Timing matters - estimating when pathologies biologically impact in Lewy body disorders

E. Matar[1,2,3], G. M. Halliday[3,4,5]

Affiliations

1 – Central Clinical School, Faculty of Medicine and Health, University of Sydney, NSW Australia

2 – Department of Neurology, Royal Prince Alfred Hospital, Camperdown NSW, Australia

3 – Brain and Mind Centre, University of Sydney, Camperdown New South Wales

4 – School of Medical Sciences, Faculty of Medicine and Health, University of Sydney, NSW Australia

5 - Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA

Corresponding Authors

Dr Elie Matar
Department of Neurology, Level 8, Main Building, Royal Prince Alfred Hospital
elie.matar@sydney.edu.au

Prof Glenda Halliday
Brain and Mind Centre 100 Mallett St Camperdown, New South Wales, 2050
glenda.halliday@sydney.edu.au

Word Count: Abstract: 266, Manuscript: 6487
References: 157
Panels: 3, **Tables:** 1, **Figures:** 3

Author Contributions – EM - Conceptualization, Writing - Original Draft, Literature Search, Figures; GMH - Conceptualization, Writing - Review and Editing

Financial Disclosures

EM receives salary and grant funding from the National Health and Medical Research Council (Investigator Grant 2008565), the University of Sydney, and the Sydney Local Health District. GMH receives salary and grant funding from National Health and Medical Research Council [Investigator Grant 1176607], Aligning Science Across Parkinson's [grants 020505, 020529], Michael J. Fox Foundation for Parkinson's Research (MJFF) and the University of Sydney. The funders were not involved in the manuscript writing process, nor in the decision to submit this manuscript. For the purpose of open access, the authors have applied a CC BY public copyright license to all Author Accepted Manuscripts arising from this submission.

Conflicts of Interest

The authors have no conflicts of interest to declare

Key words: Parkinson's disease, Dementia with Lewy bodies, REM sleep behaviour disorder, synuclein, amyloid, Alzheimer's disease, biomarkers

Abstract

Catalysed by the emergence of promising biomarkers of central α-synuclein pathology there have been concerted efforts to devise biological definitions and staging systems of Parkinson's disease (PD) and to incorporate dementia with Lewy bodies (DLB) and other Lewy body disorders. These essential (and inevitable) efforts will ultimately inform patient selection in biomarker studies and clinical trials of biologically targeted therapies. Following suit with Alzheimer's disease (AD), consensus schemas have been proposed that are anchored on the hallmark neuropathological findings in Lewy body diseases, namely the presence of characteristic abnormal α-synuclein-containing aggregates (Lewy-related pathologies or LRP) and neurodegeneration, with the latter emphasising nigrostriatal dopaminergic deficiency. Understanding the biological and temporal interrelationships between LRP, co-pathologies, cellular loss, and symptom manifestation are crucial to formulating and refining an accurate biological classification and staging system for Lewy body disorders that can assist clinical phenomenology. The early adoption of biomarkers in AD and their use in supporting specific hypotheses of the temporal ordering of the pathology has played a pivotal role in shaping treatment approaches. The ongoing debate and criticisms of such approaches notwithstanding, the pathological sequence of events that give rise to the cardinal manifestations of Lewy body disorders and their relationship to available biomarkers, has not been adequately reconciled.

Drawing on neuropathological and *in vivo* data, we highlight the importance of considering the temporal order of the primary pathological events seen in Lewy body disorders in developing biological staging and note important knowledge gaps. We contend that such issues will facilitate the adoption and refinement of biological schema for Lewy body diseases and inform the design of future studies.

Introduction

Disorders with underlying α-synuclein-rich neuronal Lewy pathology (Lewy bodies and Lewy neurites) include isolated rapid eye movement sleep behaviour disorder (iRBD), Parkinson's disease (PD) and dementia with Lewy bodies (DLB). Despite a similar core pathology, these disorders present with distinct dominant symptoms (RBD, parkinsonism or dementia, in most instances) but also have overlapping features that can occur at any time, suggesting regionality in their biological underpinnings. While the regional biology associated with iRBD is still debated, selective and at least moderate loss of dopamine (DA) neurons in the substantia nigra (SN) occurs in all patients with parkinsonism, and widespread cortical pathology occurs in cases with dementia. As α-synuclein is highly prevalent and has important normal functions in the nervous system, recognising the biological impact and timing of all of these regional pathologies is as important as recognising the similarity of one of the underlying cellular features – Lewy related pathology (LRP).

Until recently, the diagnosis of Lewy body disorders has been primarily clinical, with PD defined by the cardinal motor manifestations of bradykinesia, rigidity and tremor; and DLB by a cognitive syndrome accompanied by additional core features (motor parkinsonism, cognitive fluctuations, visual hallucinations, and RBD). Recent advances enabling the *in vivo* detection of α-synuclein pathology, together with the urgent need to diagnose these disorders early where disease-modification may be most successful, has motivated new biological classification and staging for Lewy body disorders (summarized in Panel 1). This important effort aims to define a prognostically relevant trajectory of disease based on the interrelationship between symptom onset, and biomarkers for LRP and neurodegenerative cell loss. The imminent implementation of moving to a biological disease definition brings evolving neuropathology into sharp clinical focus and necessitates a review of the data regarding the temporal ordering of these factors.

In this *Personal View* we aim to critically review and reconcile the clinical and biological evidence on the timing of pathologies in Lewy body disorders focusing on onset of symptoms, spread of LRP, and neuronal dysfunction and neurodegeneration, and discuss key factors impacting on clinical and pathological heterogeneity, such as age and co-pathology. We argue that these issues will be relevant for ongoing development and validation of any biological definition and staging of Lewy body disorders and highlight important knowledge gaps.

Search strategy and selection criteria

Key references for this Personal View were identified through searches of PubMed and MEDLINE between January 1, 1995, and July 31, 2024. Emphasis was given to studies published after January 1, 2019. Search terms included "synuclein," "Lewy pathology," "Lewy bodies," "Lewy neurites," "Parkinson's disease," "dementia with Lewy bodies," and "REM sleep behavior disorder," targeting full-length articles. Additional references were sourced from relevant papers. The final reference list was curated based on the relevance of the studies to the topics discussed in this Personal View.

When does clinical disease begin?

Most consider this question from the perspective of when a patient meets diagnostic criteria for a disorder. In the case of PD and DLB, clinical diagnostic criteria (Table 1) have been updated and optimized for clinical specificity through a reciprocal benchmarking process centred around neuropathological confirmation of the presence of LRP and SN dopaminergic cell loss. This process led to clinicopathological based diagnoses focused on the core syndromic presentations, namely motor parkinsonism linked to dopaminergic degeneration in PD, and dementia associated with cortical pathologies in DLB. In addition to these, other (primarily non-motor) signs and symptoms have gained recognition as integral features of Lewy body disorders and may be potentially more informative regarding pathophysiological progression. Onset of clinical features in Lewy body disorders is complicated by the significant heterogeneity in the range of expression, timing and onset of motor and non-motor symptoms between patients. This is epitomized by the fact that the two prototypical (PD and DLB) and more newly recognised expressions of Lewy body disease (isolated RBD; iRBD) are primarily distinguished by the relative timing of the cardinal features (RBD, parkinsonism and dementia). Here we focus on the timing of these syndromes and summarize recent efforts to understand the variability of their presentation.

Onset of isolated rapid eye movement sleep disorder (iRBD)

iRBD has come into prominence as a Lewy body syndrome which can precede the onset of parkinsonism and dementia by years, with many eventually converting to PD or DLB (and a small fraction to multiple system atrophy).[1] RBD is a parasomnia characterized by a spectrum of dream enactment behaviours associated with loss of the normal atonia of REM sleep (Table 1). A recent meta-analysis has shown that approximately 75% of iRBD patients test positive on abnormal α-synuclein assays in CSF and peripheral tissues.[2] The onset of iRBD occurs on average around age 60-65 years, with diagnosis around age 65-70 years although this can vary significantly (standard deviations of up to 10 years). Single centre studies with long follow up and high conversion rates (suggesting a cohort enriched for synucleinopathy) find an age of onset of iRBD around 62 years and an age of diagnosis around 69 years.[3] Estimates of disease onset are contaminated by recall bias with lack of polysomnographic validation and inclusion of patients with younger onset iRBD (below 50 years) which may be less likely to reflect an underlying synucleinopathy.[4] This is concordant with recent work suggesting a bimodal age of onset including early (median: 58 years, range: 38–64) and late (median: 73 years, range: 65–82), with the latter more likely having a neurodegenerative aetiology.

Mild parkinsonism occurs in iRBD prior to meeting diagnostic criteria for other Lewy body disorders. Large studies of iRBD show the mean and median intervals between baseline evaluation and conversion were 4.6 years and 8 years respectively (mean age at baseline of 65-68 years).[5,6] Motor abnormalities based on clinical examination or quantitative testing were present 5-8 years before conversion.[6] iRBD symptom onset (rather than diagnosis) was estimated up to 14 years before conversion.[7] Patients with iRBD converting to DLB were more likely to have abnormal quantitative motor testing at baseline than those converting to PD. The timing of parkinsonian symptoms between those patients did not differ, with the pattern of conversion dictated more so by the timing of dementia onset. In summary, the present evidence suggests subjective and objective motor abnormalities appear between 5-15 years in iRBD patients before conversion to PD.

Cognitive impairment occurs in iRBD prior to conversion to other Lewy body disorders. In subjects with iRBD, deviation in attention and executive function scores were present up to 10 years prior to conversion, reaching the impaired range 4-6 years before conversion to DLB and 0-2 years prior to conversion to PD.[5,8,9] Interestingly, although performance across most cognitive domains were lower in DLB-converters than PD-converters, memory and

visuospatial function declined at a faster rate to DLB (beginning 4-6 years prior).[9] In summary, the present evidence suggests cognitive impairment appears around 5 years before conversion to DLB.

It remains unclear as to what precipitates conversion to PD or DLB in those with iRBD, but a recent study using CSF biomarkers have highlighted BBB alterations and AD-related co-pathology as significant predictors of conversion from iRBD to PD and DLB respectively.[10] Understanding the natural history of iRBD and its underlying pathologies at all stages will provide crucial information for early intervention to potentially more aggressive neurodegenerative conditions to come.

Onset of Parkinsonism

Clinical disease in PD has been historically defined by the onset of levodopa responsive bradykinesia, rigidity and / or classic resting tremor (Table 1). The timing of other features is usually anchored to the presence of these cardinal features. Diagnosis occurs with a median age of onset of 60 years and average age of 65, although with variation of decades across individuals and cohorts. Recent meta-analyses reveal that α-synuclein seed amplification assays are highly sensitive (90%) for differentiating PD from non-neurodegenerative neurological controls and other parkinsonian conditions but less specific (30-50%) for differentiating PD from the α-synucleinopathy multiple system atrophy.[11,12]

As seen in iRBD, the diagnosis of PD is preceded by a variable phase of gradually progressive subtle motor impairments that have yet to cross the diagnostic threshold. In iRBD, the evidence suggests subjective and objective motor abnormalities appear between 5-15 years in iRBD patients before conversion to PD. Large multicentre cohort studies of other older adults have found mild parkinsonian signs predict emerging PD 3-14 years prior to diagnosis,[13-16] with hypokinesia/bradykinesia present from 7.5 years before PD diagnosis and tremor present as early as 14 years before PD diagnosis (becoming significantly different from controls only in the last 6.1 years).[17] Rigidity and postural abnormalities seemed to develop later and became more frequent in PD at around 3 years. In summary, the present evidence suggests mild motor abnormalities appearing between 5-14 years before the clinical diagnosis of PD.

In many patients, RBD intervenes after the diagnosis of clinically significant motor symptoms in PD. Cohort studies with longitudinal polysomnography have found that around 25% of patients with a new diagnosis of PD meet criteria for RBD at the time of diagnosis, increasing

to 52% at 6 years of follow-up.[18-20] In those with RBD, the degree of REM sleep without atonia increases independently with age as well as disease duration. This suggests that the mechanisms of RBD may relate not only to disease-progression, but also biological factors associated with aging (discussed below).

Overt cognitive symptoms in the form of 'mild cognitive impairment' (MCI), in which objective impairments are not yet sufficient to significantly impair daily function, can occur concurrently with or after the diagnosis of parkinsonism in PD,[21] although MCI is also an unstable diagnosis. Whilst those with PD and MCI are at higher risk of converting to dementia, some remain in this state indefinitely, whilst a small proportion convert back to normal function. Prospective cohort studies have shown MCI to affect 20% of PD patients at baseline, increasing to 40-50% at 5 years.[22] Large community-based studies of older adults have also shown cognitive decline to precede incident parkinsonism and PD, sometimes by up to 8 years prior and arguing its role as a prodromal symptom of PD.[17,23-25] This has been substantiated by more recent studies in prodromal PD.[24] Across cognitive domains, executive and attentional dysfunction are particularly emphasized as being most affected earlier in the course but with some differences between studies.[26] In summary, the present evidence suggests cognitive impairment up to 8 years before conversion to PD.

