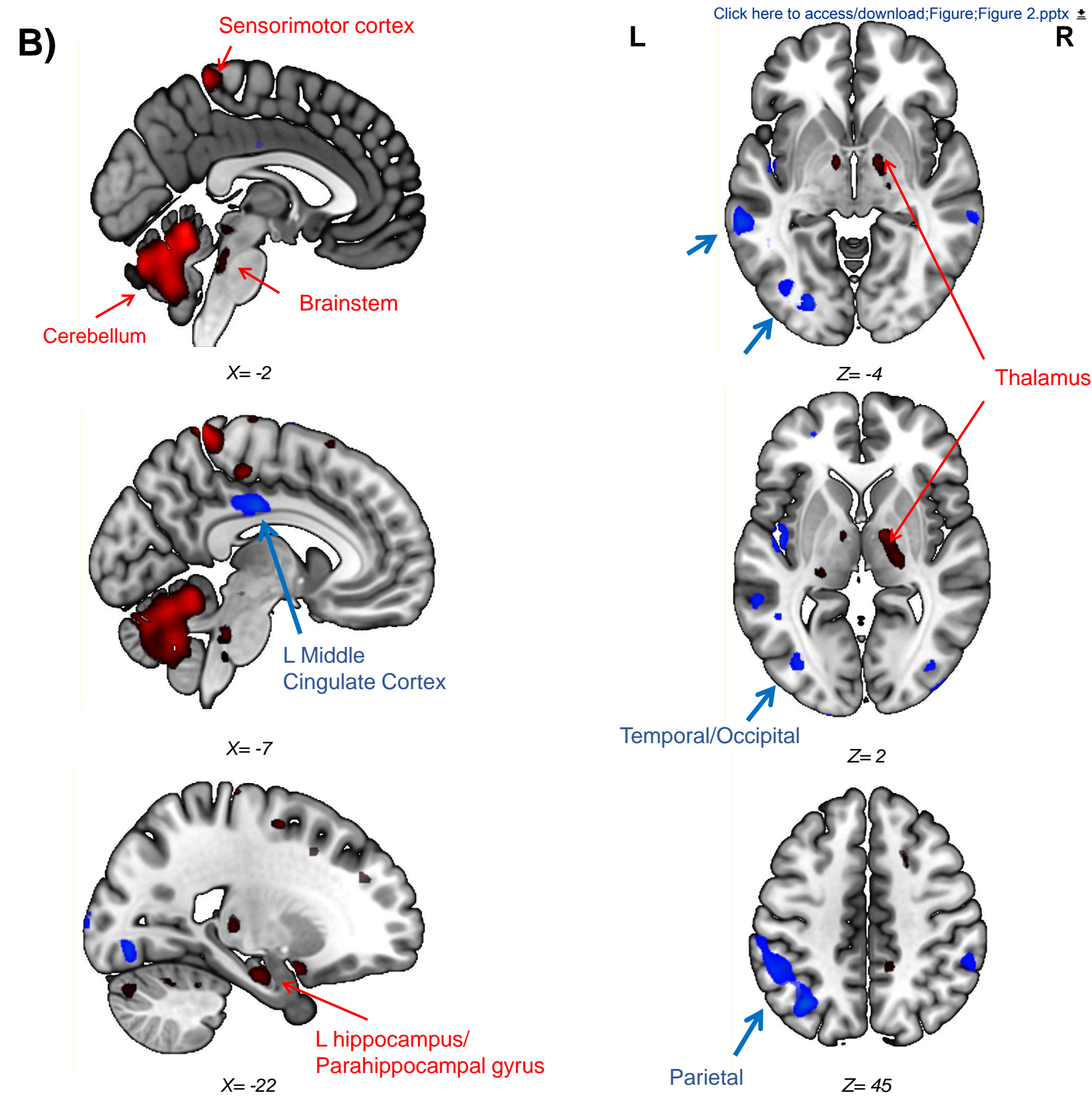