Neuropathological and genetic correlates of survival and dementia onset in synucleinopathies: a retrospective analysis

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Summary

Background Great heterogeneity exists in survival and the interval between onset of motor symptoms and dementia symptoms across synucleinopathies. We aimed to identify genetic and pathological markers that have the strongest association with these features of clinical heterogeneity in synucleinopathies.

Methods In this retrospective study, we examined symptom onset, and genetic and neuropathological data from a cohort of patients with Lewy body disorders with autopsy-confirmed α synucleinopathy (as of Oct 1, 2015) who were previously included in other studies from five academic institutions in five cities in the USA. We used histopathology techniques and markers to assess the burden of tau neurofibrillary tangles, neuritic plaques, α-synuclein inclusions, and other pathological changes in cortical regions. These samples were graded on an ordinal scale and genotyped for variants associated with synucleinopathies. We assessed the interval from onset of motor symptoms to onset of dementia, and overall survival in groups with varying levels of comorbid Alzheimer's disease pathology according to US National Institute on Aging–Alzheimer's Association neuropathological criteria, and used multivariate regression to control for age at death and sex.

Findings On the basis of data from 213 patients who had been followed up to autopsy and met inclusion criteria of Lewy body disorder with autopsy-confirmed α synucleinopathy, we identified 49 (23%) patients with no Alzheimer's disease neuropathology, 56 (26%) with low-level Alzheimer's disease neuropathology, 45 (21%) with intermediate-level Alzheimer's disease neuropathology, and 63 (30%) with high-level Alzheimer's disease neuropathology. As levels of Alzheimer's disease neuropathology increased, cerebral α-synuclein scores were higher, and the interval between onset of motor and dementia symptoms and disease duration was shorter ($p<0·0001$ for all comparisons). Multivariate regression showed independent negative associations of cerebral tau neurofibrillary tangles score with the interval between onset of motor and dementia symptoms (β –4·0, 95% CI –5·5 to –2·6; $p<0·0001$; R^2 0·22, $p<0·0001$) and with survival (–2·0, –3·2 to –0·8; 0·003; 0·15, <0·0001) in models that included age at death, sex, cerebral neuritic plaque scores, cerebral α-synuclein scores, presence of cerebrovascular disease, *MAPT* haplotype, and *APOE* genotype as covariates.

Interpretation Alzheimer's disease neuropathology is common in synucleinopathies and confers a worse prognosis for each increasing level of neuropathological change. Cerebral neurofibrillary tangles burden, in addition to α-synuclein pathology and amyloid plaque pathology, are the strongest pathological predictors of a shorter interval between onset of motor and dementia symptoms and survival. Diagnostic criteria based on reliable biomarkers for Alzheimer's disease neuropathology in synucleinopathies should help to identify the most appropriate patients for clinical trials of emerging therapies targeting tau, amyloid-β or α synuclein, and to stratify them by level of Alzheimer's disease neuropathology.

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Introduction

Parkinson's disease dementia[1] and dementia with Lewy bodies[2] are thought to be on a spectrum of clinical manifestations of underlying Lewy body disease[3] characterised by intraneuronal inclusions composed of pathological α-synuclein protein (ie, synucleinopathies).[4] Most patients with idiopathic Parkinson's disease will eventually develop dementia during the course of their illness.[5] However, the timing of the onset of dementia is highly variable, with some patients showing no signs of cognitive impairment years after the onset of Parkinson's disease.[6–8] By contrast, up to 25% of patients with de novo Parkinson's disease have mild cognitive impairment, and incident mild cognitive impairment in patients with established Parkinson's disease can rapidly progress to Parkinson's disease dementia.[9]

Furthermore, according to consensus criteria,[2] patients with dementia with Lewy bodies have dementia that precedes or occurs within a year of the onset of motor signs of parkinsonism. Although Parkinson's disease dementia and dementia with Lewy bodies are diagnostically classified by timing of symptom occurrence,

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Research in context

Evidence before this study

We searched PubMed with the terms "Lewy body dementia", "Parkinson's disease dementia", and "autopsy" for original research articles published in English from Jan 1, 2006, to April 25, 2016. We excluded non-autopsy biomarker studies and studies of α-synuclein pathology without a focus on Lewy body disorders (eg, comorbid α-synuclein pathology in Alzheimer's disease), because these studies were outside our focus. We found three studies in which the neuropathological correlates of the interval between onset of motor and dementia symptoms and survival were addressed in patients with autopsy-confirmed Parkinson's disease dementia or dementia with Lewy bodies, although the results between studies vary. A potential reason for these discrepancies between studies relates to sample size and relative frequencies of patients with Parkinson's disease dementia and dementia with Lewy bodies, because each of these studies included 55 or fewer patients with dementia with Lewy bodies. Another potential source of variability are the methods for ascertainment of burden of neurofibrillary tangles, because in two of the studies only Braak staging was used, which is largely based on topographical spread of pathology and not severity.

Added value of this study

To our knowledge, ours is one of the largest multicentre cohorts of patients with autopsy-confirmed synucleinopathies with detailed clinical, genetic, and neuropathological data to provide a systematic examination of the neuroanatomical substrate of the heterogeneity in the interval between onset of motor and dementia symptoms and survival. We classified Alzheimer's disease neuropathology into four stages of severity on the basis of neuropathological methods and criteria, and also examined continuous measures of Alzheimer's disease and α-synuclein pathology (ie, global cerebral scores). We examined a range of other common comorbid neuropathological changes in synucleinopathies, together with genetic risk polymorphisms, to provide a comprehensive assessment of neuropathology in synucleinopathies in our final multivariate models. We assessed continuous measures of the interval between onset of motor and dementia symptoms and survival, rather than the categorical clinical classification or non-specific global measures of cognition used in previous studies. We showed that increasing severity of the cortical burden of tau neurofibrillary tangle pathology was associated with a shorter time course to development of dementia and death.

