J Neuropathol Exp Neurol
Vol. 76, No. 3, March 2017, pp. 216–224
doi: 10.1093/jnen/nlx002

ORIGINAL ARTICLE

Regional Overlap of Pathologies in Lewy Body Disorders

Martí Colom-Cadena, MSc, Oriol Grau-Rivera, MD, Lluís Planellas, MD, Catalina Cerquera, MD, MSc, Estrella Morenas, MD, Sergio Helgueta, MSc, Laia Muñoz, BSc, Jaime Kulisevsky, MD, PhD, Maria Jose Martí, MD, PhD, Eduard Tolosa, MD, PhD, Jordi Clarimon, PhD, Alberto Lleó, MD, PhD, and Ellen Gelpi, MD, PhD

Abstract

Lewy body disorders (LBD) are common neurodegenerative diseases characterized by the presence of aggregated α-synuclein in Lewy bodies and Lewy neurites in the central and peripheral nervous systems. The brains of patients with LBD often display other comorbid pathologies, i.e. insoluble tau, β-amyloid aggregates, TAR DNA-binding protein 43 (TDP-43) deposits, and argyrophilic grain disease (AGD). The incidence and physiological relevance of these concurrent pathological findings remain controversial. We performed a semiquantitative detailed mapping of α-synuclein, tau, β-amyloid (Aβ), TDP-43, and AGD pathologies in 17 areas in 63 LBD cases (44 with Parkinson disease [PD], 28 with dementia, and 19 with dementia with Lewy bodies). *APOE* and *MAPT* genetic variants were also investigated. A majority of LBD cases had 2 or 3 concomitant findings, particularly Alzheimer disease-related pathology. Pathological stages of tau, β-amyloid and α-synuclein pathologies were increased in cases with dementia. Aβ score was the best correlate of the time to dementia in PD. In addition, β-amyloid deposition correlated with α-synuclein load in all groups. *MAPT* H1 haplotype did not influence any assessed pathology in PD. These results highlight the common concurrence of pathologies in patients with LBD that may have an impact on the clinical expression of the diseases.

Key Words: α-Synuclein, β-Amyloid, Copathology, Dementia with Lewy bodies, Parkinson disease, Parkinson disease dementia, Tau.

INTRODUCTION

Lewy body disorders (LBD) are a common group of neurodegenerative diseases that include Parkinson disease (PD) and dementia with Lewy bodies (DLB). They are neuropathologically characterized by the presence of aggregated α-synuclein in Lewy bodies (LBs) and Lewy neurites (LNs) in the central and peripheral nervous systems (1, 2).

There is substantial controversy concerning the correlates between the different clinical syndromes and the underlying pathologies in LBD (1–3). The recent reports that common pathologies other than α-synuclein are frequently found in the CNS of patients with LBD has led to uncertainty as to the relative contribution of each of these pathologies to the respective clinical syndrome. In particular, the neuropathological substrate of dementia has been associated with either cortical, striatal or limbic regional aggregation of α-synuclein in some studies (4–7), or with a combination of α-synuclein and hyperphosphorylated tau (pTau) and/or β-amyloid pathology load in others (8–15). The relationship between Alzheimer disease (AD)- and Lewy-related pathologies is supported by studies in cellular and animal models describing synergistic effects between α-synuclein, pTau, and β-amyloid (16–21). In human postmortem studies, there have been conflicting results showing a correlation between α-synuclein and pTau (4, 10, 22) and/or β-amyloid (4, 8,23–27), or even no correlation (28, 29). In addition, aggregates of TAR DNA-binding protein 43 (TDP-43) are often encountered in the brain of patients with LBD (10, 30–33).

Finally, genetic variants may also modulate the confluence and severity of pathologies in LBD. *APOE* ϵ4 allele and *MAPT* H1 haplotype, among others, have been associated with an increase of β-amyloid and/or α-synuclein deposition (10, 27, 34–38).