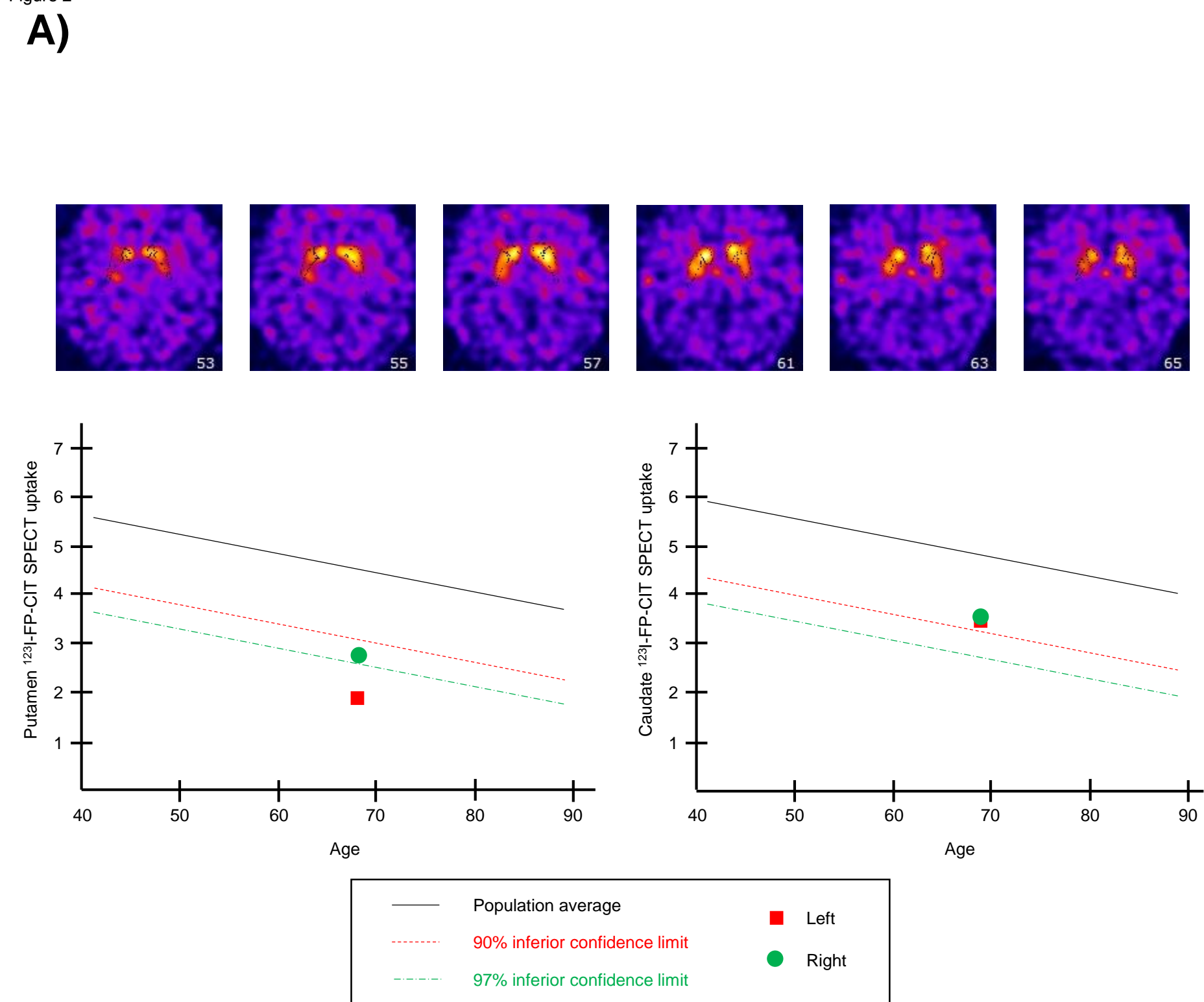