Onset of dementia

The onset of 'dementia' is defined operationally as cognitive impairment severe enough to impair social, occupational or personal life. Dementia can occur as the primary presentation in the case of DLB (Table 1), or in the setting of pre-existing PD (termed PD dementia or PDD, Table 1). Historically, the two are distinguished by an arbitrary but practical threshold known as the '1-year rule' in which dementia preceding or occurring within one year after clinically significant parkinsonism is defined as DLB. Meta-analyses of α-synuclein biomarkers including the seed amplification assay and skin biopsy[27,28] finds a diagnostic sensitivity of 90% or greater for DLB, although a recent large clinical cohort found only 72% positivity.[29]

Recent revisions of the clinical diagnostic criteria for DLB have incorporated RBD and biomarkers with the aim of increasing the specificity of the diagnosis (Table 1). Typically, dementia in DLB and PDD develops around the age of 70,[30] with an age of diagnosis between 70-75 years[31] and a similar 3 year duration of cognitive symptoms. In the Sydney multicentre study 83% of surviving patients with PD developed dementia at 20 years and the mean time

from diagnosis to dementia was 10.9 years (standard deviation of 5.5 years), consistent with other large prospective studies. As with PD, attention has turned to MCI as a prodromal stage of DLB, formalized in recent criteria as MCI with Lewy bodies (MCI-LB, Table 1). One of the largest cohort studies of MCI-LB with a mean age 67.9 ± 6.1 and a disease duration of 4 years, saw half of the patients progressing to dementia at around 3 years.[32] In summary although there is significant heterogeneity, dementia typically occurs around 70-75 years of age, with a cognitive prodrome of around 6 years and MCI 3 years prior.

By definition, the onset of clinically significant parkinsonism in DLB (defined more leniently as having at least one of the cardinal features of bradykinesia, rigidity or tremor) can occur no earlier than 1 year prior to the onset of functionally impactful cognitive symptoms. As such, studies indexing core features in patients with DLB will typically find parkinsonism occurring concurrently with or after the diagnosis. One large cohort study found the median time from onset of cognitive symptoms to parkinsonism in DLB to be 1.9 years (IQR: 0-4 years) with 20-25% of patients having motor symptoms at the time of onset of cognitive symptoms.[31] In this cohort, the median age of onset of parkinsonism was 73 years (IQR: 67-78) which was 3 years later than cognitive symptom onset (70 years, IQR: 65-75). In a cohort of patients with MCI who converted to DLB, three quarters of patients had evidence of mild parkinsonian signs at baseline suggesting such features can be present at least 3 years prior to diagnosis.

Between 75-90% of patients with DLB will also have comorbid RBD, and as a core feature in the diagnostic criteria, RBD is common at the time of diagnosis. In a recent study examining the timing of onset of core symptoms in probable DLB, 76% had RBD at first visit with an average age of onset of RBD 65 years compared to average age of onset of cognitive symptoms at 70 years. Those with RBD tended to be younger at first visit, with a shift of onset of most clinical symptoms by 4 years compared to those without RBD. In another study from the same institute examining RBD onset found RBD was more likely to precede a diagnosis of DLB (78%) than PD (42%), with most remaining cases of incident RBD occurring in the first 5 years after onset of DLB.[33] Interestingly, when extrapolating the cumulative prevalence of RBD according to age interval rather than time to diagnosis in this study, one finds that the incidence of RBD symptoms is similar in each age group irrespective of whether they developed DLB or PD suggesting possibly a closer link between RBD and age, similar to the time of dementia and age as suggested by the previous studies reviewed above.

Onset of other 'non-motor' features

Lewy body disorders manifest a broader range of clinical features formally recognized in clinical and biological criteria for established and prodromal PD and DLB (Table 1 and Panel 2) but are also observed in the general older population, suggesting these features can occur due to a variety of pathologies. These features include but are not limited to olfactory disturbance (hyposmia), autonomic dysfunction, and neuroleptic sensitivity. Many of these symptoms can precede the onset of parkinsonism and dementia by years.[1,34,35] For instance, autonomic disturbance such as constipation and orthostatic hypotension have been interpreted to imply early pathological α-synuclein involvement of autonomic nerves innervating peripheral tissues (i.e. gastrointestinal tract, heart). Constipation is highly prevalent in older adults, affecting between 20-50% of people over the age of 65 and increasing to 75% of those in aged-care facilities.[36,37] In prospective studies, a duration between first onset of constipation to PD diagnosis is 12 years,[38,39] with medical records suggesting a possibly longer lead time of up to 20 years. In patients with PD, constipation increases the likelihood of having a positive α-synuclein seeding assay,[40] although there are no studies using this assay in the elderly with constipation that do not reach criteria for a Lewy body disease.

Orthostatic hypotension (OH) is also seen frequently in PD and DLB. OH is more prevalent with age, affecting 20-30% of older adults and can interact with other age-related comorbidities and medication use.[41] However, significant neurogenic OH without a clear secondary cause is a defining feature of 'pure autonomic failure' (PAF), an entity that is regarded as a predominantly peripheral but also central α-synucleinopathy, which may also be associated with other signs of autonomic impairment of bowel, bladder, sweat and sexual function.[42] Immunohistochemical detection of cutaneous phosphorylated α-synuclein from skin biopsies is positive in 100% and 96% of patients with PAF and the non-Lewy body α-synucleinopathy MSA. PAF has a median age of onset of 65 and over 11 years converts to PD (42%, mean onset at 75 years), DLB (35%, mean onset at 78 years) and MSA (23% mean onset at 65 years).[43] More extensive longitudinal phenotyping of symptoms in PAF cohorts, akin to iRBD, will help establish differences in temporal progression of pathology in Lewy body disorders arising from this route.

Early olfactory disturbance has been observed in most (but not all) patients leading to the olfactory bulb being proposed as an initial site of pathology in Lewy body disorders (Panel 3). Hyposmia is highly prevalent in older age and depending on the detection method, affects

around 25-50% of older individuals,[44] increasing with each decade of life after 60 years.[45] Olfactory impairment has been associated with increased risk of developing PD, and PD-MCI. In a cohort of community dwelling adults, hyposmia was present up to 4 years prior to the diagnosis of PD.[38] Hyposmia is a predictor of dopaminergic imaging abnormalities in older patients. [46] In a study of patients with iRBD, hyposmia was present 10 years prior to conversion in some individuals, with onset of symptoms estimated at 22 years.[6] However, these measures were extrapolated and based on the assumption of a linear trajectory of decline which does not consider other strong interactors such as age. In patients with PD or DLB, olfactory disturbance significantly increases the odds (18.3 times) of having a positive α-synuclein seeding assay.[40,47] However, there are no studies using this assay in the elderly with olfactory dysfunction that do not reach criteria for a Lewy body disease, and impaired olfactory function correlates with MCI and AD.[48-51] Beyond neurodegenerative causes, age-related olfactory disturbance may also arise from multiple processes including viral infection, head trauma, toxins and chronic rhinosinusitis. Distinguishing these different contributions will be important to understand the role of olfactory disturbance in the relative timing of pathologies in Lewy body disorders.

Although these age-related features have been purported to occur decades prior to diagnosis of Lewy body disease, statistically significant deviations from controls in prospective cohorts have been more conservative and are not too dissimilar to the onset of more core cognitive and motor symptoms preceding DLB and PD diagnoses. While there are likely to be significant interindividual differences in the onset of and presence of these symptoms, further considerations of which factors most relate to α-synuclein positive Lewy pathologies need more empirical and data-driven approaches.

Summary of when clinical disease begins (Figure 1)

All Lewy body disorders have overlapping core symptoms (Table 1), often manifesting in milder forms, but generally present for a large part of the disease when assessed systematically (Figure 1). The syndromes can be differentiated at any time by their dominant features (sleep, motor and cognitive dysfunction) and the degree of additional features impacting on progression has led to the concepts of subtypes (Panel 2). This is best demonstrated by the lack of a hierarchy of conversion from one Lewy body syndrome to another suggesting differences in biology. While some do progress from iRBD to PD and PDD, an equal number appear immune to certain Lewy body syndromes. DLB can occur without PD or iRBD as prodromes, and PD can occur without the prodrome of iRBD. This considerable variability suggests a

regional focus of neurodegeneration impacted by genetics and environment that underpins biological subtypes. While there is a general background of mild impairments that appear in all Lewy body diseases and may represent the dysfunctions of neuronal α-synuclein aggregations, the severity of other clinical features suggests additional focal pathologies associated with these dominant features.

When does 'neurodegeneration' begin?

Although there is a general perception that you lose neurons with age, this is not true when applying accurate methods for estimating neuronal numbers.[52] Neuronal loss in Lewy body disorders is therefore disease related. Neuronal loss in Lewy body disorders has been assessed within clinical syndromes, and unfortunately there are no large postmortem studies on iRBD, particularly any quantifying neuronal loss. However, there are *in vivo* studies which suggests loss of neural tissue using a variety of structural MRI metrics and molecular imaging markers.[53] Clinicopathological studies in Lewy body positive PD and DLB cohorts confirm that symptom manifestation in these Lewy body disorders is more closely related to cell loss and/or dysfunction than to Lewy body pathology *per se*.

Loss of nigral dopaminergic neurons

Up to 15% of SN dopaminergic neurons have LRP at any time with cell loss starting prior to LRP formation and no relationship between the severity of SN cell loss and the proportion of neurons with LRP.[54-56] The severity of SN cell loss and not the severity of SN LRP is directly related to clinical motor severity.[57,58] This data suggests cell loss and LRP in the SN are not necessarily biologically coupled, consistent with some genetic forms of PD having no/limited LRP. The loss of neuromelanin pigmented cells in the SN (especially cells in the ventrolateral tier) correlates most to the severity of akinesia and rigidity in early PD and is often used as an index of progression of neurodegeneration. Cross-sectional pathological studies estimate that around 50% of cells (30-70%) in this region are lost by the time of onset of motor symptoms in PD.[57,59-62] Regression analyses based on disease duration and SN dopaminergic cell counts shows neuronal loss precedes symptom onset by 5-10 years.[57,59,62] This is consistent with 5-6 years estimated onset of neuromelanin MRI signal reduction in the ventral SN prior to PD onset in longitudinally followed clinical PD and iRBD cohorts.[63] There have been less pathological studies of SN dopaminergic cell loss over the disease course in DLB cases, even though reduced dopaminergic imaging is a diagnostic feature for DLB (Table 1). Studies of end-stage DLB compared with end-stage PD show an average loss of SN dopamine neurons of around

50% in DLB compared with >70% in PD,[64,65] suggesting less impact on the dopaminergic system and/or a slower rate of progression. These studies show that the loss of neuromelanin pigmented neurons in the SN is progressive and correlates with motor severity and not SN LRP.

PET ligands for nigral dopamine terminal loss in the putamen have been used more for clinical studies as estimates are there is up to 80% loss of these terminals at the time of motor onset of PD.[66,67] Virtually complete loss of putaminal dopaminergic terminals is seen by 4 years after diagnosis at autopsy[60] and therefore has a more prolonged preclinical phase. PET imaging of dopamine terminals in the putamen estimates a premotor phase of 10-20 years.[63] Of interest, putaminal dopamine imaging in iRBD correlates linearly and continuously with metrics of reduced muscle activity (atonia) in REM[68] with ongoing reduction linked to conversion to PD.[5] Importantly, putaminal dopamine imaging deficits are not seen in 15% of patients with DLB at presentation or in 40% of prodromal MCI-LB, suggesting a different temporal and topographic pattern of cellular vulnerability.[69-71] Direct comparison of putaminal dopamine and SN neuromelanin imaging between early and intermediate stage DLB versus PD shows a large decrease in dopamine terminals in DLB over time with less loss of SN neurons compared with PD where the large loss of dopamine terminals is related to the loss of SN neurons.[72] Furthermore, recent longitudinal work shows a more gradual loss of putaminal binding ratios in MCI-LB[73] with an estimated time to become abnormal that exceeds the average time to convert to DLB.[74] These studies suggest significant SN cell loss is more likely to lead to clinical parkinsonism and that there is variable rates of putaminal dopaminergic denervation in Lewy body disorders, decoupling dopamine terminal and cell loss biologically.

Loss of locus coeruleus noradrenergic neurons

The neuromelanin pigmented noradrenergic locus coeruleus (LC) is a predeliction site for age-related pathologies, with LRP early in Lewy body disorders and neurofibrillary pathology early in AD.[75,76] But importantly, neuromelanin MRI shows no LC loss in cognitively intact elderly between 60-80 years, confirming loss of pigmented LC neurons is a sign of pathology.[77] In AD, LC hypopigmentation is considered a neurobiological correlate of sleep-wake dysregulation[78] and MRI lesion mapping in RBD also identifies the LC as pathologically involved in RBD,[79] confirming animal based RBD models.[80] In Lewy body disorders, up to 50% of LC noradrenergic neurons have LRP with increased severity of LRP in PDD with RBD.[81] The severity of pigmented noradrenergic LC cell loss is highly variable in pathological

studies and has been related to a variety of clinical features,[82] consistent with its known wide ranging impact in regulating behaviour.[83] Hypopigmentation of the LC can occur without frank cell loss as pathological inclusions (neurofibrillary tangles in many, Lewy bodies in Lewy body disorders) significantly reduce pigment volume in LC neurons.