Implications of all the available evidence

The results of our study suggest that biomarkers of Alzheimer's disease neuropathology have important prognostic implications for clinical care and trial design in patients with synucleinopathies. Future disease-modifying therapies targeted at Alzheimer's disease might also attenuate cognitive symptoms in most patients with synucleinopathies, because the increasing severity of neurofibrillary tangles is associated with decreasing interval between onset of motor and dementia symptoms and survival. These observations require replication in prospective cohorts of living patients with validated biomarkers of underlying Alzheimer's disease neuropathology.

clinical features of cognitive and motor impairment are often indistinguishable between the two, especially later in the disease course.[1–3] While the underlying neuropathological and genetic influences on this variable expression of cognitive impairment across synucleinopathies are unknown, we previously showed that cortical α-synuclein pathology was the strongest predictor of dementia in Parkinson's disease.[6] Furthermore, in patients with a clinical diagnosis of Parkinson's disease dementia who had substantial Alzheimer's disease neuropathology, the interval from onset of Parkinson's disease to the onset of dementia was shorter than in those without Alzheimer's disease neuropathology, and thus more closely resembled the natural history of dementia with Lewy bodies.[6]

In this study, we aimed to assess whether comorbid Alzheimer's disease neuropathology (ie, neuritic plaques and tau neurofibrillary tangles) is associated with the timing of dementia onset and survival in patients with synucleinopathies.

Methods

Participants

Patients included in this retrospective study were previously recruited by local clinicians and study investigators as part of several pre-existing clinical research projects from clinical research centres associated with the Penn Udall Center for Excellence in Parkinson's Disease Research (Philadelphia, PA, USA), the Pacific Udall Center (Seattle, WA, USA, and Portland, OR, USA), the Alzheimer's Disease Core Center (Philadelphia, PA, USA), the Alzheimer's Disease Research Center (Seattle, WA, USA), the Layton Aging and Alzheimer's Disease Center (Portland, OR, USA), the Alzheimer's Disease Research Center (Pittsburgh, PA, USA), or the Sanders-Brown Center on Aging (Lexington, KY, USA).

Patients who had been followed up to autopsy at the corresponding institutional neuropathology laboratory as of Oct 1, 2015, were included if they met formal clinical criteria for either probable dementia with Lewy bodies[2] or Parkinson's disease dementia,[10] as previously described,[6] with autopsy confirmation of brainstem, transitional, or neocortical stage synucleinopathy consistent with Lewy body spectrum disorders.[2,4] One patient had a secondary neuropathological diagnosis of progressive supranuclear palsy tauopathy, which can confound the examination of cortical Alzheimer's disease tau pathology, and was thus excluded. A subset of the neuropathological and genetic

data that we present were previously reported in a smaller cohort of patients in a different analysis of Parkinson's disease dementia[6] or the frequency of genetic variants in synucleinopathies[11,12] compared with healthy controls (103 individuals who were included in at least one of these studies). All procedures were done in accordance with local institutional review board guidelines and approvals at each centre. Written informed consent for autopsy and analysis of tissue sample data was obtained for all patients, either from the patients themselves or their next of kin. Further details of clinical data collection and referral centres are in the appendix.

Procedures

EBL, PTN, RW, JK, JBL, CDK, TJM, and JQT did the neuropathological examinations; they used standard methods and the same consensus diagnostic criteria at each centre.[4,13] As part of this assessment, sections for each of seven standardly sampled cortical and limbic regions were stained[4,13] and graded on a four-point (ie, 0–3) ordinal scale[4] for neurofibrillary tangles, cored or neuritic plaques, and α-synuclein pathology (further details about staining at each centre and other pathological variables are in the appendix). The mean cerebral score for each pathological change was calculated as previously described.[6] Briefly, we used a mean of the ordinal score ratings from the seven regions as a continuous measure of cortical burden for neurofibrillary tangles, neuritic plaques, and α synuclein (ie, global cerebral score). We used Braak neurofibrillary tangles staging and the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuritic plaque score to classify Alzheimer's disease neuropathology into four groups on the basis of modified criteria: no (or negligible) Alzheimer's disease neuropathology, low-level Alzheimer's disease, intermediate-level Alzheimer's disease, and high-level Alzheimer's disease (appendix).[4,13] We also dichotomised the burden of Alzheimer's disease neuropathology into Alzheimer's-disease-neuropathology-positive (intermediate-level or high-level disease)

See Online for appendix

	High-level Alzheimer's disease (N=63)	Intermediate-level Alzheimer's disease (N=45)	Low-level Alzheimer's disease (N=56)	No Alzheimer's disease (N=49)	p value
Post-mortem interval	..	..	..	..	0·8
N	46	38	50	44	..
Time (h; IQR)	10 (5–17·5)	10·5 (5·25–16)	9 (4–15·5)	8 (5·5–14)	..
Brain weight (g; SD)	1274·0 (175·3)*†	1318·6 (122·4)	1286·4 (140·9)	1335·6 (165·6)	0·04
Braak/CERAD stage, n	B3/C2 16 B3/C3 47	B2/C2 17 B2/C3 25 B3/C1 3	B1/C1 7 B1/C2 16 B1/C3 17 B2/C0 11 B2/C1 5	B0/C0 8 B1/C0 41	..
Lewy body stage, n (%)	..	..	..	..	0·02‡
Brainstem predominant	0 (0%)	0 (0%)	1 (2%)	3 (6%)	..
Transitional–limbic	8 (13%)	3 (7%)	10 (18%)	13 (27%)	..
Neocortical–diffuse	55 (87%)	42 (93%)	45 (80%)	33 (67%)	..
Cerebrum, n; score (SD)					
Neurofibrillary tangles	61; 2·0 (0·7)*†§	44; 1·1 (0·5)*§	55; 0·6 (0·4)*	42; 0·3 (0·3)	<0·0001
Neuritic plaques	60; 2·3 (0·5)*§	45; 2·1 (0·7)*§	55; 1·3 (0·9)*	46; 0·0 (0·1)	<0·0001
α-synuclein	59; 2·0 (0·7)*§	45; 2·0 (0·6)*§	54; 1·6 (0·6)*	44; 1·3 (0·6)	<0·0001
Basal ganglia, n; score (IQR)					
Neurofibrillary tangles	46; 1 (1–1·3)*†§	39; 0 (0–1)*	48; 0 (0–0·8)	42; 0 (0–0)	<0·0001
Neuritic plaques	46; 0 (0–1)*	39; 0 (0–2)*	48; 0 (0–1)*	47; 0 (0–0)	<0·0001
α-synuclein	48; 1 (1–3)*§	40; 1 (0–2)	48; 1 (0–2)	40; 1 (0–2)	0·03
Cerebral amyloid angiopathy, n; score (IQR)	44; 1 (0–2)*†§	39; 0 (0–1)*	48; 0 (0–1)*	45; 0 (0–0)	<0·0001
Dystrophic Lewy neurites in CA2/3 hippocampus, n; score (IQR)	45; 2 (1–3)	38; 2 (1–3)	48; 2 (0·25–2)	43; 2 (1–3)	0·6
Hippocampal TDP43, n/N (%)	11/29 (38%)	8/34 (24%)	9/44 (21%)	7/43 (16%)	0·2
Hippocampal sclerosis, n/N (%)	6/61 (10%)	2/44 (5%)	2/51 (4%)	8/48 (17%)	0·1
Argyrophilic grain disease, n/N (%)	1/54 (2%)	1/41 (2%)	0/51 (0%)	1/46 (2%)	0·8
Cerebrovascular disease, n/N (%)	11/60 (18%)	6/43 (14%)	9/53 (17%)	9/48 (19%)	0·9