We previously assessed the presence of α-synuclein, pTau, β-amyloid, and TDP-43 pathologies in patients with DLB and found that a majority of cases had concomitant AD-

From the Department of Neurology, Institut d'Investigacions Biomèdiques Sant Pau - Hospital de Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain (MC-C, EM, SH, LM, JC, AL); CIBERNED, Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas, Madrid, Spain (MC-C, EM, SH, LM, JK, MJM, ET, JC, AL); Neurological Tissue Bank, Biobanc Hospital Clínic-IDIBAPS, Barcelona, Spain (OG-R, ET, EG); Parkinson's Disease and Movement Disorders Unit, Hospital Clinic, University of Barcelona, Barcelona, Spain (LP, CC, MJM); Neurology Unit, Hospital Universitario San Ignacio, School of Medicine, Pontificia Universidad Javeriana, Bogotá, Colombia (CC); and Movement Disorders Unit, Neurology Department, Hospital Sant Pau, Autonomous University of Barcelona, Universitat Oberta de Catalunya, Barcelona, Spain

Send correspondence to: Ellen Gelpi, MD, PhD, Neurological Tissue Bank, Biobanc Hospital Clínic-IDIBAPS. C/Casanova 143, planta 0, ala sur, 08036 Barcelona, Spain; E-mail: ellen.gelpi@idibaps.org Alberto Lleó, MD, PhD. Neurology Department, Hospital de Sant Pau. C/ Sant Antoni M Claret, 167, 08025, Barcelona, Spain. E-mail: alleo@santpau.cat

This work was funded in part by "CIBERNED". Part of this study was funded by a grant of the Fundació Marató de TV3 to EG (grant no. 20141610) and the Fondo de Investigación Sanitario to AL (PI14/1561). Dr Kulisevsky has received research support from CIBERNED and FIS PI 15/00962 and PI12/03005 (Instituto de Salud Carlos III), Fundació La marató de TV3 20142410 and 2910, and AGAUR SGR1203.

The authors do not have any actual or potential conflicts of interest.

Supplementary Data can be found at http://www.jnen.oxfordjournals.org.

related pathology in addition to α-synuclein pathology (39). Moreover, the best predictors of the total amount of Lewy-related pathology were β-amyloid load and the presence of the *MAPT* H1 haplotype (39). The aim of the present work was to extend that study and map the deposition pattern of 5 pathologies (α-synuclein, pTau, β-amyloid, TDP-43 and argyrophilic grain disease [AGD]) in 17 brain regions in a group of PD patients, with and without dementia, and DLB cases. We also evaluate their associations with clinical manifestations and *APOE* ϵ4 and *MAPT* H1 genetic variants.

MATERIALS AND METHODS

Standard Protocol Approvals and Patient Consents

All brain donors and/or next of kin had given written informed consent for the use of brain tissue for research. The study was approved by the local ethics committee of the Hospital de Sant Pau, Barcelona, Spain.

Human Brain Samples

Human brain samples were obtained from the Neurological Tissue Bank (NTB) of the Biobanc-Hospital Clinic-IDIBAPS in Barcelona. Brain sampling and tissue processing protocols were carried out as previously described (40) and as internationally recommended. Sixty-three patients fulfilling clinical and neuropathological criteria for PD ($n = 44$, 16 without dementia and 28 with dementia) and DLB ($n = 19$) (41, 42) were studied. Clinical data were retrospectively reviewed from the clinical charts available at the NTB and the following data were obtained: age at disease onset, age at onset of dementia in PD and age at death. Neuropathological and genetic data of the DLB cases has already been published elsewhere (36, 39).

Neuropathological Assessment

Formalin-fixed and paraffin-embedded tissue blocks from 17 brain regions were included in the study; they included 4 cortical areas (frontal, parietal, temporo-occipital adjoining the parahippocampal region, and occipital cortices including the primary visual area), 2 subcortical areas (caudate nucleus and putamen), 5 limbic areas (cingulate, hippocampus CA1 sector, amygdala, entorhinal region, and insula), and 6 brainstem areas (substantia nigra, periaqueductal grey matter, dorsal raphe, locus coeruleus, dorsal nucleus of the vagal nerve and intermediate reticular zone). The cerebellum was assessed only for β-amyloid pathology.