All studies on the LC in Lewy body disorders are cross-sectional and show that LC neurodegeneration occurs in a graded manner, with a stepwise reduction in LC neuronal counts, being most severe in DLB, followed by PDD, and least in PD.[84] Although there are no neuropathological cell loss studies of the LC in iRBD, neuromelanin MRI shows LC hypopigmentation in up to 80% of iRBD patients that is comparable to PD.[85,86] In asymptomatic older individuals, LC hypopigmentation correlates with cortical tau deposition and hippocampal volume loss.[87] Of all the brainstem aminergic nuclei, only cell loss in the LC correlates with cognitive decline, with higher densities of LC tangles and Lewy bodies and greater hypopigmentation over time associated with more rapid cognitive decline.[88,89] In PD, LC hypopigmentation using neuromelanin MRI is associated with both motor and non-motor symptoms, with reduction linked to RBD, cognitive decline and orthostatic hypotension.[90,91] Assessment of the timing of LC cell loss in relation to SN cell loss in PD without AD changes shows preclinical SN cell loss (see above), while LC cell loss begins around the time of PD motor diagnosis, and those with cognitive impairment have a more rapid loss of LC neurons.[62] While LC hypopigmentation occurs in iRBD to the same extent as PD,[86] the reduction may relate to severity of pathology rather than cell loss as LC cell loss in PD occurs at motor diagnosis,[62] and LC cell loss in those with cognitive decline starts at the onset of MCI diagnosis.[92]

Unlike the dopamine system discussed above where there is a correlation between the loss of nigrostriatal dopamine terminals and dopamine neurons, there is a disconnect between the loss of noradrenergic terminals and LC neurons,[93] making assessment of current noradrenergic PET ligands less predictive as a measure of the loss of this noradrenergic system. Of note, a recent metanalysis[94] showed considerable differences between CSF and PET biomarkers of noradrenaline dysfunction that require further research and correlative pathological studies.

Loss of basal forebrain cholinergic neurons

The cholinergic basal forebrain (chBF) neurons provide the cholinergic innervation for the human cerebral cortex and are well known to make neurofibrillary tangles then degenerate in

dementia.[95] The cholinergic system remains the main target of symptomatic therapy for early AD as well as PDD and DLB. Up to 40% of chNB neurons having LRP in Lewy body disorders,[62] and were the first neurons in which LRP was first identified by Friedrich Lewy[96].

Seminal work in post-mortem tissue demonstrated significant chBF cell loss in PD and DLB with more severe loss occurring in dementia stages.[96-101] Assessment of the timing of chBF cell loss in relation to SN cell loss in PD without AD changes shows preclinical SN cell loss (see above), while chBF cell loss begins around a decade after the onset of PD motor diagnosis in association with dementia.[62,102] There is more severe LRP in chBF neurons in PDD and greater loss chBF neurons in PDD and DLB than in AD, with no difference between Lewy body disorders with dementia.[102,103]

Like the noradrenergic system in Lewy body disorders, there is a disconnect between the loss of cholinergic markers and the degeneration of chBF neurons, making pathological assessment of cholinergic system *in vivo* less predictive. In postmortem studies, significantly reduced neocortical cholinergic markers (80-85%) occur in all PD patients even though there is limited chBF cell loss in the same cases.[102] However, these chBF neurons have significant LRP in these PD cases. *In vivo* cholinergic changes are seen in *de novo* PD patients without cognitive impairment[104] and iRBD,[105] being predictive of cognitive decline.[106-108] PD-MCI and PDD are distinguished from PD by a loss of cholinergic markers in the hippocampus and increased LRP in both the hippocampus and chBF neurons.[102,103] *In vivo* cholinergic PET and SPECT studies show a greater reduction of cortical cholinergic tracer binding which correlates with chBF volume loss in PD-MCI, PDD and DLB.[109] Limited longitudinal molecular imaging studies suggest a continuous pattern of chBF loss from prodromal to established DLB,[110] consistent with the start of degeneration in this system at the onset of cognitive decline.

Loss of hippocampal neurons

Only a few studies have assessed hippocampal neuronal loss, but a comparison of DLB versus PD shows selective neuronal loss of the presubiculum (projecting to frontal cortex) in DLB with preservation of the hippocampus in PD despite hippocampal LRP in both.[111] This is consistent with volumetric MRI studies showing hippocampal and parahippocampal gyrus atrophy in DLB and not PD with atrophy in DLB associated with ß-amyloid deposition,[112,113] The degree of hippocampal atrophy is similar in MCI-LB versus MCI due to AD,[114] although the subregions involved differ – CA and adjacent subiculum degenerates in AD and not DLB,

whereas the presubiculum in the parahippocampus and the perirhinal cortex degenerates in DLB.[115]

Other degenerative changes

Converging data from pathological and imaging studies suggest that synaptic and axonal loss likely precedes cellular loss.[116,117] A range of white matter and grey matter abnormalities are visualized in all Lewy body diseases, but the degree of abnormalities observed is far less and more variable compared to other neurodegenerative disorders where structural imaging has more diagnostic value. Standardizing these changes across studies and defining onset points prior to clinical diagnosis has not been performed across Lewy body disorders, and not compared to other neurodegenerative diseases with overlapping clinical features.

Lewy body deposition – subtypes and staging

LRP is observed at onset and in various neuron types to various degrees in Lewy body disorders[76] with multiple lines of evidence showing that α-synuclein (and other age-related aggregating proteins) can propagate to neighbouring cells,[118,119] a concept leveraged to develop α-synuclein seeding assays.[120] Importantly, neuronal loss and LRP in general are unrelated in all animal and human studies with the spread of α-synuclein Lewy pathologies requiring neurons to be intact.[121] This has important implications that separate the neurodegeneration described above from the spread of α-synuclein pathology. It is likely that α-synuclein pathology leads to dysfunction and its spread contributes to disease progression. This has been captured in a number of staging systems for PD and DLB (Panel 3 and Figure 2).

Few studies have assessed the temporal relationship between symptoms and regional LRP. The longitudinal Sydney multicentre study of PD followed participants with PD to post-mortem over a period of 20 years assessing and stratifying participants at time of death according to duration of disease.[122] Three main patterns of pathological progression were identified (Figure 2): 1) a proportion of patients had early cognitive symptoms representing a DLB syndrome and all had prominent diffuse neocortical Lewy pathology at death (average disease duration of 5 years), 2) PD cases with an average onset of 60 years and a slow pathological progression which have the LRP pattern predicted by Braak staging depending on the duration of their disease, and 3) an older onset PD progressing to dementia within 5-10 years with limbic and neocortical LRP and the time to dementia and survival negatively modified by a higher AD burden. In the typical PD cases, the average time between brainstem and limbic Lewy

pathology was 13 years with neocortical pathology occurring on average 5 years later. A more recent neuropathological study[123] validating three data-driven clinical subtypes of PD[124] found three similar subtypes: 1) a slowly progressing mild-motor group starting at 58 (SD 12) years, 2) an intermediate group starting at 65 (SD 10) years and 3) the fast-progressing diffuse malignant group starting at 70 (SD 6) years. As identified in the Sydney Multicentre Study,[125] the subtypes develop dementia at similar mean ages (mild motor 72.5 years; intermediate, 73.2 years; and diffuse malignant, 73.8 years respectively). Recent machine learning based data-driven modelling applied also identified three similar spatio-temporal patterns of LRP progression with the slow progressing subtype starting with LRP in the LC (Figure 2).[126] The concept of two PD phenotypes and a DLB phenotype at autopsy is consistent with the concept of pathological subtypes of PD with a\ more limbic/cortical driven 'brain-first' or brainstem focused 'body-first' hypothesis.[127,128]

In the last few years there have been a few studies comparing α-synuclein seeding assay positivity to disease staging in autopsy confirmed cohorts.[129-132] These have shown that sensitivity for the α-synuclein seeding assay is highest for diffuse (neocortical) and limbic LRP (98%) and lower in amygdala predominant (14-40%) and brainstem predominant (17%-40%) cases. One of the proposed reasons for these differences is that the seeding capability may reflect the 'virulence' or propensity of certain α-synuclein strains to spread regionally in the brain. Indeed, kinetics of the α-synuclein seeding assay has been shown to predict cognitive decline in PD,[133] and faster decline in cognitively impaired and unimpaired individuals from any cause.[134,135]

The importance of age and co-pathology in the expression and timing of symptoms

Co-pathology is frequently seen in cases of Lewy body disease at post-mortem, particularly amyloid plaques and neurofibrillary tangles (associated with AD).[136-139] In patients with preclinical, prodromal and established clinical AD, α-synuclein assay positivity was seen in 30% and associated with visuospatial deficits and additional behavioural disturbances including visual hallucinations. A recent comprehensive retrospective study of 1647 autopsied individuals found that clinical Lewy body disease was associated with the most heterogenous combination of comorbid neurodegenerative and age-related pathologies,[139] with more

pathologies with older age, increased disease durations and *APOE4* allele status. Age-associated AD pathology is most frequently seen in Lewy body disorders (Figure 2), observed in a third of PD cases and over 70% of cases with DLB.[140,141] AD pathology is greater on average in DLB than PDD[142] and influences the phenotypic expression of DLB (masking core features resulting in reduced diagnostic sensitivity).[143] Together, these findings suggest the need to consider age-associated co-pathologies within biological models and definitions of Lewy body disorders (Figure 2). α-Synuclein assay positivity may be simply indexing a secondary co-pathology, driven by another primary pathology – as is often seen in amygdala-predominant cases. Alternatively, disease staging and prognosis in α-synucleinopathy may be modified by comorbid pathology, which is more likely in older age groups. Whether co-pathology differentially affects the temporal progression of LRP and regional cell loss is unclear. The development and use of additional biomarkers, with topographic specificity and/or the ability to quantify total burden of all pathologies will be a vital need in future studies assessing the primacy of one detected neuropathology over another.

Key knowledge gaps and future studies

We have emphasized significant temporal heterogeneity in expression of cell loss and LRP across different vulnerable cell populations. Importantly, the variable timing of these pathologies appears to be influenced by age-related co-pathologies and reflect other undisclosed vulnerability and resistance factors. Over the last two decades, three broad subtypes of LRP pathology progression have been determined, which align imperfectly with clinical subtypes. Inherently missing from this body of work are subtypes incorporating cell loss (beyond only dopaminergic) and synaptic change. Recent advances in molecular and MRI imaging techniques have also enabled assessment of the noradrenergic and cholinergic cell groups among others, reflecting vulnerability of cellular populations that give rise to the variety of sleep, neuropsychiatric, autonomic and cognitive symptoms. Clinical deficits seem to more closely relate to cell loss rather than Lewy body spread alone. Future *in vivo* and post-mortem detailed characterization of cellular change beyond just the dopaminergic system in well phenotyped, longitudinally followed patients will be necessary to delineate a true biological staging scheme of these disorders. Studies assessing axonal integrity (white matter) as well as grey matter changes with MRI which can reflect synaptic loss or degeneration of neurons or glia will be an important complement to the above and may be more useful markers of earliest pathological change. In addition to more precisely determining a patient's subtype and

trajectory, determining the individual thresholds between biomarkers that demarcate synaptic dysfunction for the above systems compared to cellular loss will allow delineation of a window of opportunity for which disease-modifying therapies may be beneficial. To understand the true natural history of Lewy body disease, prospective cohorts should also examine RBD, pure autonomic failure, older-onset psychiatric disorders among others. Finally acknowledging and understanding the interaction of age and age-related pathologies which modify and influence the clinical expression of these will allow a more accurate and holistic assessment of a patient's trajectory, and potential response to therapies targeting other proteinopathies such as amyloid lowering therapies.

Implications for current biological staging systems

The implications of the above data for currently proposed 'biological' staging systems cannot be ignored and are represented in Figure 3. The main message is that multiple different brain regions and neuropathologies are involved in the different Lewy body disorders, which are broadly predictive of particular clinical features. Acknowledging the existence of current pathological 'subtypes' using additional biomarkers for known co-pathologies would seem a minimal effort. The order and timing of the progression of all the pathologies should be factored into any biological staging scheme. Based on current evidence, all pathologies present at highly variable times in the different Lewy body phenotypes (see Figure 3) with pathological distribution and severity potentiated by co-pathologies and increasing age. Validation of all neurodegenerative biomarkers (multiple proteins and cell loss in multiple regions) in Lewy body patients at different ages will go a long way towards refining biological staging and help to better delineate the expected timing that a patient will transition between different 'stages'. Until such multiple robust biomarkers become readily available, clinical clues such as the duration of symptoms and age of different symptom onset (minimum RBD, parkinsonism, dementia) may remain the best proxies for inferring large underlying biological changes and should not be ignored. But for full biological definitions we also need to consider different degrees of cell loss which may not only represent better clinical phenotypes but may also assist in identifying the type and timing of the pathogenic processes (e.g. oxidative stress, lysosomal and mitochondrial dysfunction etc) impacting these more devastating changes. Assessment of all these biologies at different stages of disease are necessary so that they can be targeted in a timely manner.[144] Ultimately, we contend that given the highly heterogenous and

multidimensional interaction between the timing of these biological processes a more complex conceptual framework of the biology is required.

Conflicts of Interest

The authors have no conflicts of interest to disclose.