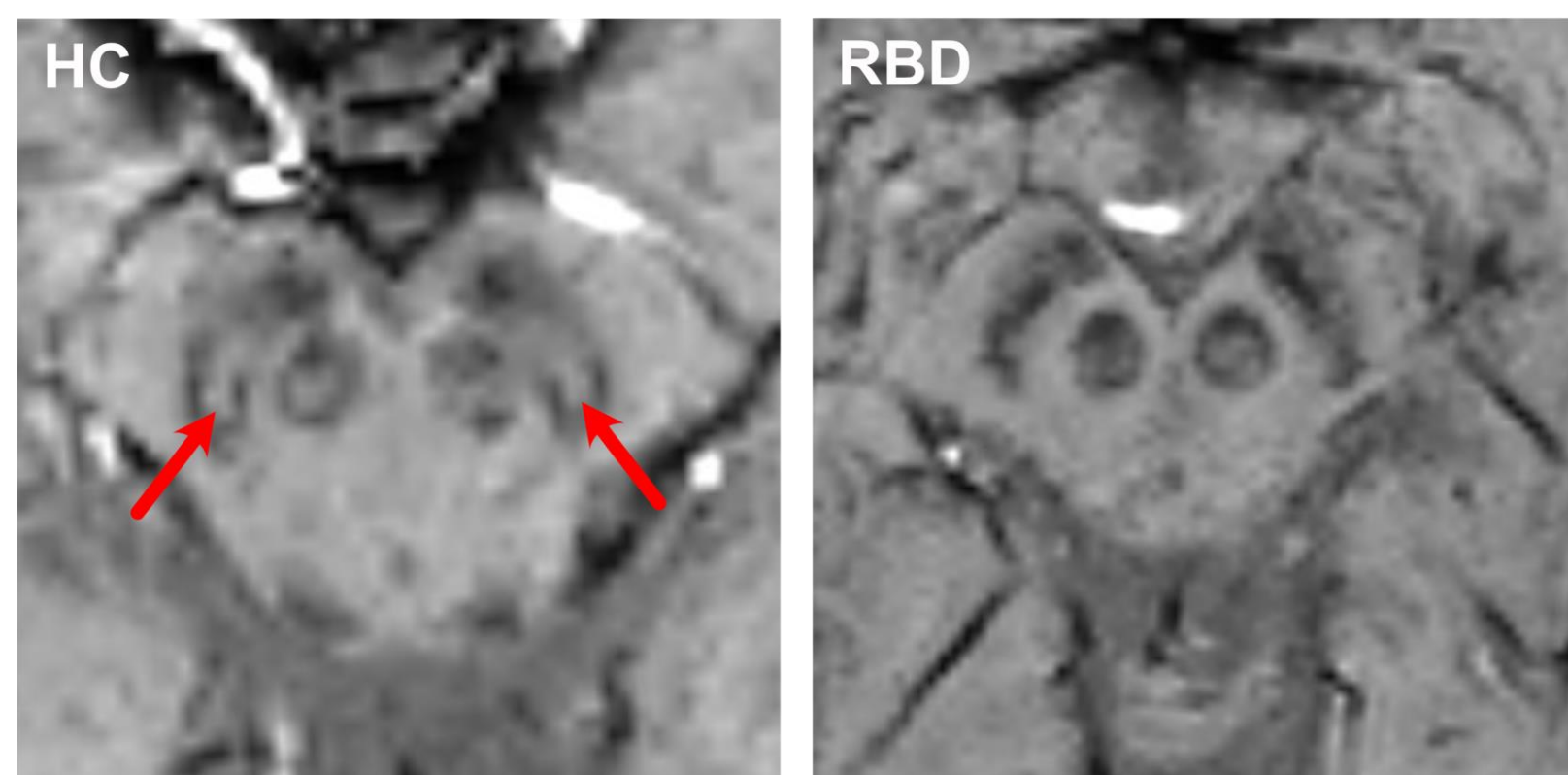
Figure 1