A modified Bielschowsky silver impregnation method was performed on paraffin sections in selected brain areas to evaluate neuritic plaques (48, 50). Immunohistochemistry was performed on 5-μm-thick sections on an automated stainer (DAKO Autostainer Plus, Glostrup, Denmark) using the following primary antibodies and their corresponding pretreatments: anti-β-amyloid (DAKO, clone 6F/3D, dilution 1:400, pre-treatment with formic acid 98% for 5 minutes followed by boiling ethylenediamine tetraacetic acid (EDTA) pH9 for 20 minutes in microwave), anti-phosphorylated tau (pTau, Thermo Scientific, Waltham, MA, clone AT8, dilution 1:2000, pre-treatment with citrate buffer pH6 boiling for 20 minutes in microwave), anti-tau 3-repeat isoform (RD3) (Millipore, Billerica, MA, clone 8E6/C11, dilution 1:1000, pre-treatment with formic acid 98% for 5 minutes followed by boiling in citrate buffer pH6 for 20 minutes in microwave), anti-tau 4-repeat isoform (RD4) (Millipore, clone 1E1/A6, dilution 1:50, pre-treatment with formic acid 98% for 5 minutes followed by boiling in citrate buffer pH6 for 20 minutes in microwave), anti-α-synuclein (Novocastra, Newcastle, UK, clone KM51, dilution 1:500, pre-treatment with formic acid 98% for 5 minutes followed by boiling in EDTA pH9 for 20 minutes in microwave), and anti-TAR DNA-binding protein 43 (TDP-43) (Abnova, Taipei, Taiwan, clone 2E2-D3, dilution 1:500, pre-treatment with citrate buffer pH6 boiling for 20 minutes in microwave). Reaction was visualized using the EnVision+ system peroxidase procedure (Dako).

The densities of α-synuclein, pTau, and β-amyloid pathologies were assessed in a 100× magnification field (3.88 mm^2), as previously described (39). In brief and as illustrated in Figure 1, the density of AT8-immunoreactive structures (neurofibrillary tangles [NFT], neuropil threads [NT] and pretangles [pT]) was assessed separately and semiquantitatively as follows: 0: absent; 1: isolated (NT) or 1–2 aggregates (NFT + pT); 2: mild or 3–6 aggregates; 3: moderate or 7–10 aggregates; 4: severe or >10 aggregates. A final stage of neurofibrillary pathology was given according to the Braak staging system (43, 44); when appropriate, primary age-related tauopathy (PART) criteria were also applied (45). When the presence of AGD was suspected, immunohistochemistry for 4-repeat and 3-repeat tau isoforms was performed and a Saito stage was given (46).

The density of α-synuclein immunoreactive structures (Lewy bodies defined as intracytoplasmic round α-synuclein-reactive aggregates (LBs), Lewy neurites (LNs) and diffuse and punctate cytoplasmic α-synuclein staining (CAS) was assessed separately and semiquantitatively as follows: 0: absent; 1: isolated (LN) or 1–2 aggregates (LB + CAS); 2: mild or 3–6 aggregates; 3: moderate or 7–10 aggregates; 4: severe or >10 aggregates. A final stage of LB pathology was given according to Braak criteria (47); LB type pathology was given according to McKeith criteria (42). Although these classifications should strictly only be applied to a specific disease (eg LB Braak stages to PD and McKeith types to DLB), we applied both criteria to all cases for the purpose of comparison.

The density of β-amyloid-immunoreactive structures (diffuse plaques, primitive plaques defined as a compact β-amyloid structure without a clear core, and mature or cored plaques described when the core was differentiated) (Fig. 1) was assessed separately and semiquantitatively as follows: 0: absent; 1: isolated: 1–5 plaques; 2: mild: 6–15 plaques; 3: moderate: 16–30 plaques: 4: severe >30 plaques. Staging of AD-related plaque pathology was performed according to Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria (neuritic plaque score) (48) and amyloid phases were assessed according to Thal et al. (49). The National Institute on Aging-Alzheimer's Association Guidelines for neuropathologic assessment of AD were applied and a final ABC score was assigned (50). Cerebral amyloid angiop-