References

1. Savica R, Boeve BF, Logroscino G. Epidemiology of alpha-synucleinopathies: from Parkinson disease to dementia with Lewy bodies. *Handb Clin Neurol* 2016; **138**: 153-8.
2. Iftikhar IH, AlShimemeri S, Rabah H, Rao ST, BaHammam AS. Alpha-synuclein pathology in isolated rapid eye movement sleep behaviour disorder: a meta-analysis. *Journal of sleep research* 2024: e14204.
3. Iranzo A, Fernández-Arcos A, Tolosa E, et al. Neurodegenerative disorder risk in idiopathic REM sleep behavior disorder: study in 174 patients. *PLoS One* 2014; **9**(2): e89741.
4. Matar E, McCarter SJ, St Louis EK, Lewis SJG. Current Concepts and Controversies in the Management of REM Sleep Behavior Disorder. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics* 2021; **18**(1): 107-23.
5. 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.
6. 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.
7. Schenck CH, Boeve BF, Mahowald MW. Delayed emergence of a parkinsonian disorder or dementia in 81% of older men initially diagnosed with idiopathic rapid eye movement sleep behavior disorder: a 16-year update on a previously reported series. *Sleep Med* 2013; **14**(8): 744-8.
8. 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.
9. Joza S, Hu MT, Jung KY, et al. Prodromal dementia with Lewy bodies in REM sleep behavior disorder: A multicenter study. *Alzheimers Dement* 2024; **20**(1): 91-102.

10. Fernandes M, Maio S, Eusebi P, et al. Cerebrospinal-fluid biomarkers for predicting phenoconversion in patients with isolated rapid-eye movement sleep behavior disorder. *Sleep* 2024; **47**(1).
11. Grossauer A, Hemicker G, Krismer F, et al. α-Synuclein Seed Amplification Assays in the Diagnosis of Synucleinopathies Using Cerebrospinal Fluid-A Systematic Review and Meta-Analysis. *Movement disorders clinical practice* 2023; **10**(5): 737-47.
12. Zheng Y, Li S, Yang C, Yu Z, Jiang Y, Feng T. Comparison of biospecimens for α-synuclein seed amplification assays in Parkinson's disease: A systematic review and network meta-analysis. *Eur J Neurol* 2023; **30**(12): 3949-67.
13. Lerche S, Seppi K, Behnke S, et al. Risk factors and prodromal markers and the development of Parkinson's disease. *J Neurol* 2014; **261**(1): 180-7.
14. Mahlknecht P, Gasperi A, Willeit P, et al. Prodromal Parkinson's disease as defined per MDS research criteria in the general elderly community. *Mov Disord* 2016; **31**(9): 1405-8.
15. Mahlknecht P, Stockner H, Marini K, et al. Midbrain hyperechogenicity, hyposmia, mild parkinsonian signs and risk for incident Parkinson's disease over 10 years: A prospective population-based study. *Parkinsonism Relat Disord* 2020; **70**: 51-4.
16. de Lau LM, Koudstaal PJ, Hofman A, Breteler MM. Subjective complaints precede Parkinson disease: the rotterdam study. *Arch Neurol* 2006; **63**(3): 362-5.
17. Darweesh SK, Verlinden VJ, Stricker BH, Hofman A, Koudstaal PJ, Ikram MA. Trajectories of prediagnostic functioning in Parkinson's disease. *Brain* 2017; **140**(2): 429-41.
18. Zimansky L, Muntean ML, Leha A, Mollenhauer B, Trenkwalder C, Sixel-Döring F. Incidence and Progression of Rapid Eye Movement Behavior Disorder in Early Parkinson's Disease. *Movement disorders clinical practice* 2021; **8**(4): 534-40.
19. Ferri R, Aricò 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).
20. Sixel-Döring F, Trautmann E, Mollenhauer B, Trenkwalder C. Rapid eye movement sleep behavioral events: a new marker for neurodegeneration in early Parkinson disease? *Sleep* 2014; **37**(3): 431-8.
21. Wood KL, Myall DJ, Livingston L, et al. Different PD-MCI criteria and risk of dementia in Parkinson's disease: 4-year longitudinal study. *NPJ Parkinson's disease* 2016; **2**: 15027.
22. Aarsland D, Batzu L, Halliday GM, et al. Parkinson disease-associated cognitive impairment. *Nature reviews Disease primers* 2021; **7**(1): 47.
23. Darweesh SKL, Wolters FJ, Postuma RB, et al. Association Between Poor Cognitive Functioning and Risk of Incident Parkinsonism: The Rotterdam Study. *JAMA Neurology* 2017; **74**(12): 1431-8.

24. Pan C, Li Y, Ren J, et al. Characterizing mild cognitive impairment in prodromal Parkinson's disease: A community-based study in China. *CNS Neurosci Ther* 2022; **28**(2): 259-68.
25. Foubert-Samier A, Helmer C, Le Goff M, et al. Cognitive and functional changes in prediagnostic phase of Parkinson disease: A population-based study. *Parkinsonism Relat Disord* 2020; **79**: 40-6.
26. Bock MA, Vittinghoff E, Bahorik AL, Leng Y, Fink H, Yaffe K. Cognitive and Functional Trajectories in Older Adults With Prediagnostic Parkinson Disease. *Neurology* 2023; **100**(13): e1386-e94.
27. Peña-Bautista C, Kumar R, Baquero M, et al. Misfolded alpha-synuclein detection by RT-QuIC in dementia with lewy bodies: a systematic review and meta-analysis. *Frontiers in molecular biosciences* 2023; **10**: 1193458.
28. Gibbons CH, Levine T, Adler C, et al. Skin Biopsy Detection of Phosphorylated α-Synuclein in Patients With Synucleinopathies. *Jama* 2024; **331**(15): 1298-306.
29. Brockmann K, Quadalti C, Lerche S, et al. Association between CSF alpha-synuclein seeding activity and genetic status in Parkinson's disease and dementia with Lewy bodies. *Acta Neuropathol Commun* 2021; **9**(1): 175.
30. Sim J, Li H, Hameed S, Ting SKS. Clinical Manifestations of Early-Onset Dementia With Lewy Bodies Compared With Late-Onset Dementia With Lewy Bodies and Early-Onset Alzheimer Disease. *JAMA Neurol* 2022; **79**(7): 702-9.
31. Choudhury P, Graff-Radford J, Aakre JA, et al. The temporal onset of the core features in dementia with Lewy bodies. *Alzheimers Dement* 2022; **18**(4): 591-601.
32. van de Beek M, van Steenoven I, Ramakers IHGB, et al. Trajectories and Determinants of Quality of Life in Dementia with Lewy Bodies and Alzheimer's Disease. *Journal of Alzheimer's Disease* 2019; **70**: 389-97.
33. Stang CD, Mullan AF, Hajeb M, et al. Timeline of Rapid Eye Movement Sleep Behavior Disorder in Overt Alpha-Synucleinopathies. *Ann Neurol* 2021; **89**(2): 293-303.
34. Per B, Jacob H, Katrine A, et al. Neuropathological evidence of body-first vs. brain-first Lewy body disease. *Neurobiology of Disease* 2021; **161**: 105557.
35. Braak H, Del Tredici K. Neuropathological Staging of Brain Pathology in Sporadic Parkinson's disease: Separating the Wheat from the Chaff. *J Parkinsons Dis* 2017; **7**(s1): S71-s85.
36. Kinnunen O. Study of constipation in a geriatric hospital, day hospital, old people's home and at home. *Aging (Milan, Italy)* 1991; **3**(2): 161-70.
37. Salari N, Ghasemianrad M, Ammari-Allahyari M, Rasoulpoor S, Shohaimi S, Mohammadi M. Global prevalence of constipation in older adults: a systematic review and meta-analysis. *Wiener klinische Wochenschrift* 2023; **135**(15-16): 389-98.

38. Ross GW, Petrovitch H, Abbott RD, et al. Association of olfactory dysfunction with risk for future Parkinson's disease. *Ann Neurol* 2008; **63**(2): 167-73.
39. Abbott RD, Petrovitch H, White LR, et al. Frequency of bowel movements and the future risk of Parkinson's disease. *Neurology* 2001; **57**(3): 456-62.
40. Venuto CS, Herbst K, Chahine LM, Kieburtz K. Predicting Cerebrospinal Fluid Alpha-Synuclein Seed Amplification Assay Status from Demographics and Clinical Data. *medRxiv : the preprint server for health sciences* 2024.
41. Ricci F, De Caterina R, Fedorowski A. Orthostatic Hypotension: Epidemiology, Prognosis, and Treatment. *Journal of the American College of Cardiology* 2015; **66**(7): 848-60.
42. Coon EA, Singer W, Low PA. Pure Autonomic Failure. *Mayo Clinic proceedings* 2019; **94**(10): 2087-98.
43. Millar Vernetti P, Norcliffe-Kaufmann L, Palma J-A, et al. Phenoconversion in pure autonomic failure: a multicentre prospective longitudinal cohort study. *Brain* 2024; **147**(7): 2440-8.
44. Yang J, Pinto JM. The Epidemiology of Olfactory Disorders. *Current otorhinolaryngology reports* 2016; **4**(2): 130-41.
45. Karpa MJ, Gopinath B, Rochtchina E, et al. Prevalence and neurodegenerative or other associations with olfactory impairment in an older community. *Journal of aging and health* 2010; **22**(2): 154-68.
46. Jennings D, Siderowf A, Stern M, et al. Imaging prodromal Parkinson disease: the Parkinson Associated Risk Syndrome Study. *Neurology* 2014; **83**(19): 1739-46.
47. Coughlin DG, MacLeod KR, Middleton JS, et al. Association of CSF α-Synuclein Seeding Amplification Assay Results With Clinical Features of Possible and Probable Dementia With Lewy Bodies. *Neurology* 2024; **103**(3): e209656.
48. Roberts RO, Christianson TJ, Kremers WK, et al. Association Between Olfactory Dysfunction and Amnestic Mild Cognitive Impairment and Alzheimer Disease Dementia. *JAMA Neurol* 2016; **73**(1): 93-101.
49. Wilson RS, Arnold SE, Schneider JA, Tang Y, Bennett DA. The relationship between cerebral Alzheimer's disease pathology and odour identification in old age. *J Neurol Neurosurg Psychiatry* 2007; **78**(1): 30-5.
50. Beach TG, Adler CH, Zhang N, et al. Severe hyposmia distinguishes neuropathologically confirmed dementia with Lewy bodies from Alzheimer's disease dementia. *PLoS One* 2020; **15**(4): e0231720.
51. Thomas AJ, Hamilton CA, Barker S, et al. Olfactory impairment in mild cognitive impairment with Lewy bodies and Alzheimer's disease. *Int Psychogeriatr* 2021: 1-8.
52. von Bartheld CS. Myths and truths about the cellular composition of the human brain: A review of influential concepts. *J Chem Neuroanat* 2018; **93**: 2-15.

53. Grimaldi S, Guye M, Bianciardi M, Eusebio A. Brain MRI Biomarkers in Isolated Rapid Eye Movement Sleep Behavior Disorder: Where Are We? A Systematic Review. *Brain sciences* 2023; **13**(10).
54. Milber JM, Noorigian JV, Morley JF, et al. Lewy pathology is not the first sign of degeneration in vulnerable neurons in Parkinson disease. *Neurology* 2012; **79**(24): 2307-14.
55. Parkkinen L, O'Sullivan SS, Collins C, et al. Disentangling the relationship between lewy bodies and nigral neuronal loss in Parkinson's disease. *J Parkinsons Dis* 2011; **1**(3): 277-86.
56. Greffard S, Verny M, Bonnet AM, Seilhean D, Hauw JJ, Duyckaerts C. A stable proportion of Lewy body bearing neurons in the substantia nigra suggests a model in which the Lewy body causes neuronal death. *Neurobiol Aging* 2010; **31**(1): 99-103.
57. Greffard S, Verny M, Bonnet AM, et al. Motor score of the Unified Parkinson Disease Rating Scale as a good predictor of Lewy body-associated neuronal loss in the substantia nigra. *Arch Neurol* 2006; **63**(4): 584-8.
58. Buchman AS, Shulman JM, Nag S, et al. Nigral pathology and parkinsonian signs in elders without Parkinson disease. *Ann Neurol* 2012; **71**(2): 258-66.
59. Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain* 1991; **114 (Pt 5)**: 2283-301.
60. Kordower JH, Olanow CW, Dodiya HB, et al. Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. *Brain* 2013; **136**(Pt 8): 2419-31.
61. Ma SY, Röyttä M, Rinne JO, Collan Y, Rinne UK. Correlation between neuromorphometry in the substantia nigra and clinical features in Parkinson's disease using disector counts. *J Neurol Sci* 1997; **151**(1): 83-7.
62. Huynh B, Fu Y, Kirik D, Shine JM, Halliday GM. Comparison of Locus Coeruleus Pathology with Nigral and Forebrain Pathology in Parkinson's Disease. *Mov Disord* 2021; **36**(9): 2085-93.
63. Biondetti E, Santin MD, Valabrègue R, et al. The spatiotemporal changes in dopamine, neuromelanin and iron characterizing Parkinson's disease. *Brain* 2021; **144**(10): 3114-25.
64. Piggott MA, Marshall EF, Thomas N, et al. Striatal dopaminergic markers in dementia with Lewy bodies, Alzheimer's and Parkinson's diseases: rostrocaudal distribution. *Brain* 1999; **122 (Pt 8)**: 1449-68.
65. Piggott MA, Perry EK, Marshall EF, et al. Nigrostriatal dopaminergic activities in dementia with Lewy bodies in relation to neuroleptic sensitivity: comparisons with Parkinson's disease. *Biol Psychiatry* 1998; **44**(8): 765-74.
66. Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K, Seitelberger F. Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. *J Neurol Sci* 1973; **20**(4): 415-55.