p values correspond to four-group comparisons. CERAD=Consortium to Establish a Registry for Alzheimer's Disease. The appendix lists the exact p values denoted by the footnotes. *p<0·05 compared with the no Alzheimer's disease group. †p<0·05 compared with the intermediate-level Alzheimer's disease group. ‡p<0·05 for linear trend association in categorical variables. §p<0·05 compared with the low-level Alzheimer's disease group.

Table 1: **Neuropathological data in patients with synucleinopathies, stratified by burden of Alzheimer's disease pathology**

	High-level Alzheimer's disease (N=63)	Intermediate-level Alzheimer's disease (N=45)	Low-level Alzheimer's disease (N=56)	No Alzheimer's disease (N=49)	p value
APOE ε4					Add 0·3; dom 0·1
n	61	42	56	49	
0	24 (39%)	20 (48%)	30 (54%)	30 (61%)	
1	30 (49%)	19 (45%)	23 (41%)	18 (37%)	
2	7 (11%)	3 (7%)	3 (5%)	1 (2%)	
MAPT H1 haplotype					Add 0·9; rec 0·7
n	59	42	53	48	
H2/H2	1 (2%)	2 (5%)	1 (2%)	1 (2%)	
H1/H2	19 (32%)	14 (33%)	13 (25%)	15 (31%)	
H1/H1	39 (66%)	26 (62%)	39 (74%)	32 (67%)	
SNCA rs356219 genotype					Add 0·6; dom 0·9
n	59	42	53	48	
GG	16 (27%)	9 (21%)	15 (28%)	13 (27%)	
GA	32 (54%)	30 (71%)	29 (55%)	28 (58%)	
AA	11 (19%)	3 (7%)	9 (17%)	7 (15%)	
GBA E326K genotype					Add N/A; dom 0·03*
n	47	26	35	32	
GG	47 (100%)	26 (100%)	31 (89%)	28 (88%)	
GA	0 (0%)	0 (0%)	4 (11%)	4 (13%)	
AA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
GBA mutation					0·03*
n	45	34	39	39	
Mutation	1 (2%; N370S)	2 (6%; 1 N370S and 1 Rec1)	6 (15%; 1 A456P, 1 L444P, 3 N370S, and 1 S196P)	8 (21%; 3 N370S, 1 R463C, 1 R163X, 1 R359X, 1 Rec1, and 1 V394L)	

Data are n (%). p values correspond to four-group comparison (χ^2 contingency analysis). Add=additive genetic model (ie, 0 *vs* 1 *vs* 2 copies). Dom=dominant genetic model (0 *vs* 1 or 2 copies). Rec=recessive genetic model (0 or 1 *vs* 2 copies). N/A=not applicable (no homozygous risk allele patients). *p<0·05 for linear trend association in categorical variables (a precise list of p values is provided in the appendix).

***Table 2*: Genetic data in patients with synucleinopathies, stratified by burden of Alzheimer's disease pathology**

and Alzheimer's-disease-neuropathology-negative (no or low-level disease) groups, as described previously,[6] to test the diagnostic accuracy of the interval from onset of motor symptoms to onset of dementia.

We used standard techniques to isolate DNA from peripheral blood before death or frozen brain samples post mortem.[6] Samples were genotyped for common single nucleotide polymorphisms in genes previously associated with synucleinopathies[11,14]—apolipoprotein E (*APOE* ε2, ε3, and ε4 via rs429358/rs7412), tau (*MAPT* H1 haplotype via risk allele A at rs1800547), and α synuclein (*SNCA* risk allele G at rs356219)—by TaqMan assay (Life Technologies, Waltham, MA, USA). In addition, the entire *GBA* coding region and all intron–exon boundaries were sequenced with PCR to detect known pathogenic mutations and the coding single nucleotide polymorphism p.E326K risk allele G at rs2230288 as described.[15] Missing data from cases with no or insufficient DNA samples were omitted from our analysis. All genotyping was done by CPZ at the Pacific Udall Center (Seattle, WA, USA).

Statistical analyses

We analysed continuous variables with parametric or non-parametric univariate tests as appropriate, and compared categorical variables with a χ^2 test analysis. To test the diagnostic accuracy for underlying pathology of use of an interval of 1 year or less between onset of motor and dementia symptoms,[2] which distinguishes dementia with Lewy bodies from Parkinson's disease dementia, we used receiver-operating characteristic (ROC) curve analyses for advanced α-synuclein pathology (neocortical stage *vs* brainstem or limbic stages) or Alzheimer's disease pathology (Alzheimer's-disease-neuropathology-positive *vs* Alzheimer's-disease-neuropathology-negative disease). Correlations of global cerebral neurofibrillary tangles, neuritic plaques, and α-synuclein scores with clinical features (age at death, interval between onset of motor symptoms and dementia symptoms, and survival) were done via a Pearson correlation.

We used multivariate linear regression to test the independent associations of Alzheimer's-disease-related

pathology (ie, cerebral neurofibrillary tangles and neuritic plaques) and other genetic and pathological features with survival and interval between onset of motor symptoms and dementia as the dependent variables in base models controlling for age at death and sex. We used Bayesian information criteria to derive final models.[16] In the absence of an external validation sample, the final model was validated by a bootstrap procedure (ie, internal validation).[17] We report mean β estimates with 95% CIs from a bootstrapping random sampling procedure with 1000 bootstrap samples (appendix).

All analyses were two-tailed (α=0·05). We adjusted correlation analyses to α=0·003 to correct for multiple comparisons and reduce the likelihood of false-positive discovery. We used SPSS (v23.0) or Stata (v12.1) for all analyses.