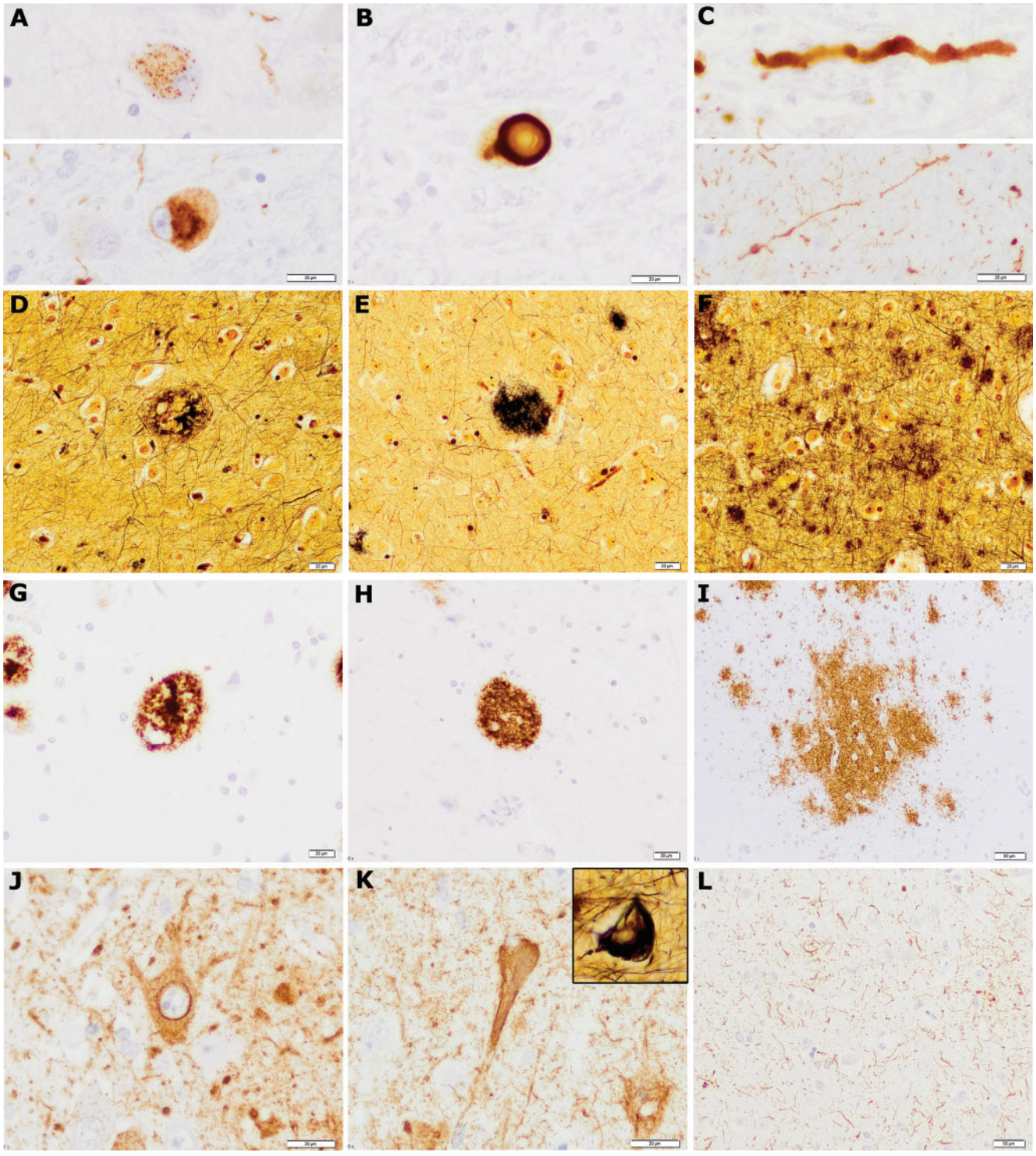

FIGURE 1. Types of pathological aggregates. **(A–C)** Assessment of α-synuclein immunoreactive structures. Punctate (**A,** upper panel) and diffuse (**A,** lower panel) cytoplasmic α-synuclein staining (CAS). Lewy bodies are defined as round intracytoplasmic aggregates **(B)**. Thick Lewy neurites (**C,** upper panel) and thin Lewy neurites (**C,** lower panel). **(D–F)** Bielschowsky silver impregnation showing different types of plaques including neuritic plaque (**D),** primitive plaque **(E),** and a diffuse plaque **(F). (G–I)** Assessment of β-amyloid immunoreactive structures. Mature or cored plaques are described when the core is differentiated **(G)**, primitive plaques are defined as a compact β-amyloid structure without a clear core **(H)** and diffuse deposits **(I)**. **(J–L)** Assessment of AT8-immunoreactive structures include pretangles **(J)**, neurofibrillary tangle (**K,** inset shown by Bielschowsky silver impregnation), and neuropil threads **(L).**

athy (CAA) with or without capillary involvement was reported as present or absent in each region and staged according to Vonsattel et al. in stages 1–3 (51). TDP-43 protein aggregates were reported as present or absent in frontotemporal and limbic regions.

For each subject, a total α-synuclein, pTau, and β-amyloid pathology score was given as a sum of the 17 areas. Scores ranged from 0 to 68 for each of the 3 types of pTau, α-synuclein or β-amyloid aggregate, and from 0 to 204 for total pTau, α-synuclein, or β-amyloid pathology (representing the sum of all type of aggregates). Neuropathological evaluation was carried out by 2 of the authors (M.C.C., E.G.) on a multiheaded microscope. Both evaluators were blinded to clinical and genetic data.

Genetic Analyses

DNA for *MAPT* haplotypes and *APOE* genotypes determination was extracted from frozen brain tissue and was processed as previously described (52).