67. Scherman D, Desnos C, Darchen F, Pollak P, Javoy-Agid F, Agid Y. Striatal dopamine deficiency in Parkinson's disease: role of aging. *Ann Neurol* 1989; **26**(4): 551-7.
68. Zoetmulder M, Nikolic M, Biernat H, Korbo L, Friberg L, Jennum P. Increased Motor Activity During REM Sleep Is Linked with Dopamine Function in Idiopathic REM Sleep Behavior Disorder and Parkinson Disease. *J Clin Sleep Med* 2016; **12**(6): 895-903.
69. McKeith I, O'Brien J, Walker Z, et al. Sensitivity and specificity of dopamine transporter imaging with 123I-FP-CIT SPECT in dementia with Lewy bodies: a phase III, multicentre study. *Lancet Neurol* 2007; **6**(4): 305-13.
70. van der Zande JJ, Booij J, Scheltens P, Raijmakers PG, Lemstra AW. [(123)]FP-CIT SPECT scans initially rated as normal became abnormal over time in patients with probable dementia with Lewy bodies. *Eur J Nucl Med Mol Imaging* 2016; **43**(6): 1060-6.
71. Thomas AJ, Donaghy P, Roberts G, et al. Diagnostic accuracy of dopaminergic imaging in prodromal dementia with Lewy bodies. *Psychol Med* 2019; **49**(3): 396-402.
72. Okitsu M, Sugaya K, Nakata Y, et al. Degeneration of nigrostriatal dopaminergic neurons in the early to intermediate stage of dementia with Lewy bodies and Parkinson's disease. *J Neurol Sci* 2023; **449**: 120660.
73. Durcan R, Roberts G, Hamilton CA, et al. Serial Nigrostriatal Dopaminergic Imaging in Mild Cognitive Impairment With Lewy Bodies, Alzheimer Disease, and Age-Matched Controls. *Neurology* 2023; **101**(12): e1196-e205.
74. Hamilton CA, Matthews FE, Donaghy PC, et al. Progression to Dementia in Mild Cognitive Impairment With Lewy Bodies or Alzheimer Disease. *Neurology* 2021; **96**(22): e2685-e93.
75. Braak H, Thal DR, Ghebremedhin E, Del Tredici K. Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. *J Neuropathol Exp Neurol* 2011; **70**(11): 960-9.
76. Attems J, Toledo JB, Walker L, et al. Neuropathological consensus criteria for the evaluation of Lewy pathology in post-mortem brains: a multi-centre study. *Acta Neuropathol* 2021; **141**(2): 159-72.
77. Giorgi FS, Lombardo F, Galgani A, et al. Locus Coeruleus magnetic resonance imaging in cognitively intact elderly subjects. *Brain Imaging Behav* 2022; **16**(3): 1077-87.
78. Van Egroo M, van Someren EJW, Grinberg LT, Bennett DA, Jacobs HIL. Associations of 24-Hour Rest-Activity Rhythm Fragmentation, Cognitive Decline, and Postmortem Locus Coeruleus Hypopigmentation in Alzheimer's Disease. *Ann Neurol* 2024; **95**(4): 653-64.
79. Yuan T, Zuo Z, Xu J. Neuroanatomical Localization of Rapid Eye Movement Sleep Behavior Disorder in Human Brain Using Lesion Network Mapping. *Korean journal of radiology* 2023; **24**(3): 247-58.

80. Boeve BF, Silber MH, Saper CB, et al. Pathophysiology of REM sleep behaviour disorder and relevance to neurodegenerative disease. *Brain* 2007; **130**(Pt 11): 2770-88.
81. Dugger BN, Murray ME, Boeve BF, et al. Neuropathological analysis of brainstem cholinergic and catecholaminergic nuclei in relation to rapid eye movement (REM) sleep behaviour disorder. *Neuropathol Appl Neurobiol* 2012; **38**(2): 142-52.
82. Kalaitzakis ME, Gentleman SM, Pearce RK. Disturbed sleep in Parkinson's disease: anatomical and pathological correlates. *Neuropathol Appl Neurobiol* 2013; **39**(6): 644-53.
83. Poe GR, Foote S, Eschenko O, et al. Locus coeruleus: a new look at the blue spot. *Nat Rev Neurosci* 2020; **21**(11): 644-59.
84. Tilley BS, Patel SR, Goldfinger MH, Pearce RKB, Gentleman SM. Locus Coeruleus Pathology Indicates a Continuum of Lewy Body Dementia. *J Parkinsons Dis* 2021; **11**(4): 1641-50.
85. Ehrminger M, Latimier A, Pyatigorskaya N, et al. The coeruleus/subcoeruleus complex in idiopathic rapid eye movement sleep behaviour disorder. *Brain* 2016; **139**(Pt 4): 1180-8.
86. 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.
87. Engels-Domínguez N, Riphagen JM, Van Egroo M, et al. Lower Locus Coeruleus Integrity Signals Elevated Entorhinal Tau and Clinical Progression in Asymptomatic Older Individuals. *Ann Neurol* 2024.
88. Kelly SC, He B, Perez SE, Ginsberg SD, Mufson EJ, Counts SE. Locus coeruleus cellular and molecular pathology during the progression of Alzheimer's disease. *Acta Neuropathologica Communications* 2017; **5**(1): 8.
89. Bueichekú E, Diez I, Kim CM, et al. Spatiotemporal patterns of locus coeruleus integrity predict cortical tau and cognition. *Nature aging* 2024; **4**(5): 625-37.
90. Sommerauer M, Fedorova TD, Hansen AK, et al. Evaluation of the noradrenergic system in Parkinson's disease: an 11C-MeNER PET and neuromelanin MRI study. *Brain* 2018; **141**(2): 496-504.
91. Laurencin C, Lancelot S, Brosse S, et al. Noradrenergic alterations in Parkinson's disease: a combined 11C-yohimbine PET/neuromelanin MRI study. *Brain* 2024; **147**(4): 1377-88.
92. Wilson RS, Nag S, Boyle PA, et al. Neural reserve, neuronal density in the locus ceruleus, and cognitive decline. *Neurology* 2013; **80**(13): 1202-8.
93. Doppler CEJ, Kinnerup MB, Brune C, et al. Regional locus coeruleus degeneration is uncoupled from noradrenergic terminal loss in Parkinson's disease. *Brain* 2021; **144**(9): 2732-44.
94. Lancini E, Haag L, Bartl F, et al. Cerebrospinal fluid and positron-emission tomography biomarkers for noradrenergic dysfunction in neurodegenerative diseases: a systematic review and meta-analysis. *Brain communications* 2023; **5**(3): fcad085.

95. Giacobini E, Cuello AC, Fisher A. Reimagining cholinergic therapy for Alzheimer's disease. *Brain* 2022; **145**(7): 2250-75.
96. Liu AK, Chang RC, Pearce RK, Gentleman SM. Nucleus basalis of Meynert revisited: anatomy, history and differential involvement in Alzheimer's and Parkinson's disease. *Acta Neuropathol* 2015; **129**(4): 527-40.
97. Whitehouse PJ, Hedreen JC, White CL, 3rd, Price DL. Basal forebrain neurons in the dementia of Parkinson disease. *Ann Neurol* 1983; **13**(3): 243-8.
98. Perry EK, Curtis M, Dick DJ, et al. Cholinergic correlates of cognitive impairment in Parkinson's disease: comparisons with Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 1985; **48**(5): 413-21.
99. Perry EK, Haroutunian V, Davis KL, et al. Neocortical cholinergic activities differentiate Lewy body dementia from classical Alzheimer's disease. *Neuroreport* 1994; **5**(7): 747-9.
100. Perry EK, Irving D, Kerwin JM, et al. Cholinergic transmitter and neurotrophic activities in Lewy body dementia: similarity to Parkinson's and distinction from Alzheimer disease. *Alzheimer Dis Assoc Disord* 1993; **7**(2): 69-79.
101. Ruberg M, Ploska A, Javoy-Agid F, Agid Y. Muscarinic binding and choline acetyltransferase activity in Parkinsonian subjects with reference to dementia. *Brain Res* 1982; **232**(1): 129-39.
102. Hall H, Reyes S, Landeck N, et al. Hippocampal Lewy pathology and cholinergic dysfunction are associated with dementia in Parkinson's disease. *Brain* 2014; **137**(Pt 9): 2493-508.
103. Liu AKL, Chau TW, Lim EJ, et al. Hippocampal CA2 Lewy pathology is associated with cholinergic degeneration in Parkinson's disease with cognitive decline. *Acta Neuropathol Commun* 2019; **7**(1): 61.
104. van der Zee S, Kanel P, Gerritsen MJJ, et al. Altered Cholinergic Innervation in De Novo Parkinson's Disease with and Without Cognitive Impairment. *Mov Disord* 2022; **37**(4): 713-23.
105. Kantarci K, Nedelska Z, Chen Q, et al. Longitudinal atrophy in prodromal dementia with Lewy bodies points to cholinergic degeneration. *Brain communications* 2022; **4**(2): fcac013.
106. Ray NJ, Kanel P, Bohnen NI. Atrophy of the Cholinergic Basal Forebrain can Detect Presynaptic Cholinergic Loss in Parkinson's Disease. *Ann Neurol* 2023; **93**(5): 991-8.
107. Ray NJ, Bradburn S, Murgatroyd C, et al. In vivo cholinergic basal forebrain atrophy predicts cognitive decline in de novo Parkinson's disease. *Brain* 2018; **141**(1): 165-76.
108. Tan C, Nawaz H, Lageman SK, et al. Cholinergic Nucleus 4 Degeneration and Cognitive Impairment in Isolated Rapid Eye Movement Sleep Behavior Disorder. *Mov Disord* 2023; **38**(3): 474-9.
109. Okkels N, Horsager J, Labrador-Espinosa M, et al. Severe cholinergic terminal loss in newly diagnosed dementia with Lewy bodies. *Brain* 2023; **146**(9): 3690-704.

110. Staer K, Iranzo A, Terkelsen MH, et al. Progression of brain cholinergic dysfunction in patients with isolated rapid eye movement sleep behavior disorder. *Eur J Neurol* 2023.
111. Harding AJ, Lakay B, Halliday GM. Selective hippocampal neuron loss in dementia with Lewy bodies. *Ann Neurol* 2002; **51**(1): 125-8.
112. de Schipper LJ, Hafkemeijer A, van der Grond J, Marinus J, Henselmans JML, van Hilten JJ. Regional Structural Hippocampal Differences Between Dementia with Lewy Bodies and Parkinson's Disease. *J Parkinsons Dis* 2019; **9**(4): 775-83.
113. Mak E, Donaghy PC, McKiernan E, et al. Beta amyloid deposition maps onto hippocampal and subiculum atrophy in dementia with Lewy bodies. *Neurobiol Aging* 2019; **73**: 74-81.
114. Firbank MJ, Durcan R, O'Brien JT, et al. Hippocampal and insula volume in mild cognitive impairment with Lewy bodies. *Parkinsonism Relat Disord* 2021; **86**: 27-33.
115. Delli Pizzi S, Franciotti R, Bubbico G, Thomas A, Onofrj M, Bonanni L. Atrophy of hippocampal subfields and adjacent extrahippocampal structures in dementia with Lewy bodies and Alzheimer's disease. *Neurobiol Aging* 2016; **40**: 103-9.
116. Uchihara T, Giasson BI. Propagation of alpha-synuclein pathology: hypotheses, discoveries, and yet unresolved questions from experimental and human brain studies. *Acta Neuropathol* 2016; **131**(1): 49-73.
117. Wong YC, Luk K, Purtell K, et al. Neuronal vulnerability in Parkinson disease: Should the focus be on axons and synaptic terminals? *Mov Disord* 2019; **34**(10): 1406-22.
118. Luk KC, Kehm V, Carroll J, et al. Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science (New York, NY)* 2012; **338**(6109): 949-53.
119. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nature medicine* 2008; **14**(5): 504-6.
120. Concha-Marambio L, Pritzkow S, Shahnawaz M, Farris CM, Soto C. Seed amplification assay for the detection of pathologic alpha-synuclein aggregates in cerebrospinal fluid. *Nature protocols* 2023; **18**(4): 1179-96.
121. Ulusoy A, Musgrove RE, Rusconi R, et al. Neuron-to-neuron α-synuclein propagation in vivo is independent of neuronal injury. *Acta Neuropathologica Communications* 2015; **3**(1): 13.
122. Halliday G, Hely M, Reid W, Morris J. The progression of pathology in longitudinally followed patients with Parkinson's disease. *Acta Neuropathol* 2008; **115**(4): 409-15.
123. De Pablo-Fernández E, Lees AJ, Holton JL, Warner TT. Prognosis and Neuropathologic Correlation of Clinical Subtypes of Parkinson Disease. *JAMA Neurol* 2019; **76**(4): 470-9.
124. Fereshtehnejad SM, Zeighami Y, Dagher A, Postuma RB. Clinical criteria for subtyping Parkinson's disease: biomarkers and longitudinal progression. *Brain* 2017; **140**(7): 1959-76.