Role of the funding source

The funder of the study had no role in study design; data collection, analysis, or interpretation; or writing of this Article. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

213 patients with synucleinopathies meeting clinical criteria for Parkinson's disease dementia or dementia with Lewy bodies were selected for this study. Survival data were missing for two patients, and data for the interval between motor and dementia symptoms for 27 patients. Missing individual neuropathological and genetic variable data are presented in tables 1 and 2.

Classification of cases of synucleinopathy based on stages of Alzheimer's disease neuropathology[4,13] showed that 49 patients (23%) had negligible or no Alzheimer's disease, 56 patients (26%) had low-level Alzheimer's disease, 45 patients (21%) had intermediate-level Alzheimer's disease, and 63 (30%) had high-level Alzheimer's disease (table 1). Neurofibrillary tangles and neuritic plaque scores in the cerebrum and basal ganglia increased across groups with increasing levels of Alzheimer's disease neuropathology, as did cerebral

	High-level Alzheimer's disease (N=63)	Intermediate-level Alzheimer's disease (N=45)	Low-level Alzheimer's disease (N=56)	No Alzheimer's disease (N=49)	p value
Clinical phenotype frequency					<0·0001*
Parkinson's disease dementia	17 (27%)	22 (49%)	37 (66%)	39 (80%)	
Dementia with Lewy bodies	46 (73%)	23 (51%)	19 (34%)	10 (20%)	
Sex					0·09
Women	25 (40%)	12 (27%)	15 (27%)	9 (18%)	
Men	38 (60%)	33 (73%)	41 (73%)	40 (82%)	
Age at motor onset					<0·0001
n	50	42	52	49	
Years (SD)	73·1 (7·9)†‡§	69·6 (8·0)§	66·6 (10·2)§	59·6 (11·1)	
Age at dementia onset					0·005
n	61	43	54	48	
Years (IQR)	76·0 (69–81)§	73·0 (67–79)	74·5 (70–80)§	70·5 (60·5–76)	
Interval between onset of motor and dementia symptoms					<0·0001
n	48	40	50	48	
Years (SD)	1·1 (6·1)‡§	2·8 (6·8)‡§	7·1 (7·7)	8·6 (8·2)	
Age at death					0·001
n	63	45	56	49	
Years (IQR)	81 (75–86)§	79·1 (73·5–84)§	79 (73·3–82·7)§	75·0 (66·5–80)	
Interval between onset of dementia and death					0·2
n	61	43	53	48	
Years (SD)	5 (3–7)	5 (3–7)	3 (2–7)	6 (3–7)	
Survival					<0·0001
n	61	45	56	49	
Years (SD)	8·0 (5·2)†‡§	10·0 (5·6)§	11·5 (6·9)§	14·9 (6·8)	

Normally distributed variables are presented as mean (SD), non-parametric variables are presented as median (IQR), and categorical variables presented as frequency (%). p values correspond to four-group comparisons. The appendix lists the exact p values denoted by the footnotes. *p<0·001 for linear trend association in categorical variables. †p≤0·06 compared with intermediate-level Alzheimer's disease group. ‡p≤0·01 compared with low-level Alzheimer's disease group. §p≤0·01 compared with the no Alzheimer's disease group.

***Table 3*: Clinical characteristics of patients with synucleinopathies, stratified by burden of Alzheimer's disease pathology**

amyloid angiopathy scores (p<0·0001 for all comparisons; table 1). Assessment of α-synuclein pathology across increasing levels of Alzheimer's disease revealed a stepwise increase in α-synuclein stage (p=0·018), cerebral α-synuclein score (p<0·0001), and basal ganglia α-synuclein score (p=0·03). No significant differences were noted between groups for the other neuropathological changes examined (table 1).

Examination of genetic variants previously associated with synucleinopathies[11,12,14] showed decreasing numbers of heterozygous patients carrying the *GBA* pE326K risk allele or *GBA* mutation with increasing levels of Alzheimer's disease neuropathology (p=0·03 for both; table 2). Single nucleotide polymorphisms in *MAPT* and *SNCA* were similar across groups. The frequency of the *APOE* ε4 allele was not associated with the four levels of increasing Alzheimer's disease neuropathology, but in a dichotomous comparison of Alzheimer's-disease-neuropathology-positive and Alzheimer's-disease-neuropathology-negative groups, patients with one or more copies of *APOE* ε4 were more frequent (p=0·04) in the Alzheimer's-disease-neuropathology-positive group (appendix).

We noted an increasing number of patients with a clinical diagnosis of dementia with Lewy bodies (as opposed to Parkinson's disease dementia) with increasing levels of Alzheimer's disease neuropathology (p<0·0001; table 3). 18 (18%) of 98 patients with dementia with Lewy bodies) never developed clinical motor parkinsonism during the course of their disease. Similar to the entire cohort of patients with dementia with Lewy bodies, 16 (89%) of these individuals without motor symptoms had intermediate to high levels of Alzheimer's disease neuropathology, and 15 (83%) were in the neocortical Lewy body stage. Despite the significantly higher frequency of dementia with Lewy bodies in the Alzheimer's-disease-neuropathology-positive group (appendix), we noted no clear delineation of the Alzheimer's disease neuropathology groups according to clinical phenotype (figure 1A). ROC curve analysis showed that diagnostic accuracy of the 1-year motor–dementia interval, which clinically distinguishes dementia with Lewy bodies from Parkinson's disease dementia, for α-synuclein neocortical stage (area under curve 0·67) and Alzheimer's disease neuropathology (area under curve 0·72) was poor (figure 1B, 1C).

Increasing levels of Alzheimer's disease neuropathology were associated with older age at onset of motor symptoms (p<0·0001), dementia (p=0·005), and death (p=0·001), with significant differences between the Alzheimer's disease groups and the no Alzheimer's disease group (table 3). The interval from the onset of dementia to death did not differ significantly between groups. Increasing severity of Alzheimer's disease was also associated with a stepwise decrement in interval between motor and dementia symptom onset and survival (figure 2), and these differences were significant when the intermediate-level and high-level Alzheimer's disease groups were compared with the no Alzheimer's disease and low-level Alzheimer's disease groups (table 3). There was also a similar stepwise association of increasing CERAD (neuritic plaques) and Braak (neurofibrillary tangles) stages with a shorter interval between motor symptom and dementia onset and survival, with a greater number of significant differences between individual Braak stages than CERAD stages (figure 2; appendix).