Statistical Analyses

The Mann–Whitney U or Kruskal–Wallis with Dunn's analysis post-hoc tests were used to compare differences in neuropathological measures and clinical variables between groups. Differences in categorical variables were compared between groups by Chi-squared test. Nonparametric Spearman rho correlation coefficient was used to analyze correlations between the total scores of each pathologic aggregate and between clinical variables. Statistical significance was set at 5% ($\alpha = 0.05$). All data were analyzed using the Statistical Package for the Social Sciences version 19.0 (SPSS Inc., Chicago, IL).

RESULTS

Demographic, Clinical, and Genetic Characteristics of LBD Cases

Demographic, clinical and genetic data are shown in the Table. Disease duration was shorter in the DLB group compared with both PD and PD with dementia (PDD) groups ($p < 0.001$). Age at onset of disease was significantly later in DLB compared to PDD ($p = 0.011$). PDD cases had significantly shorter disease duration after dementia onset compared with DLB cases ($p = 0.001$). The 3 clinical groups had similar frequencies of *MAPT* H1H1 genotype. PDD and DLB groups had a higher frequency of *APOE* ϵ4 allele compared with the PD group.

Confluence of Multiple Pathologies in LBD

To assess the number of coexistent pathologies other than α-synuclein in LBD, the presence of pTau (Braak NFT stage ≥III), β-amyloid (Thal phase ≥2), AGD (Saito stage ≥II) and presence of TDP-43 pathology was calculated for each case (Fig. 2). Only 25% of PD and 5.26% of DLB cases had no copathologies. The majority of cases from all groups had 1 or 2 coexisting pathologies and 17.9% of PDD and 21% of DLB cases had 3 overlapping pathologies. Only 1 PDD

TABLE. Demographic, Clinical, Genetic, Neuropathological Diagnoses and Stages of Cases