125. Reid WG, Hely MA, Morris JG, Loy C, Halliday GM. Dementia in Parkinson's disease: a 20-year neuropsychological study (Sydney Multicentre Study). *J Neurol Neurosurg Psychiatry* 2011; **82**(9): 1033-7.
126. Mastenbroek SE, Vogel JW, Collij LE, et al. Disease progression modelling reveals heterogeneity in trajectories of Lewy-type α-synuclein pathology. *Nature communications* 2024; **15**(1): 5133.
127. Borghammer P. The brain-first vs. body-first model of Parkinson's disease with comparison to alternative models. *J Neural Transm (Vienna)* 2023; **130**(6): 737-53.
128. Rolinski M, Szewczyk-Krolikowski K, Tomlinson PR, et al. REM sleep behaviour disorder is associated with worse quality of life and other non-motor features in early Parkinson's disease. *J Neurol Neurosurg Psychiatry* 2014; **85**(5): 560-6.
129. Hall S, Orrù CD, Serrano GE, et al. Performance of αSynuclein RT-QuIC in relation to neuropathological staging of Lewy body disease. *Acta Neuropathol Commun* 2022; **10**(1): 90.
130. Arnold MR, Coughlin DG, Brumbach BH, et al. α-Synuclein Seed Amplification in CSF and Brain from Patients with Different Brain Distributions of Pathological α-Synuclein in the Context of Co-Pathology and Non-LBD Diagnoses. *Ann Neurol* 2022; **92**(4): 650-62.
131. Samudra N, Fischer DL, Lenio S, et al. Clinicopathological correlation of cerebrospinal fluid alpha-synuclein seed amplification assay in a behavioral neurology autopsy cohort. *Alzheimers Dement* 2024; **20**(5): 3334-41.
132. Levin J, Baiardi S, Quadalti C, et al. α-Synuclein seed amplification assay detects Lewy body co-pathology in autosomal dominant Alzheimer's disease late in the disease course and dependent on Lewy pathology burden. *Alzheimers Dement* 2024; **20**(6): 4351-65.
133. Brockmann K, Lerche S, Baiardi S, et al. CSF α-synuclein seed amplification kinetic profiles are associated with cognitive decline in Parkinson's disease. *NPJ Parkinson's disease* 2024; **10**(1): 24.
134. Quadalti C, Palmqvist S, Hall S, et al. Clinical effects of Lewy body pathology in cognitively impaired individuals. *Nature medicine* 2023; **29**(8): 1964-70.
135. Palmqvist S, Rossi M, Hall S, et al. Cognitive effects of Lewy body pathology in clinically unimpaired individuals. *Nature medicine* 2023; **29**(8): 1971-8.
136. Kovacs GG, Milenkovic I, Wöhrer A, et al. Non-Alzheimer neurodegenerative pathologies and their combinations are more frequent than commonly believed in the elderly brain: a community-based autopsy series. *Acta Neuropathol* 2013; **126**(3): 365-84.
137. Kovacs GG, Alafuzoff I, Al-Sarraj S, et al. Mixed brain pathologies in dementia: the BrainNet Europe consortium experience. *Dement Geriatr Cogn Disord* 2008; **26**(4): 343-50.
138. Jellinger KA, Attems J. Neuropathological evaluation of mixed dementia. *J Neurol Sci* 2007; **257**(1-2): 80-7.

139. Robinson JL, Xie SX, Baer DR, et al. Pathological combinations in neurodegenerative disease are heterogeneous and disease-associated. *Brain* 2023; **146**(6): 2557-69.
140. Irwin DJ, Grossman M, Weintraub D, et al. Neuropathological and genetic correlates of survival and dementia onset in synucleinopathies: a retrospective analysis. *Lancet Neurol* 2017; **16**(1): 55-65.
141. Irwin DJ, Hurtig HI. The Contribution of Tau, Amyloid-Beta and Alpha-Synuclein Pathology to Dementia in Lewy Body Disorders. *J Alzheimers Dis Parkinsonism* 2018; **8**(4): 444.
142. Jellinger KA, Korczyn AD. Are dementia with Lewy bodies and Parkinson's disease dementia the same disease? *BMC Med* 2018; **16**(1): 34.
143. Ferman TJ, Aoki N, Boeve BF, et al. Subtypes of dementia with Lewy bodies are associated with α-synuclein and tau distribution. *Neurology* 2020; **95**(2): e155-e65.
144. Espay AJ, Brundin P, Lang AE. Precision medicine for disease modification in Parkinson disease. *Nat Rev Neurol* 2017; **13**(2): 119-26.
145. Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord* 2015; **30**(12): 1591-601.
146. Emre M, Aarsland D, Brown R, et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Mov Disord* 2007; **22**(12): 1689-707; quiz 837.
147. McKeith IG, Boeve BF, Dickson DW, et al. Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium. *Neurology* 2017; **89**(1): 88-100.
148. Höglinger GU, Adler CH, Berg D, et al. A biological classification of Parkinson's disease: the SynNeurGe research diagnostic criteria. *Lancet Neurol* 2024; **23**(2): 191-204.
149. Simuni T, Chahine LM, Poston K, et al. A biological definition of neuronal α-synuclein disease: towards an integrated staging system for research. *Lancet Neurol* 2024; **23**(2): 178-90.
150. Mestre TA, Fereshtehnejad SM, Berg D, et al. Parkinson's Disease Subtypes: Critical Appraisal and Recommendations. *J Parkinsons Dis* 2021; **11**(2): 395-404.
151. Selikhova M, Williams DR, Kempster PA, Holton JL, Revesz T, Lees AJ. A clinico-pathological study of subtypes in Parkinson's disease. *Brain* 2009; **132**(Pt 11): 2947-57.
152. Morenas-Rodriguez E, Sala I, Subirana A, et al. Clinical Subtypes of Dementia with Lewy Bodies Based on the Initial Clinical Presentation. *J Alzheimers Dis* 2018; **64**(2): 505-13.
153. Mombelli S, Leitner C, D'Este G, et al. A data-driven approach to neuropsychological features in isolated REM behaviour disorder: A latent class analysis. *Journal of neuropsychology* 2023; **17**(1): 161-79.
154. Seger A, Ophey A, Doppler CEJ, et al. Clinical subtypes in patients with isolated REM sleep behaviour disorder. *NPJ Parkinson's disease* 2023; **9**(1): 155.
155. Leverenz JB, Hamilton R, Tsuang DW, et al. Empiric refinement of the pathologic assessment of Lewy-related pathology in the dementia patient. *Brain Pathol* 2008; **18**(2): 220-4.

156. Attems J, Walker L, Jellinger KA. Olfactory bulb involvement in neurodegenerative diseases. *Acta Neuropathol* 2014; **127**(4): 459-75.

157. Kalaitzakis ME, Graeber MB, Gentleman SM, Pearce RK. The dorsal motor nucleus of the vagus is not an obligatory trigger site of Parkinson's disease: a critical analysis of alpha-synuclein staging. *Neuropathol Appl Neurobiol* 2008; **34**(3): 284-95.

Table 1 Summaries of the clinical diagnostic criteria for the main Lewy-body associated conditions and their prodromes

Parkinson's disease[145] (Postuma et al., 2015)	**Parkinson's disease dementia[146] (Emre et al., 2007)**	**Dementia with Lewy bodies[147] (McKeith et al., 2017)**	**Isolated REM sleep behaviour disorder (AASM, 2023)**	**MCI-Lewy bodies (McKeith et al., 2020)**
MDS criteria for clinically established PD requires: Core features: Bradykinesia with at least one of rest tremor or rigidity At least 2 supportive features which may include: Clear and dramatic beneficial response to dopaminergic therapy; levodopa-induced dyskinesia; rest tremor; olfactory loss or cardiac sympathetic denervation on cardiac scintigraphy No exclusionary criteria or red flags which suggest an alternative diagnosis.	MDS criteria for probable PDD requires: Dementia syndrome occurring in the context of an established diagnosis of PD involving more than one cognitive domain and severe enough to impair daily life. Typical profile of cognitive deficits including impairment in at least two of the four core cognitive domains (attention, executive, visuo-spatial functions, and impaired free recall memory which usually improves with cueing) The presence of at least one of apathy, depressed or anxious mood, delusions, hallucinations,	Probable DLB requires: Dementia syndrome with cognitive impairment sufficient to impair daily life. At least two of four core clinical features: - Cognitive fluctuations - Visual Hallucinations - Motor parkinsonism (bradykinesia, rigidity or tremor) - REM sleep behaviour disorder *or* One core clinical feature and at least one indicative biomarker: - Reduced DAT uptake in basal ganglia demonstrated by SPECT or PET imaging. - PSG confirmation of REM sleep without atonia - Reduced uptake on ^{123}I-MIBG myocardial scintigraphy	As per the ICSD-3-TR, a diagnosis of RBD requires: Repeated episodes of sleep-related vocalization and/or complex motor behaviours Behaviours are documented by polysomnography to occur during REM sleep or, based on clinical history of dream enactment, are presumed to occur during REM sleep Loss of REM sleep atonia (REM sleep without atonia) on polysomnography 'Isolated' RBD requires a diagnosis of RBD that occurs in the absence of a clear secondary cause (e.g. meeting criteria for a concurrent neurological condition).	A diagnosis of probable MCI-LB requires both of: MCI defined as concern of cognitive decline in a patient with preserved or minimally affected functional independence and objective impairment of one or more cognitive domains Additionally, two or more core clinical features: - Cognitive fluctuations - Visual Hallucinations - Motor parkinsonism (bradykinesia, rigidity or tremor) - REM sleep behaviour disorder *or* One core clinical feature and at least one proposed biomarker: - Reduced DAT uptake in basal ganglia demonstrated by SPECT or PET imaging. - PSG confirmation of REM sleep without atonia

	excessive daytime somnolence. No features that suggest an alternative diagnosis, including an uncertain interval between motor and cognitive symptoms (including where dementia preceded or occurred within 1 year of PD diagnosis)	1 year rule: Dementia precedes or occurs within 1 year of the onset of motor symptoms. Possible DLB: Dementia as above with one core clinical feature alone or at least one biomarker without a core clinical feature.		- Reduced uptake on ^{123}I-MIBG myocardial scintigraphy Possible MCI-LB: MCI as above with one core clinical feature or at least one biomarker without a core clinical feature.

DAT, dopamine transporter; DLB, dementia with Lewy bodies; ICSD-3; International Classification of Sleep Disorders, 3rd Edition; MCI, mild cognitive impairment; MDS, Movement Disorders Society; MIBG, metaiodobenzylguanidine; PD, Parkinson's disease; PDD, Parkinson's disease dementia; PET, positron emission tomography; RBD, rapid eye movement sleep behaviour disorder; REM, rapid eye movement; SPECT, single photon emission computed tomography.

Table 2 Outstanding Research Questions and Proposed Directions

Knowledge Gap	Proposed research directions (example)
How do co-pathologies influence the timing and progression of symptoms?	*In vivo* assessment of the interaction of AD-related changes (e.g., amyloid and tau) and vascular pathologies with age and symptom manifestation in longitudinally followed Lewy body disorder cohorts. Correlate with post-mortem pathologies.
Can pathological staging and subtyping of Lewy body disorders be better informed by patterns of cellular loss?	Characterization of noradrenergic, cholinergic, and other less-studied cellular changes (loss) simultaneously in large autopsy cohorts
What are the regional influences on the timing and progression of pathologies in Lewy body disorders?	Longitudinal structural, functional and molecular imaging assessing different neurotransmitter systems to map regional progression of neurodegeneration
How does aging impact the timing, progression, and expression of Lewy body disorders?	Detailed characterization and record-keeping of the age of onset of different symptoms, examining the interaction of aging with biomarker timing and clinical progression across Lewy body disorders.
What is the temporal relationship between synaptic and axonal changes and cellular loss?	Application of novel synaptic markers and simultaneous assessment of white matter changes, grey matter atrophy and molecular imaging markers of neurotransmitter systems in longitudinally followed community-based and prodromal disease cohorts
What is the timing and impact of other pathologies, such as neuroinflammation and blood-brain barrier changes, on disease progression?	Assessment of other emerging plasma and imaging biomarkers assessing different pathophysiological processes in longitudinally followed cohorts to determine their importance across different timepoints and subtypes of Lewy body disease
What are the pathological correlates of early non-motor symptoms in Lewy body disorders?	Prospective studies with accurate measurement of non-motor symptoms in community based and prodromal

	cohorts alongside assessment of α-synuclein biomarkers, co-pathology, and multimodal imaging
Can we use biomarker to define optimal therapeutic windows for disease modification in Lewy body disorders?	Use complementary in vivo biomarkers (blood and imaging) with post-mortem validation to establish biomarker thresholds distinguishing synaptic dysfunction from cellular loss, thereby identifying intervention intervals.