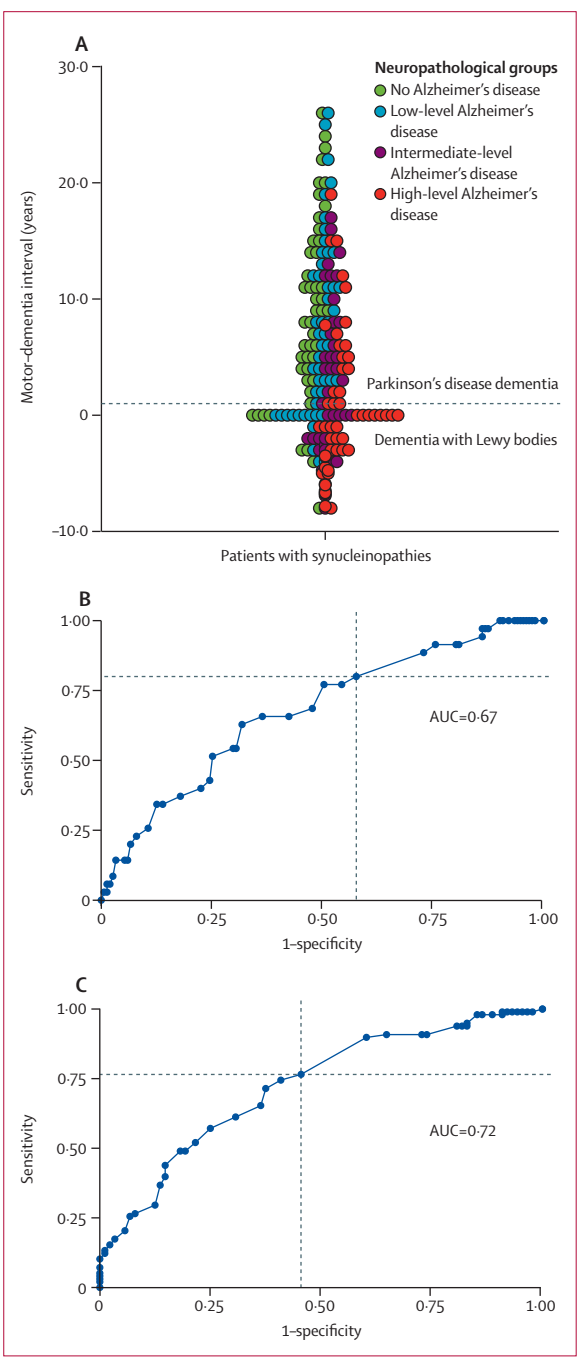

***Figure 1*: Diagnostic accuracy of interval between onset of motor and dementia symptoms to distinguish between synucleinopathies**
(A) Distribution of neuropathological Alzheimer's disease groups among Parkinson's disease dementia and dementia with Lewy bodies clinical phenotypes in relation to the 1-year interval (dotted line) between motor and dementia symptom onset used in current diagnostic criteria. (B) ROC curve analysis of diagnostic accuracy of use of the interval between onset of motor and dementia symptoms to distinguish neocortical stage of synucleinopathy. AUC is 0·67 (p=0·05), sensitivity is 80%, and specificity is 42% on the basis of the 1-year rule (intersection of dashed lines). (C) ROC curve analysis of diagnostic accuracy of the interval between onset of motor and dementia symptoms to distinguish patients with substantial Alzheimer's disease pathology. AUC is 0·72 (p=0·0004), sensitivity is 76% and specificity is 55% on the basis of the 1-year rule. ROC=receiver operating characteristic. AUC=area under curve.