EOG-right
EOG-left
F4-A1
C4-A1
O2-A1
F3-A2
C3-A2
O1-A2
Chin
ECG
TA-right
TA-left
FDS-right
FDS-left

10 s

Figure 1_no annotations
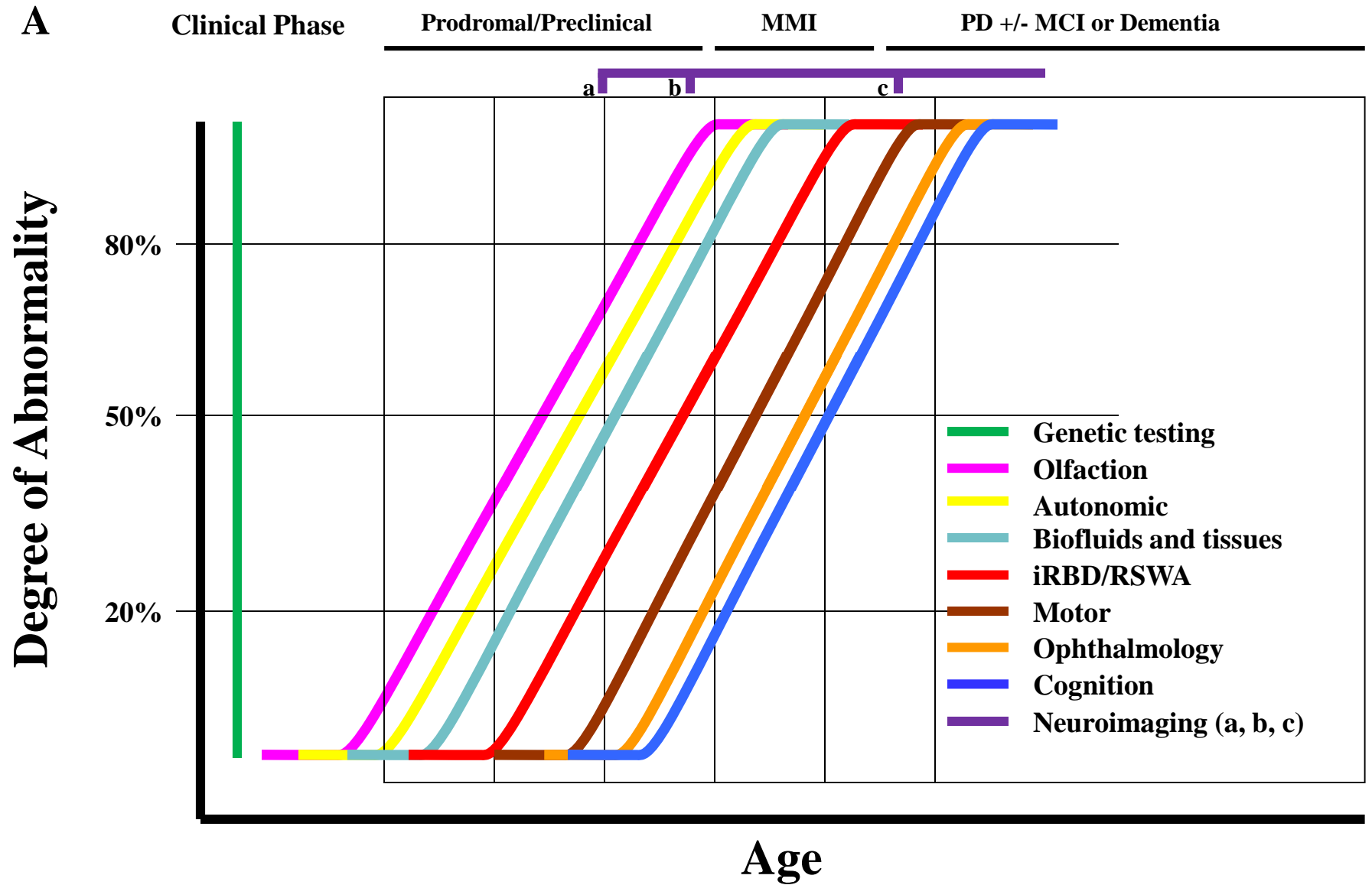