Panels

Panel 1 A summary of new biological classification and staging schemes for Lewy body disorders

Motivated by the availability of α-synuclein based biomarkers, two new biologically anchored diagnostic schemas for Lewy body disorders were recently proposed for research purposes, namely the SynNeurGe classification and the Neuronal Synuclein Disease-Integrated Staging System (NSD-ISS)[148,149]. This represents an important and inevitable shift from a clinical to a biological definition of PD and DLB. Both systems are centred largely around the three same anchors of α-synuclein positivity (S), neurodegeneration or neural dysfunction (N) and genetic susceptibility (G). The NSD-ISS uniquely also includes an explicit staging system denoting biological and clinical progression, with the purpose of being adopted into clinical trials especially for early pre-clinical stages of PD and DLB. Clinical features are not mandated for a diagnosis, although are important for staging.

NSD-ISS

This definition introduces a new definition for 'Neuronal Synuclein Disease', for those who are deemed to have pathological α-synuclein-related disease (namely Lewy body disorders). Presence of a fully penetrant pathogenic mutation in *SNCA*, the gene that encodes α-synuclein, or confirmation of α-synuclein positivity (S+) using the CSF seed amplification assay are obligatory for a diagnosis of NSD. Once satisfying either of these, the second anchor denotes the presence of neurodegeneration (N+), which is defined solely by abnormal quantitative dopamine transporter imaging (namely, Ioflupane I-123 single photo emission computed tomography or SPECT). An integrated staging system is subsequently proposed based on these biological anchors and clinical markers assuming a common sequence of clinical and pathological progression across Lewy body phenotypes. Stage 0 is defined by the presence of fully penetrant pathogenic variants in the *SNCA* gene, stage 1A is S+ but N-, and stage 1B is S+ and N+, all without functional impairment. Stage 2 is defined by the presence of early or mild clinical signs or symptoms without functional impairment which can include motor changes, hyposmia, mild cognitive impairment, constipation, dysautonomia, depression and anxiety. Stage 2B requires S+ and N+ in addition to clinical features. Stages 3 to 6 are defined as both S+ and N+, with increasing severity of functional impairment from slight, to mild, moderate and severe. Anchors for clinical progression have yet to be fully proposed or validated at the time of writing.

SynNeurGe

The SynNeurGe is a biological classification (but also definition) of Parkinson's disease based on the three anchors above. The two main diagnostic categories are Parkinson's disease or Parkinson's type synucleinopathy' depending on the presence or absence of markers of neurodegeneration / neuronal dysfunction respectively. There is no explicit distinction between PD and DLB. It accounts for both sporadic and genetic forms of PD which are synucleinopathy based, as well as non-synucleinopathy based genetic PD (such as *Parkin* variant carriers). S+ on α-synuclein seed amplification assay on CSF or skin, as well as using skin immunohistochemistry or immunofluorescence is accepted. N+ neurodegeneration includes abnormal uptake using dopaminergic SPECT or positron emission tomography (PET), or the PD-related glucose metabolic pattern on 18-fluorodeoxyglucose (18-FDG PET), or cardiac sympathetic denervation detected using cardiac scintigraphy. A number of investigational biomarkers are also offered, with view to their inclusion once sufficient evidence is reached. A wider range of genetic variants are classified according to penetrance and permit pre-clinical diagnosis of PD without evidence of synucleinopathy or clinical features. An optional designation is provided for clinical signs across motor, olfactory, autonomic, sleep and cognitive domains as being probably or possibly related to the associated α-synucleinopathy. No explicit staging scheme is proposed, noting the need for further evidence.

Panel 2 Clinical heterogeneity and subtypes

There is considerable heterogeneity in the range of expression, timing and onset of motor and non-motor symptoms between individual patients with Lewy body disorders. This has fuelled extensive hypothesis- and data-driven subtyping efforts resulting in a range of classification schemes mostly stratified by presence of motor and non-motor features, in addition to genetic predisposition among other factors. Some of these subtypes have been shown to have potential biological and prognostic relevance and have been linked to different biomarkers and neuropathological correlates. However, there are inconsistencies across studies due to choice of subtyping methods, the type of study (cross-sectional or prospective), the baseline sample characteristics and the range of clinical and biological markers studied. Moreover, the accurate determination of onset of non-diagnostic features in addition to cardinal diagnostic features is lacking, and separating iRBD, PD and DLB diminishes the potential to ascertain the features that are indicative of the continuum of clinical subtypes known to occur within Lewy body disorders beyond sleep, motor and cognitive presentations. Of course, to be certain in any biologically underpinnings of clinical subtypes, pathological diagnostic confirmation is

critical, particularly given the higher rate of misdiagnosis of DLB. Retrospective determination of clinical subtypes derived from pathological subtypes may be more informative in this regard.

Clinical subtypes in Parkinson's disease

A recent systematic review of 38 PD subtyping studies identified critical shortcomings relating to the methodological quality, lack of prospective or external validation, and uncertainty regarding the stability of the subtypes.[150] Regardless, data-driven subtyping studies investigating large multicentre comprehensively phenotyped prospective cohorts of early or *de novo* PD have advantage over earlier, and hypothesis driven single variable (e.g. motor, age) subtyping techniques. These studies have converged on that findings that PD may be divided in at least two groups according to the speed of progression of cognitive and motor decline (progression). Based on clinical features alone, faster progressing PD patients tend to have at baseline a higher degree of olfactory disturbance, greater degree of motor and non-motor severity including presence of RBD, and greater degree of measurable cognitive impairment. In most studies, patients in the faster progressing cohort also tend to have a higher age at baseline. In studies combining clinical features with *in vivo* biomarkers, faster progressing patients tended to associate with greater structural atrophy, higher burden of AD co-pathology and striatal dopaminergic denervation. Neuropathological studies validating clinical subtypes has also demonstrated that faster progressing subtypes are associated with a higher burden of beta-amyloid, neurofibrillary tangle burden and neocortical Lewy body load.[122,151]

Clinical subtypes in dementia with Lewy bodies and isolated REM sleep behaviour disorder

Fewer data-driven clinical phenotyping studies exist in DLB and iRBD, and most are cross-sectional. One study using onset of core features to stratify patients found one group with a longer duration of symptoms to DLB diagnosis, an older age group that presented with psychotic features, and short time to diagnosis, as well as a group with early presentation of motor features.[152] One iRBD study using neuropsychological features identified three types on severity, with those moderately impaired having a high risk of conversion to another Lewy body disease and greater impairment in the visuospatial domain.[153] Another iRBD study found two subtypes using a greater diversity of measures, a late-onset aggressive subtype (higher non-motor symptom burden) and an early-onset benign subtype.[154] Again, these and similar studies demonstrate distinct variations in duration and age at onset of symptoms in DLB and iRBD patients akin to PD patients.

Panel 3

Braak Staging for PD

In this system (Figure 2), Lewy-related pathology (LRP) is posited to begin initially in the caudal medulla, specifically the dorsal motor nucleus of vagus (DMN) and adjoining intermediate zone as well as the olfactory nucleus (Stage 1). Subsequent stages see more rostral accumulation of LRP in structures including the caudal raphe nuclei, gigantocellular reticular nucleus and coeruleus-subcoeruleus complex (Stage 2), followed by midbrain including the substantia nigra pars compacta (SNc) and ventral tegmental area (Stage 3), then further involvement of amygdala (olfactory and basolateral portions), basal forebrain, tuberomammillary nucleus, intralaminar nuclei of the thalamus and temporal mesocortex (stage 4) and finally higher order association and primary neocortex (Stage 5-6). These stages were shown to correlate broadly with presence of PD symptoms, including presymptomatic stages (1-2), early symptomatic stages (3-4) with onset of motor symptoms, and late symptomatic stages (5-6) which often correlates with established PD and also cognitive involvement (especially stage 6).

McKeith criteria for DLB

The McKeith criteria for DLB pathology (Figure 2) - proposed in 1996 and refined over two decades - uses semi-quantitative scoring of LRP in prespecified regions, recognizing three main distributions including a brainstem predominant, limbic (transitional) and diffuse neocortical distribution. The update in 2017 included amygdala-predominant and olfactory bulb only categories. Unique to the McKeith system, the pattern of LRP is then combined with the degree of co-existing AD pathology, to determine the likelihood that the pathological findings are associated with a DLB syndrome. It should be noted that brainstem, amygdala and olfactory restricted cases are assigned with a low probability of a DLB syndrome. Unlike the Braak staging system, the temporal nature of the McKeith staging criteria is less explicit and is more suited to a classification scheme. Other systems and iterations of this criteria have been proposed[155,156] addressing limitations relating to the semi-quantitative nature of the scoring and improving inter-rater reliability.

Unified Staging System for Lewy body pathology

The Braak staging system has been particularly influential, but also subject to much criticism. In particular up to 42% of cases in some pathological series of Lewy body cases could not be classified according to the Braak staging scheme, as DLB cases were often included. Many such cases had

prominent involvement of the amygdala, often in the presence of AD co-pathology. However, rarer cases with midbrain and cortical involvement but without DMN involvement[157] have also been identified. The Unified Staging System proposed by Beach and colleagues (Figure 2) found that the olfactory nucleus is the most common 'isolated' site of pathology, and proposed that olfactory restricted LRP represented stage I. From here, two divergent stages (Stage IIa and IIb) reflecting brainstem or limbic predominant involvement. Thereafter both brainstem and limbic regions are expected to be involved (Stage III), followed finally by neocortical spread (stage IV). Under this system, cases of DLB with AD would be over-represented in the limbic predominant (amygdala) pathway, and patients need not have olfactory involvement to be classified as Stage II.

New data driven staging

Very recently, a machine learning-based disease progression model (Ordinal Subtyping Stage and Inference model) was applied to ten regional neuropathological Lewy body density scores obtained from 814 brain donors with LRP representing one of the most comprehensive data-driven staging schemes to date[126]. The cohort overlapped with those used to generate the Unified Staging System for Lewy body pathology, and the two staging schemes were highly aligned. The study found three inferred trajectories of LRP (S1, S2 and S3) characterized by differing clinicopathological diagnoses and progression. The S1 subtype (61% of donors) was limbic LRP dominated with early involvement of the olfactory bulb and tract, amygdala and transentorhinal cortex which had concentrated LRP. Most S1 cases also had AD with dementia. The S2 subtype (21%) also began in the olfactory bulb and tract, but unlike S1, was followed by medulla with similar progression to the Braak staging scheme. S2 had higher LRP load than the other subtypes, and more AD pathologically than the S3 subtype. The S3 subtype (18% of donors) differed by starting in the locus coeruleus and having more rapid spread of LRP that remained low in abundance. Nigral cell loss and motor impairment was more severe in S3 compared to the S2 brainstem subtype. Clinical DLB diagnosis was found in all subtypes and related most to AD and low LRP pathology. Unfortunately, data regarding age of onset or duration of any clinical diagnosis was not included, precluding subtype comparisons based with respect to these variables.