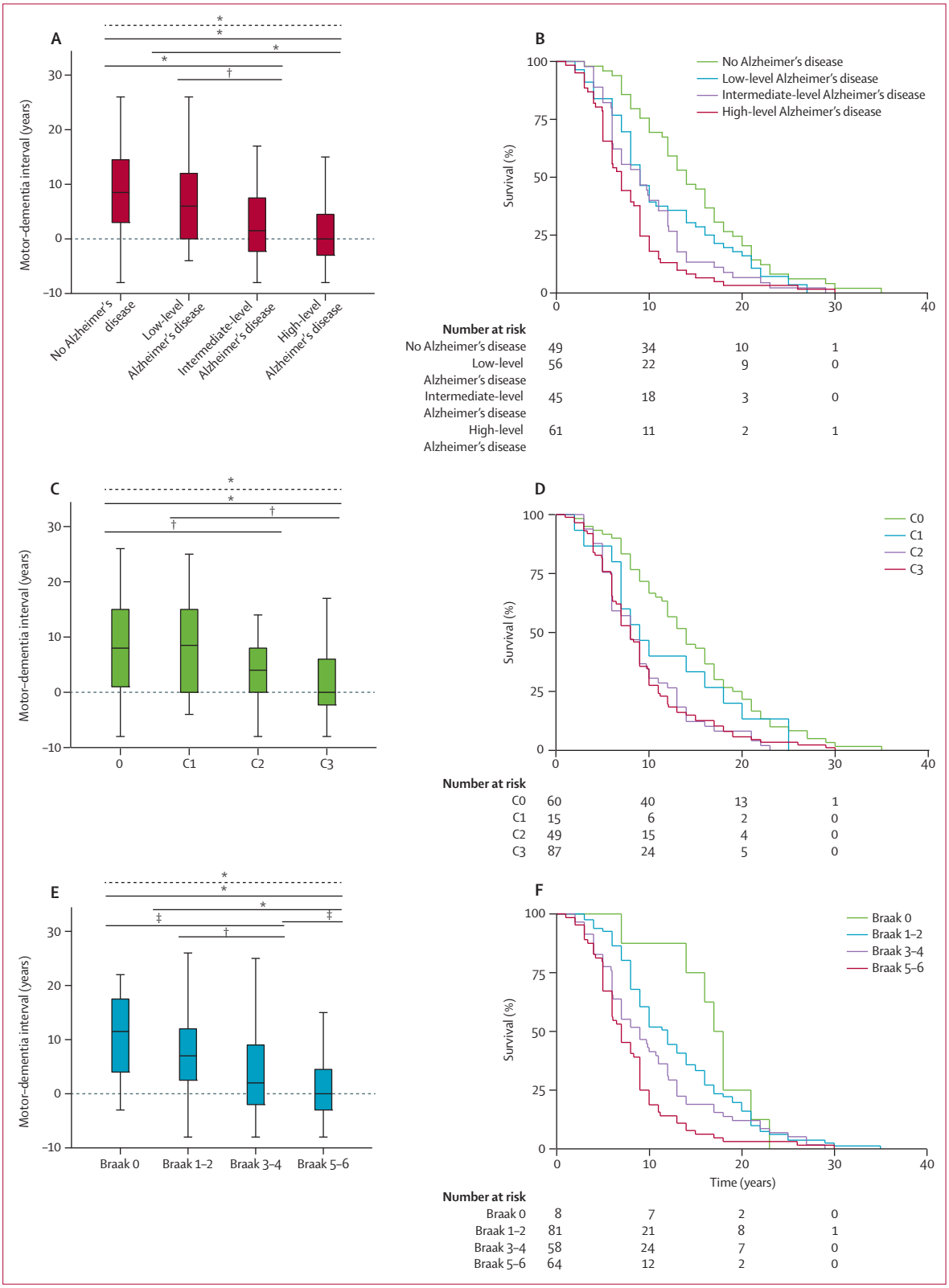

Figure 2: **Comparison of interval between motor and dementia symptoms and survival in patients with synucleinopathies stratified by neuropathological group**
(A) Box plot of interval between onset of motor and dementia symptoms and total Alzheimer's disease neuropathological change and (B) Kaplan-Meier curve of survival by total Alzheimer's disease neuropathological change. (C) Box plot of CERAD stages of neuritic plaque pathology and (D) Kaplan-Meier curve of survival by CERAD stages of neuritic plaque pathology. (E) Box plot of Braak stages of tau neurofibrillary tangles and (F) Kaplan-Meier curve of survival by Braak stages of neurofibrillary tangles. Dashed lines represent across-groups comparison (one-way ANOVA) and solid lines represent post-hoc individual group comparisons (independent *t* tests). The exact p values referred to in the footnotes are listed in the appendix. CERAD=Consortium to Establish a Registry for Alzheimer's Disease. *p<0·001. †p<0·01. ‡p≤0·05.

Global cerebral neurofibrillary tangles, neuritic plaques, and α-synuclein scores were all moderately positively correlated with each other (r=0·4 to 0·6, p<0·0001) and moderately negatively correlated with the interval between motor and dementia symptoms and survival (r=–0·3 to –0·4, p<0·0001 for both; figure 3). Global cerebral pathology scores did not correlate with age at death (data not shown).

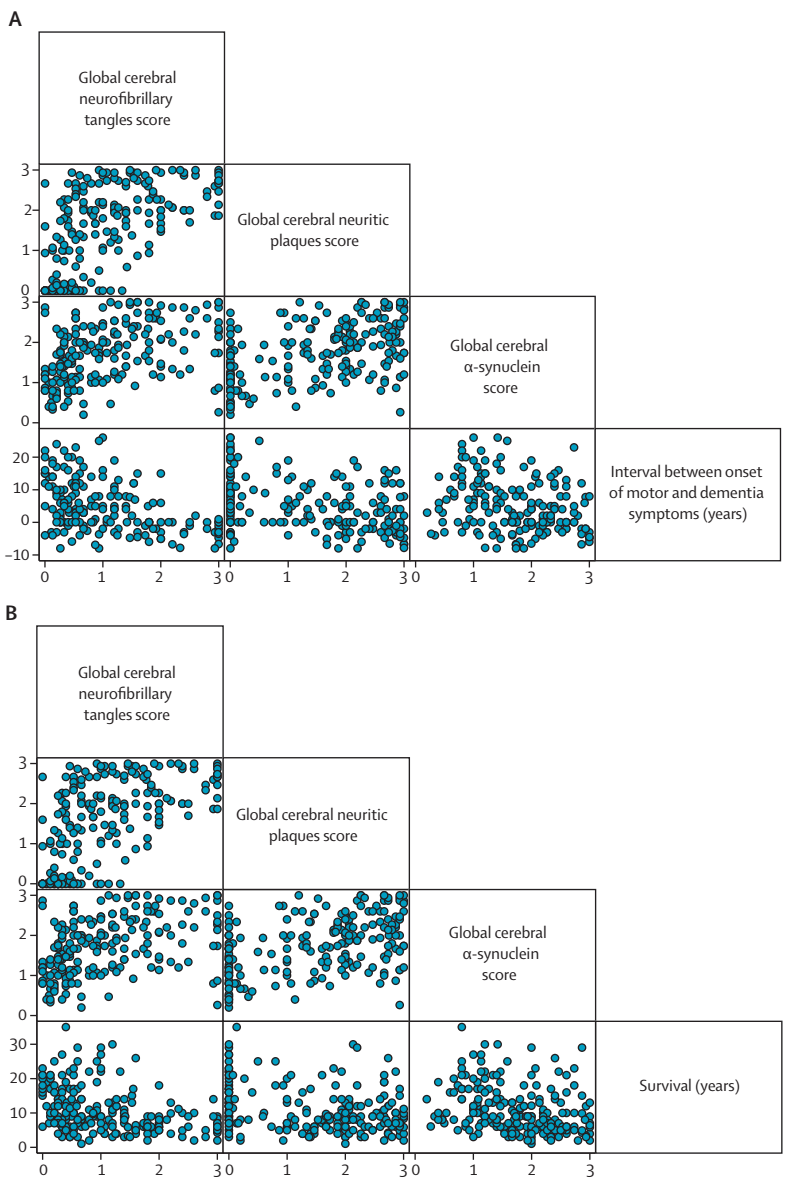

***Figure 3*: Comparison of global cerebral neuropathology scores with the interval between motor and dementia symptoms and survival**

In both panels, the scatterplot matrices show individual patient data correlations for each variable row–column combination. All global cerebral pathology scores are correlated with each other. Cerebral neurofibrillary tangles: cerebral neuritic plaques (r=0·6, p<0·0001), cerebral neurofibrillary tangles: cerebral α-synuclein (r=0·4, p<0·0001), cerebral neuritic plaques: cerebral α-synuclein (r=0·4, p<0·0001). (A) Comparison of global cerebral neuropathology scores with interval between onset of motor and dementia symptoms. Negative correlations: cerebral neurofibrillary tangles (r=–0·4, p<0·0001), cerebral neuritic plaques (r=–0·4, p<0·0001), cerebral α synuclein (r=–0·3, p=0·0001). (B) Comparison of global cerebral neuropathology scores with survival. Negative correlations: cerebral neurofibrillary tangles (r=–0·4, p<0·0001), cerebral neuritic plaques (r=–0·3, p<0·0001), and cerebral α synuclein (r=–0·3, p=0·0001).