Figure 2

Figure 3

Figure 3

B

C

Clinical Phase   Prodromal/Preclinical   PAF   MSA

Degree of Abnormality

80%

50%

20%

a   b

Age

Genetic testing
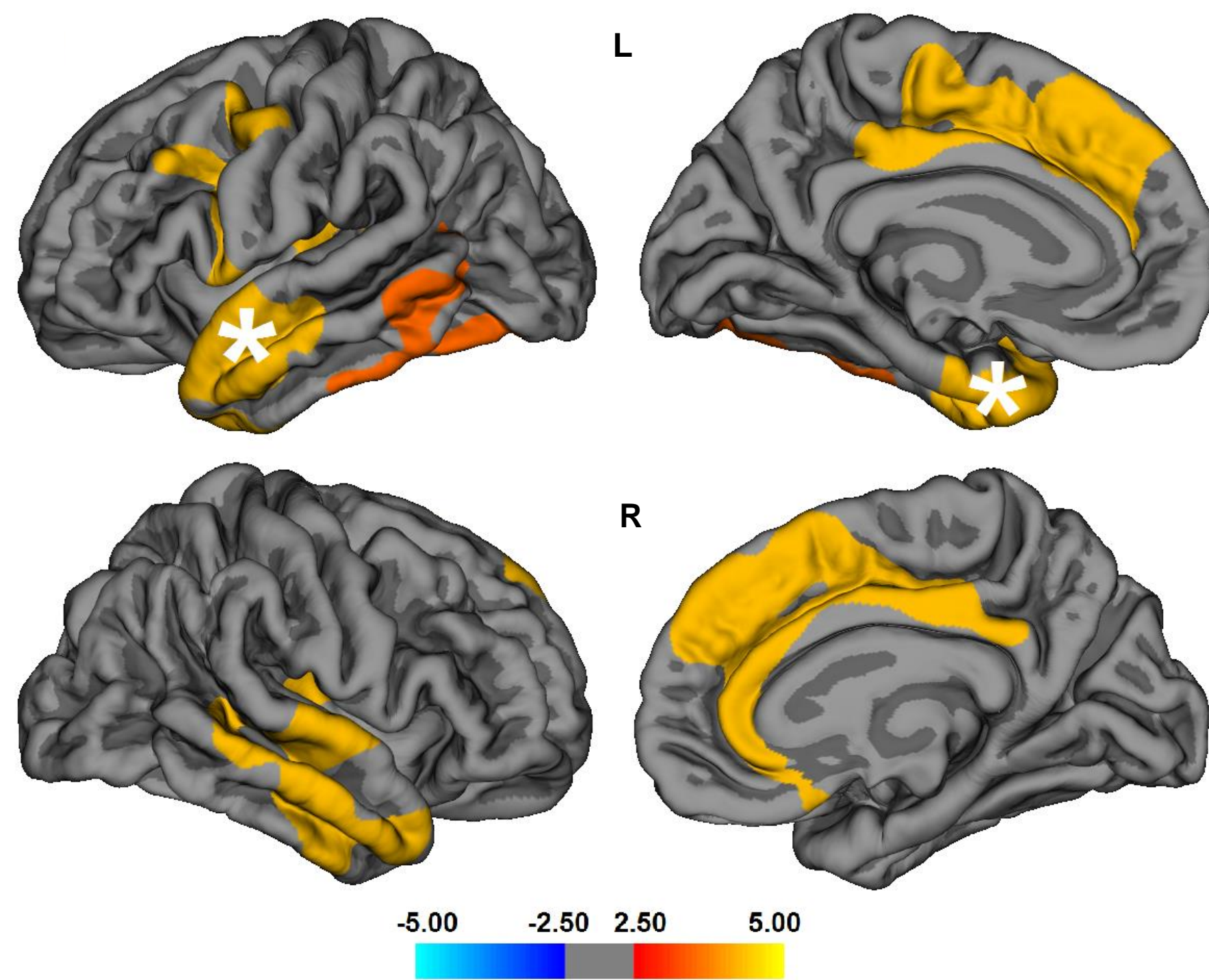Olfaction
Autonomic
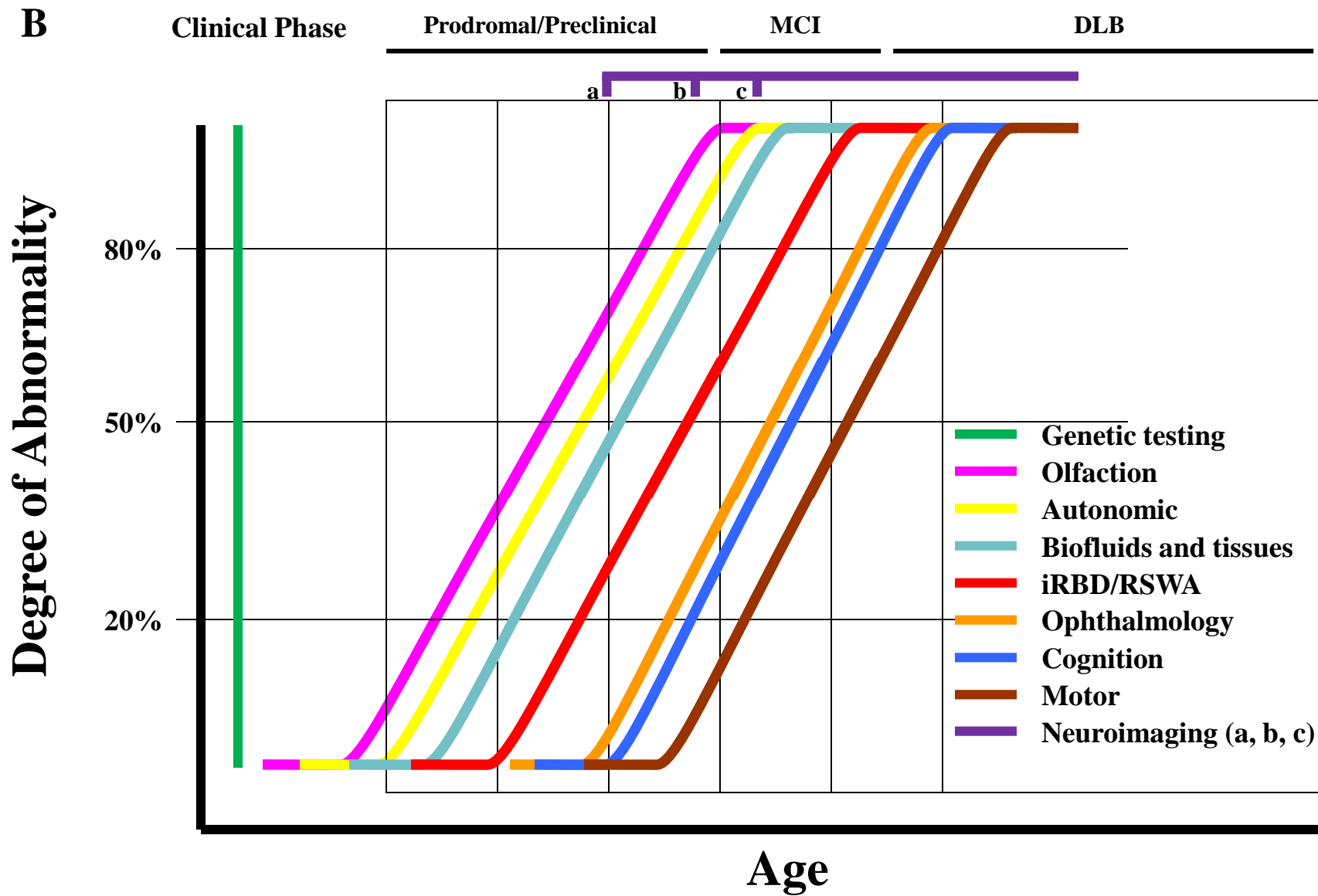Biofluids and tissues
iRBD/RSWA
Motor
Ophthalmology