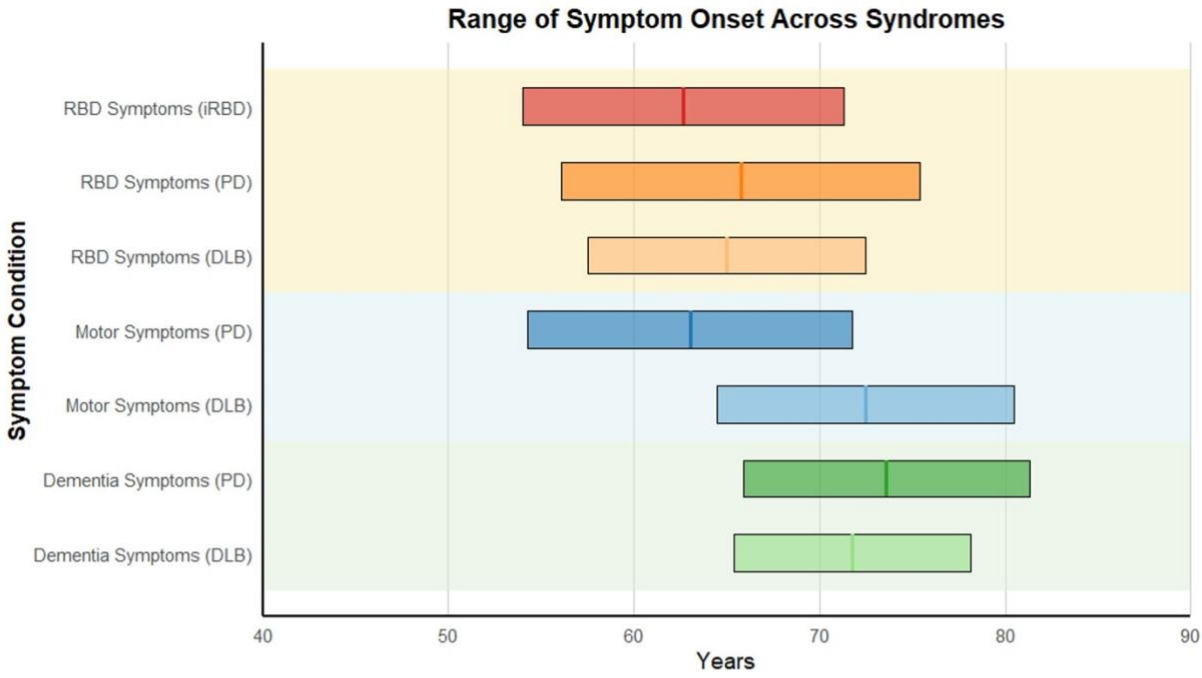
Figure Captions

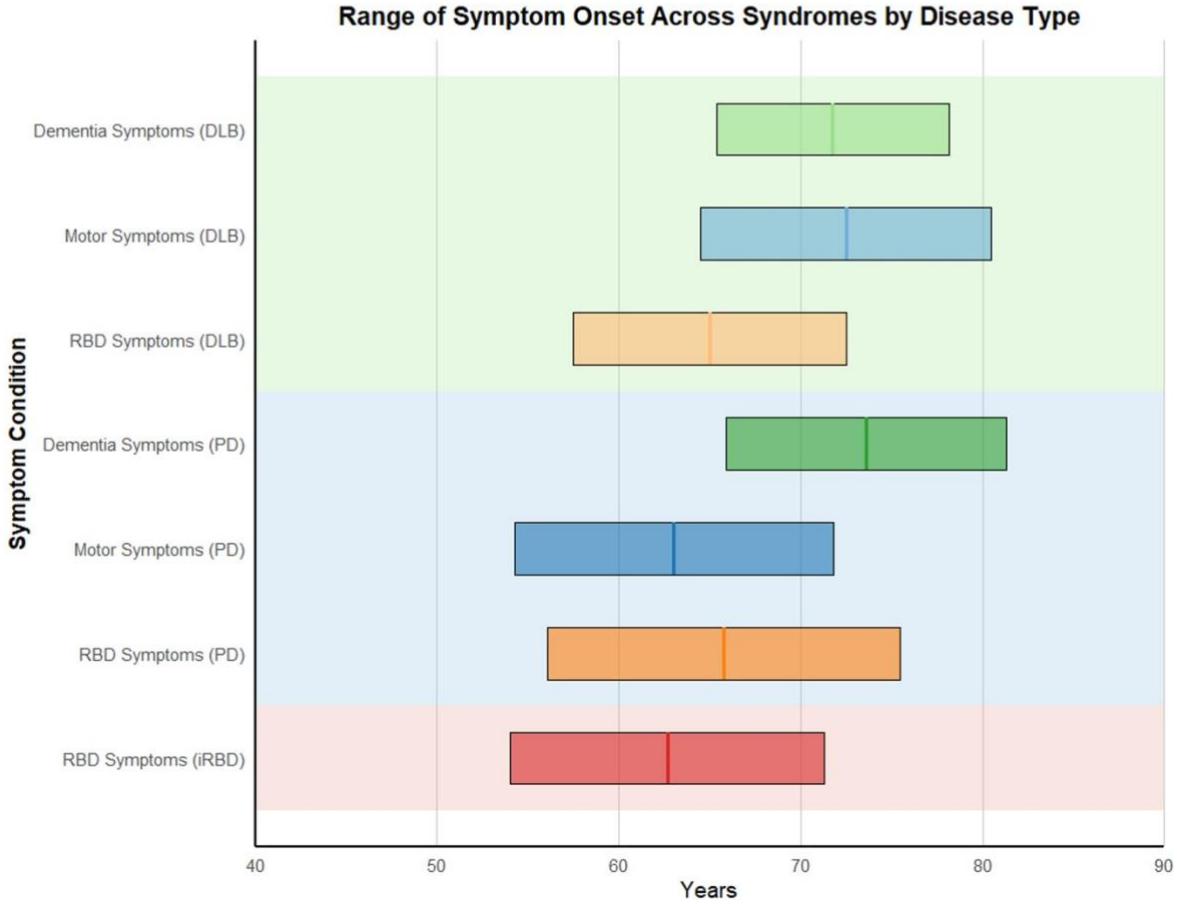
Figure 1. Mean (line) and standard deviation (bars) of age and timing of the onset of dementia, motor parkinsonism and REM sleep behaviour disorder across cohorts of patients with Parkinson's disease, dementia with Lewy bodies and isolated REM sleep behaviour disorder. Top Panel shows the onset of symptoms stratified by the type of symptom, whilst the Bottom Panel shows onset of symptoms stratified based on diagnosis. The diagram highlights similarity in the age of dementia onset across diagnostic categories, and likewise similar timing of REM sleep behaviour disorder which has a high variation of age of onset. Motor symptom onset seems to be the most distinct across different clinical groups. DLB, dementia with Lewy bodies; iRBD, isolated REM sleep behaviour disorder; PD, Parkinson's disease; RBD, REM sleep behaviour disorder.

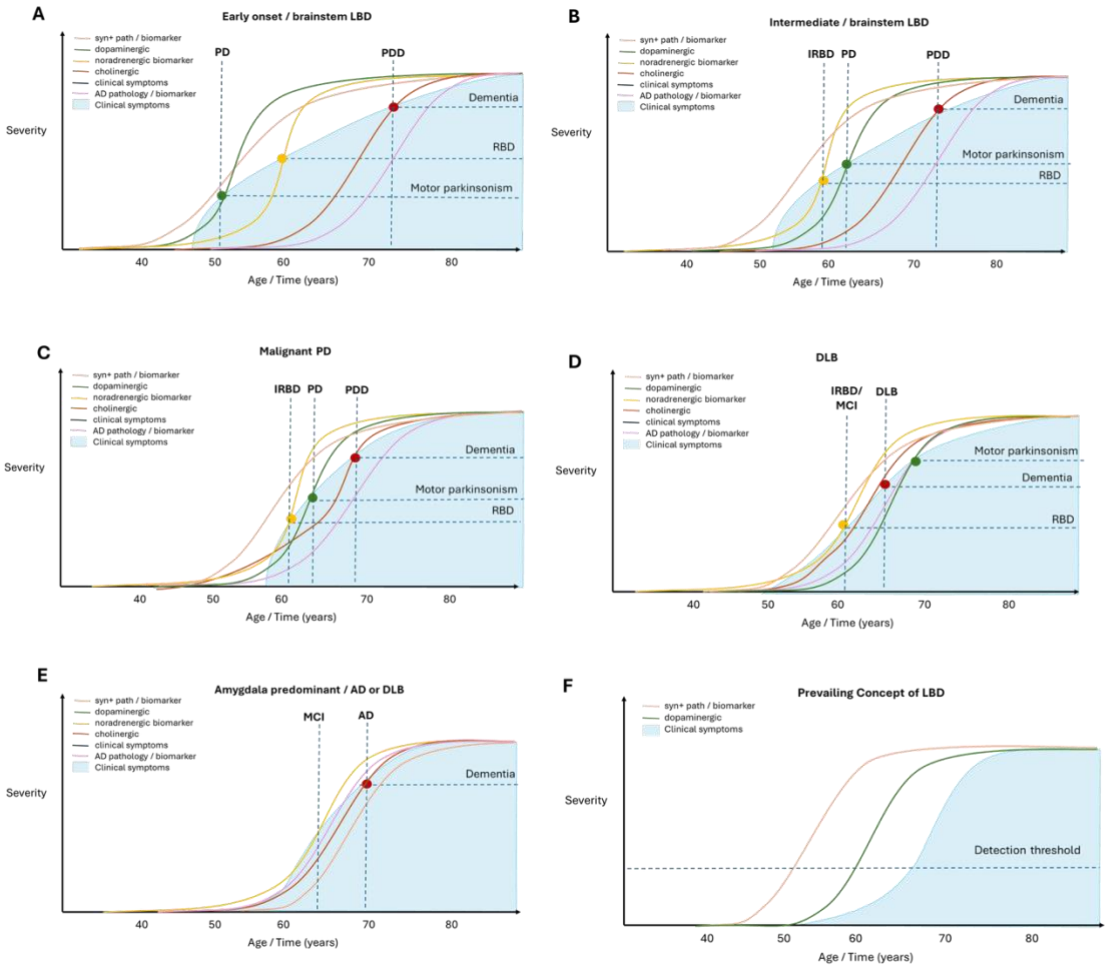
Figure 2. History and evolution of a selection of pathological staging and subtyping schemes over the last 2 decades (summarized in Panel 3 and the main text), including the Braak staging scheme in 2003, and the McKeith criteria proposed in 1996 and refined in 2005 (third consensus report of the international dementia with Lewy bodies consortium) and again 2017. These share the common classification of Lewy related pathology (LRP) from brainstem, limbic and neocortex, with the Braak staging scheme proposing six ordered stages beginning in the dorsal motor nucleus of vagus in the medulla (Stage 1) through to higher order association and primary neocortex (Stage 5-6). The Unified Staging System proposed by Beach and colleagues published in 2009 reported two divergent stages reflecting brainstem or limbic predominant pathways. Around this time there was also reporting of pathological subtypes published by Halliday and colleagues (see main text) in 2008 which correlated with a slowly progressing mild-motor group with limited co-pathology, an intermediate older onset PD group developing dementia with co-pathology and a fast-progressing diffuse malignant group with a high rate of AD co-pathology. In recent years, data-driven subtyping and staging has emerged, highlighting three subtypes, including a limbic predominant (S1) subtype with high rates of AD, as well as two brainstem predominant subtypes, including S2 beginning in the olfactory bulb and tract, followed by medulla with similar progression to the Braak staging scheme, and ending with a higher LRP load and more AD pathologically than the S3 subtype which started in the locus coeruleus with earlier spread of LRP that remained low in abundance.

Figure 3. Hypothetical case examples highlighting the heterogeneity of the temporal ordering of biological changes reflected in their respective biomarkers, namely synuclein positivity or spread, dopaminergic, cholinergic (e.g. PET markers or basal forebrain atrophy), noradrenergic (e.g. PET or neuromelanin MRI) cell changes, age, and AD co-pathology (e.g. Amyloid-beta). This also coincides with different timing of clinical expressions and trajectories (blue shading). A) Demonstrates an example of a PD case which at the time of diagnosis will likely have some synuclein pathology (noting that it may coincide closely with dopaminergic cell loss), as well minimal impact of other neuromodulatory symptoms with slow progression, developing dementia after 10-20 years. This would broadly align with a 'mild motor' PD clinical phenotype, and a staging of pathology akin to a brainstem predominant, or 'early onset' phenotype. B) Reflects a more intermediate subtype with older age of onset, mild cholinergic and moderate noradrenergic involvement. This may or may not be preceded by RBD, and again may be a brainstem predominant phenotype. C) Represents a more rapid PD course, with somewhat older onset, shorter duration between motor and cognitive symptoms, higher noradrenergic and cholinergic pathology and most likely RBD, and higher AD biomarker pathology. Such a case may be akin to a 'diffuse malignant' and bear semblance to DLB, with a rapid phenotype, and early limbic and neocortical involvement in staging. D) Represents an example of DLB, noting that dopaminergic abnormalities may be more gradual and follow after presence of dementia. In these cases, noradrenergic deficits are established and often RBD is present to a higher degree, as is significant AD co-pathology which may synergistically interact to enhance changes in all biomarkers. This would be akin to an early limbic subtype. E) shows a case that may be diagnosed with AD with some Lewy body pathology, manifested by cholinergic loss and cognitive symptoms with high AD related pathology. Although not clinically a Lewy body disorder, this case may still be classified under current schema or 'neuronal synuclein disease' in early stages and demonstrates the importance of considering clinical symptoms and other neurodegenerative pathologies. Although simplified (for instance detail of autonomic symptoms, or other pathologies such as cerebrovascular), these curves highlight important concepts for trial designs aiming to use biological staging, and are more advantageous over highly simplified schematics suggesting a singular relationship of synuclein positivity, followed by dopaminergic loss and clinical expression (F). The figure aims to place into context differences in the 'snapshot' of potential biomarkers that may be present at the time of diagnosis for individuals with Lewy body disorders. Although the exact biomarkers for

each pathology are not specified, it does highlight the importance of using parametric rather than dichotomous biomarker status in phenotyping individuals with Lewy body disease, and other symptoms such as early cognitive changes, visual hallucinations and fluctuations can be superimposed on the trajectories based on the interaction of synuclein positivity, noradrenergic and cholinergic changes. We argue that the curves above highlight important implications for clinical trial design and recruitment.


Range of Symptom Onset Across Syndromes
Symptom Condition
RBD Symptoms (iRBD)
RBD Symptoms (PD)
RBD Symptoms (DLB)
Motor Symptoms (PD)
Motor Symptoms (DLB)
Dementia Symptoms (PD)
Dementia Symptoms (DLB)
40
50
60
70
80
90
Years


Range of Symptom Onset Across Syndromes by Disease Type
Symptom Condition
Dementia Symptoms (DLB)
Motor Symptoms (DLB)
RBD Symptoms (DLB)
Dementia Symptoms (PD)
Motor Symptoms (PD)
RBD Symptoms (PD)
RBD Symptoms (iRBD)
40
50
60
70
80
90
Years


A
Early onset / brainstem LBD
syn+ path / biomarker
dopaminergic
noradrenergic biomarker
cholinergic
clinical symptoms
AD pathology / biomarker
Clinical symptoms
PD
PDD
Dementia
RBD
Motor parkinsonism
Severity
40
50
60
70
80
Age / Time (years)
B
Intermediate / brainstem LBD
IRBD
PD
PDD
Dementia
Motor parkinsonism
RBD
C
Malignant PD
IRBD
PD
PDD
D
DLB
IRBD/
MCI
DLB
E
Amygdala predominant / AD or DLB
MCI
AD
F
Prevailing Concept of LBD
Detection threshold