Univariate linear regression models with the interval between onset of motor symptoms and dementia symptoms as the dependent variable showed significant associations with *APOE* genotype (p=0·04) and continuous measures of cerebral neurofibrillary tangles, neuritic plaques, and α-synuclein pathology (p<0·0001; appendix). Our final multivariate model, in which we controlled for age at death, sex, and other neuropathological or genetic variables, showed a negative association with cerebral neurofibrillary tangles score (β –4·0, 95% CI –5·5 to –2·6; p<0·0001) and a positive association with age at death (0·2, 0·02 to 0·3; 0·05; model R^2 0·22; p<0·0001; appendix).

Univariate linear regression models with survival as the dependent variable showed that *APOE* genotype (p=0·003) and continuous measures of cerebral neurofibrillary tangles, neuritic plaques, and α-synuclein pathology all had significant (p<0·0001) associations (appendix). Our final multivariate model showed an independent negative association with cerebral neurofibrillary tangles score (β –2·0, 95% CI –3·2 to –0·8; p=0·003; model R^2 0·15; p<0·0001; appendix). Examination of interaction terms for cerebral neurofibrillary tangles score with cerebral α-synuclein score or cerebral neuritic plaques score, and for *APOE* with cerebral neuritic plaques score or cerebral neurofibrillary tangles score were not significant and did not optimise Bayesian information criteria values of either model.

Discussion

We analysed the contribution of Alzheimer's disease neuropathology to onset of dementia and to survival in synucleinopathies through group-wise comparisons of four levels of severity of Alzheimer's disease neuropathology.[4] Our retrospective analysis in a large cohort of patients with synucleinopathies for whom detailed clinical, pathological, and genetic information were available showed accumulation of neurofibrillary tangles—from both a continuous measure of cerebral neurofibrillary tangles burden score and sequential categorical Braak neurofibrillary tangles stage—to be the strongest correlate of a decreased interval between onset of motor symptoms and onset of dementia and of overall survival. Indeed, patients in the high-level Alzheimer's disease group had, on average, a 7-year shorter interval between onset of motor and dementia symptoms and overall survival than did the no Alzheimer's disease group (table 3).

Other studies have also shown a shorter interval between onset of motor symptoms and dementia symptoms and shorter overall survival in patients with Parkinson's disease and comorbid Alzheimer's disease neuropathology,[8,18,19] and a similar negative association of cerebral neurofibrillary tangles, neuritic plaques, and α-synuclein pathology with the interval between onset of motor symptoms and dementia symptoms across

synucleinopathies.[7] However, additional research suggests that amyloid-β plaques[8,20]—or a summation of neurofibrillary tangles, amyloid β, and α-synuclein pathological changes[21]—were the strongest correlates of a shorter interval between onset of motor and dementia symptoms in synucleinopathies. There are several potential reasons for these discrepancies. In our univariate analyses, the global cerebral neuritic plaques score was negatively associated with both the interval between onset of motor and dementia symptoms and survival (p<0·0001), but after accounting for neurofibrillary tangles, this association was not significant (appendix). Our measure of amyloid-β accumulation included neuritic plaques only, rather than all forms of amyloid-β plaques, and thus we might have underrepresented the contribution from amyloid β.

Our multicentre cohort is one of the largest reported series of clinically characterised patients with roughly equal numbers of patients with Parkinson's disease dementia and dementia with Lewy bodies, which allowed for comparisons across four levels of Braak stages and CERAD scores and a continuous measure of global cerebral neurofibrillary tangles and neuritic plaques scores. By contrast, previous studies included limited assessments of neurofibrillary tangle pathology—Braak stages were collapsed for dichotomous comparisons,[8] or only categorical Braak neurofibrillary tangles stages[20] were examined. Additionally, samples were small, with relative imbalances of patients with Parkinson's disease dementia and those with dementia with Lewy bodies,[20,21] or samples of patients included only patients with Parkinson's disease with or without dementia.[8] Although in this study we focused on patients with synucleinopathies who developed dementia (either Parkinson's disease dementia or dementia with Lewy bodies) and did not include patients with Parkinson's disease who underwent autopsy before the onset of dementia, we have previously published findings in a series[6] of patients with Parkinson's disease before dementia (autopsy-confirmed), most of whom had a low level of tau pathology (35 [80%] of 44 cases had Braak tau stages 0–2—ie, tau pathology restricted to the hippocampus only), which further reinforces our findings here. However, our results will need replication in an independent cohort.

We found that age at death was independently associated with a longer interval between onset of motor symptoms and dementia symptoms (appendix), reflecting an overall longer disease course in patients with a long interval between onset of motor and dementia symptoms. Thus, age-related factors can also contribute to discrepancies between this study and previous work, although in our multivariate regression correcting for age at death and sex, we noted a significant independent association of global cerebral neurofibrillary tangles scores with a shorter interval (p<0·0001) and survival (p=0·003).

Although we did not include age at onset in our models because age at death more closely reflects the effects of ageing on the measures of pathology detected at autopsy, age at onset might influence clinical phenotype in patients with synucleinopathies. A prospective autopsy study[22] of patients with idiopathic Parkinson's disease (the Sydney multicentre Parkinson's disease study) showed three clinicopathological subtypes defined partly by age at onset. Patients with Parkinson's disease who were younger at disease onset were more likely to survive longer without dementia and had less comorbid Alzheimer's disease neuropathology than were patients who were older at onset, who had shorter survival and higher burdens of secondary Alzheimer's neuropathology. This study[22] also detected a small subgroup of six patients that had typical dopa-responsive Parkinson's disease and early dementia that met clinical criteria for dementia with Lewy bodies, with a rapid disease course and high burdens of both α-synuclein and amyloid plaque neuropathology.