Cognition
Neuroimaging (a, b)
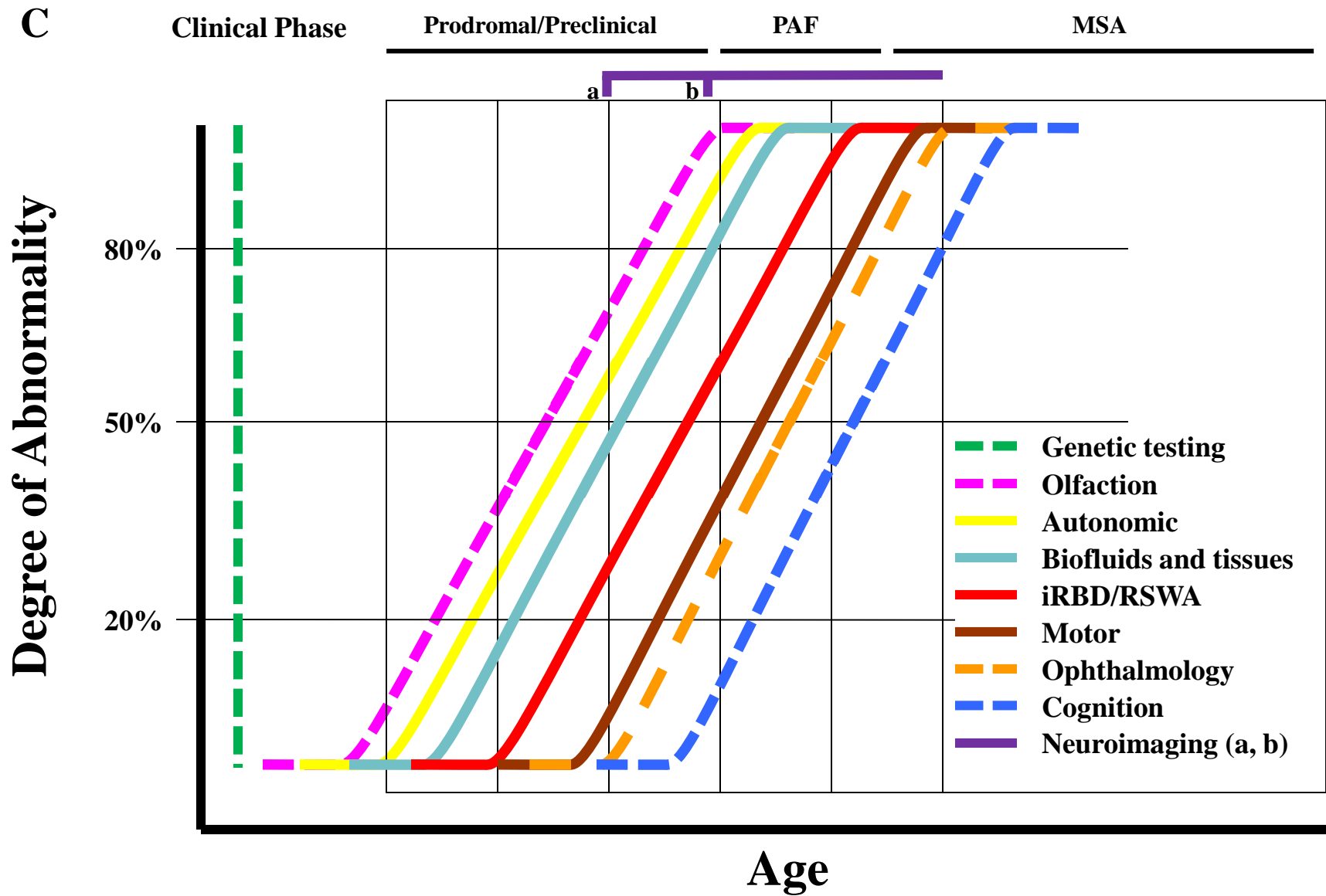
Biomarkers of Conversion to Alpha-Synucleinopathy in Isolated REM Sleep Behaviour Disorder