These clinicopathological subgroups of patients are similar to those included in our study and those included in our previously published study[6] of patients with Parkinson's disease without dementia. Although important for providing novel insights into the progression of typical idiopathic Parkinson's disease, the Sydney study[22] does not include all forms of clinical dementia with Lewy bodies, many of which are not associated with typical dopa-responsive parkinsonism at onset. Indeed, 18% of the dementia with Lewy body patients in our cohort here did not develop clinical Parkinsonism at all during their disease course. Thus, the Sydney study and our work provide complementary views of synucleinopathies and, taken together, show a complex relation between ageing, α synuclein, and Alzheimer's disease neuropathology. To more clearly define the clinicopathological subtypes of synucleinopathies, future studies should quantify neuropathological burden parametrically, and in-vivo CSF or imaging biomarkers of tau, amyloid β, and α-synuclein pathology should be used in conjunction with detailed clinical data in patients who are prospectively followed up to autopsy.

Nonetheless, our data suggest that the consequences of Alzheimer's disease neuropathology—particularly neurofibrillary tangles burden—are not solely an artifact of the ageing process, but are instead central to pathogenesis in most patients with synucleinopathies. We found that patients with increasing Alzheimer's disease neuropathology also had increasing α-synuclein pathology (table 1) and a strong correlation between global cerebral neurofibrillary tangles, neuritic plaques, and α-synuclein scores (figure 3), which suggests a synergistic effect of mixed Alzheimer's disease and α-synuclein neuropathology. We and others have previously shown that a high burden of Alzheimer's disease neuropathology is associated with advanced α-synuclein pathology in Lewy body disorders;[6,8] CSF markers of tau and α-synuclein pathologies also seem to

be highly correlated in Parkinson's disease.[23] These findings echo in-vitro evidence for cross-fibrillisation of tau and α-synuclein fibrils[24] and conformational strains of pathological α synuclein that coinduce α-synuclein and neurofibrillary tangles pathology.[25] Future research is needed to elucidate further these potential mechanisms of coaccelerated pathology.

Other, less common comorbid pathological changes could be contributing to dementia in synucleinopathies. 15–20% of our cohort had comorbid cardiovascular disease or limbic TDP43 pathology, with no clear association with Alzheimer's disease neuropathology, the interval between onset of motor and dementia symptoms, or disease duration. The clinical significance of these and other less common comorbid pathologies (eg, argyrophilic grain disease, hippocampal sclerosis) in our cohort is not certain, and we cannot rule out a contribution of these pathologies to phenotypic diversity of synucleinopathies on an individual patient level. Additional work will need to assess these rare, comorbid pathologies in a larger cohort.

Another contributing factor to clinical heterogeneity in synucleinopathies emerges from our genetic analyses. We noted a higher frequency of *GBA* mutations and pE326K polymorphism, both of which confer risk of synucleinopathies,[12,26] in the low-level Alzheimer's disease and no Alzheimer's disease neuropathology groups compared with patients with intermediate or high levels of Alzheimer's disease neuropathology (table 2), and previously reported a similar high frequency of *GBA* mutations in a cohort of patients with α-synuclein pathology but without comorbid Alzheimer's disease.[12] We previously showed that *APOE* genotype has an independent effect on the odds of dementia in Parkinson's disease[6] and noted a higher frequency of the *APOE* ε4 allele in both Alzheimer's-disease-neuropathology-positive and Alzheimer's-disease-neuropathology-negative synucleinopathies compared with cognitively healthy controls.[11] Univariate analyses in this study showed that the presence of one or more *APOE* ε4 allele was associated with a shorter interval between onset of motor and dementia symptoms and survival (appendix), but these associations were not significant in the final multivariate models, which included cerebral neurofibrillary tangles and neuritic plaques scores. Further work is needed to elucidate the mechanisms by which *APOE* and *GBA* polymorphisms affect α-synuclein pathology.

We did not find evidence for a definitive pathological substrate to support the categorical clinical distinction between Parkinson's disease dementia and dementia with Lewy bodies (figure 1), despite the findings of higher cortical neurofibrillary tangles, neuritic plaques, and α-synuclein pathology in patients with dementia with Lewy bodies compared with Parkinson's disease dementia (appendix). However, neuropathological assessment at autopsy might not accurately identify pathological differences that could potentially occur earlier in the disease course. We noted a consistent interval from dementia onset to death of roughly 3–6 years across neuropathological (table 3; appendix) and clinical groups (appendix). Previous studies in Parkinson's disease have shown a poor prognostic association of cognitive impairment in Parkinson's disease,[27,28] with a stereotypical decline to admission to care facilities and death 3–5 years after dementia onset.[29] Thus, the timing of dementia in synucleinopathies is an important prognostic factor.

Our study has several limitations, which are inherent to a retrospective autopsy investigation. Clinical data were missing for some patients, but we had data for survival and the interval between onset of motor and dementia symptoms for more than 87% of the cohort. All patients were referred to academic centres that specialise in movement disorders or dementia, or both, so a referral bias favouring atypical or more severe phenotypes could be present. Replication of our findings in an independent prospective cohort is needed to interpret further the generalisability of results. However, we used a bootstrapped random patient selection procedure to reduce overfitting of our models. Finally, the neuropathological data were gathered at autopsy from multiple centres, where different staining procedures were used. However, we used standardised methods to merge multicentre data, which provides reliability across participating institutions and helps to reduce inter-laboratory variability.[4,13] The neuropathological methods and criteria we used here have also been validated in a large multicentre study.[30]

On the basis of the strong associations of Alzheimer's disease neuropathology (more specifically, neurofibrillary tangles burden) with the interval between motor symptom and dementia onset and overall survival, we suggest that future diagnostic criteria for synucleinopathies incorporate biomarkers for neurofibrillary tangles and neuritic plaques for an individualised approach to diagnosis of underlying complex molecular pathology and to identify patients at greatest risk for rapid decline. In addition, future clinical trials targeting α-synuclein aggregation and propagation might benefit from stratification of analyses based on Alzheimer's-disease-related biomarker profiles and *APOE* genotype. Finally, our findings suggest that emerging therapies directed at the mitigation of pathological tau and amyloid β could potentially slow the degenerative process and onset of cognitive difficulties in most synucleinopathies.

Contributors

DJI, MG,DW, HIH, JED, TJM, CPZ, and JQT designed the study. DJI and SXX analysed data; all other authors gathered data. DJI drafted the Article and all other authors assisted with revisions and approved the final version.

Declaration of interests

DJI reports travel stipends from GE Healthcare and Inventiv Health and personal fees from Weston Brain Institute. HIH reports royalties from UpToDate. EBL reports personal fees from GLG Consulting, the

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