Supplementary material

Contents

Search Terms

1. Neurophysiology

("RBD" OR "REM sleep behavior disorder" OR "REM sleep behaviour disorder") AND ("REM sleep without atonia" OR "RWA" OR "RSWA" OR "neurophysiology" OR "polysomnography" OR "EEG" OR "electrophysiology").

2. Motor Function- ("iRBD" OR "REM sleep behaviour disorder" OR "RBD") AND ("motor" OR "upper extremity" OR "gait" OR "alpha-synucleinopathy" OR "Parkinsonism" OR "prodromal" OR "electromyography" OR "movement" OR "bradykinesia" OR "lower extremity" OR "speech")

3. Cognition- ("REM sleep behavior disorder" OR "REM sleep behaviour disorder") AND ("cognition OR "cognitive impairment" or "dementia" or "mild cognitive impairment")

4. Olfaction- ("RBD" OR "iRBD" OR "REM sleep behaviour disorder") AND ("olfaction" OR "hyposmia" OR "smell")

5. Ophthalmic Function – ("REM sleep behavior disorder") AND "("vision" OR "visual discrimination: OR "colour discrimination: OR "optical coherence tomography" OR "retina")

6. Autonomic Function- ("RBD" OR "iRBD" OR "REM sleep behaviour disorder") AND ("autonomic" OR "HRV" OR "MIBG" OR "tilt" OR "sudomotor" OR "vagal" OR "dysautonomia").

7. Biofluids- ("idiopathic RBD" OR "isolated RBD" OR "REM sleep without atonia") AND ("blood" OR "serum" OR "plasma" OR "CSF" OR "biofluid" OR "circulating" OR "synucleinopathy" OR "nasal swab").

8. Neuroimaging- ("RBD OR REM sleep behavior disorder) AND ("SPECT" OR "PET" OR "MRI").

9. Tissue Biopsy- ("REM sleep behavior disorder" OR "iRBD" OR "Parkinson's OR "synucleinopathies) AND ("tissue biopsy" OR "salivary gland biopsy" OR "colon/gastrointestinal biopsy" OR "skin biopsy" OR "p-alpha-synuclein deposits/tissue p-alpha-synuclein deposits").

10. **Genetics**- ("RBD" OR REM sleep behavior disorder) AND ("genetics" OR "gene" OR "genomics" OR "GBA" OR "LRRK2" OR "SNCA" OR "MAPT" OR "APOE" OR "TMEM175").
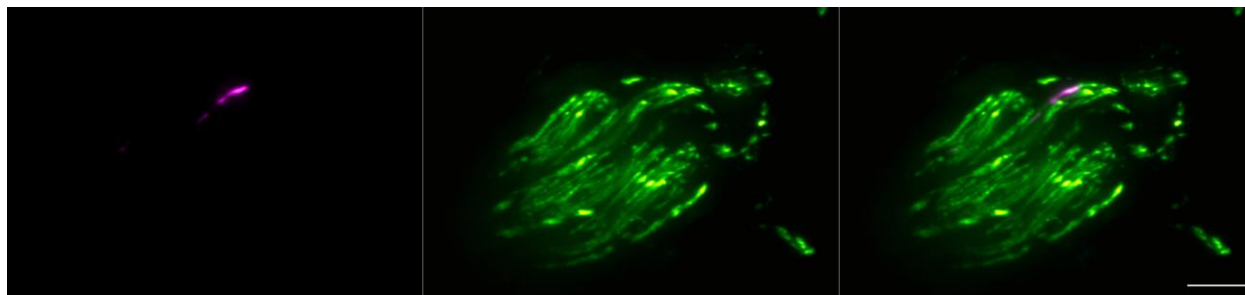
Supplementary figure 1. Example of phosphorylated α-synuclein (pSyn) deposition in a skin biopsy of an iRBD patient. The double immunofluorescence reveals A) a dermal nerve fiber positive for pSyn (anti-α-synuclein phospho-Ser129-antibody, shown in magenta; B) a nerve bundle staining green with anti-protein-gene-product-9.5-antibody; C) co-localization of A) and B), allowing for greater specificity of pSyn staining (scale bar = 20µm). *Source: Anastasia Kutzkina, University of Würzburg*