	PD (n = 16)	PDD (n = 28)	DLB (n = 19)	PD-PDD	PD-DLB	PDD-DLB
				p value	p value	p value
Gender (% female)	56.3	35.7	26.3	0.186	0.072	0.498
Age at onset (years ± SD)	62.8 ± 10.2	60 ± 9.8	68.4 ± 6	0.592	0.198	0.011*
Age at death (years ± SD)	79.5 ± 6.5	78 ± 6.2	76.7 ± 5.6	0.675	0.349	0.765
Duration of disease (years ± SD)	17.2 ± 8.1	17.4 ± 7.4	7.9 ± 3.2	0.965	0.000**	0.000**
Dementia onset from motor symptoms (years ± SD)	–	13 ± 7.1	–	–	–	–
Duration of disease from the appearance of dementia (years ± SD)	–	4.4 ± 4.9	7.9 ± 3.2	–	–	0.001**
MAPT H1H1 genotype (%)	56.3	60.7	52.6	0.772	0.830	0.582
APOE ϵ4 allele (%)	6.7	32.1	36.8	0.059	0.039*	0.739
Brain weight (g ± SD)	1228 ± 148	1247 ± 111	1263 ± 207	0.643	0.582	0.731
McKeith types (% neocortical)	50	85.7	100	0.008**	0.008**	0.088
LB Braak stage (25th, 75th percentile)	5 (4, 5)	5 (5, 5)	5 (5, 5)	0.024*	0.004**	0.033*
Thal amyloid *β* phase (25th, 75th percentile)	2 (1, 3)	4 (3, 5)	4 (3, 5)	0.000**	0.004**	0.625
CERAD plaque score (% C)	0	14.3	31.6	0.000**	0.001**	0.113
CAA (% 1, 2 or 3)	37.6	57.1	57.9	0.215	0.318	0.960
NFT Braak stage (25th, 75th percentile)	II (II, III)	III (II, IV)	V (III, V)	0.041*	0.000**	0.003**
PART (% possible or definite)	62.5	17.9	15.8	0.003**	0.018*	0.855
Saito AGD stage (% II or III)	25	21.4	10.5	0.424	0.492	0.787
TDP-43 pathology in limbic areas (%)	0	21.4	21.1	0.049*	0.051	0.976

p values of Mann–Whitney U or χ^2 statistical tests are shown.

AGD, argyrophilic grain disease; CAA, cerebral amyloid angiopathy; LB, Lewy bodies; LBD, Dementia with Lewy bodies; LN, Lewy neurites; NFT, neurofibrillary tangles; PART, primary age-related tau pathology; PD, Parkinson disease; PDD, Parkinson disease with dementia; SD, standard deviation; TDP-43, TAR DNA-binding protein 43; *$p < 0.05$; **$p < 0.01$; –, not applicable.

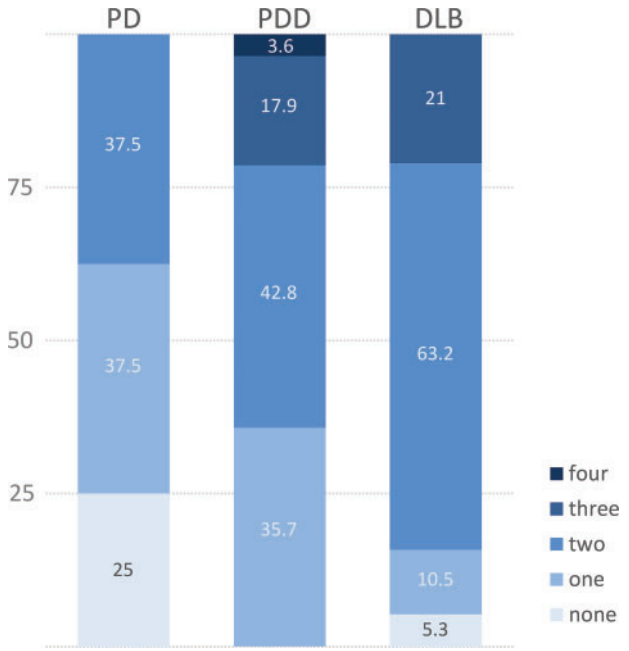

FIGURE 2. Number of coexisting pathologies in LBD. Cases in the PD, PDD, or DLB groups were grouped according to the number of coexisting pathologies (other than α-synuclein). Numbers of copathologies are expressed as a percentage of total cases in each group (represented by the numbers inside columns). Coexisting pathologies included pTau with Braak NFT stage ≥III, β-amyloid with Thal phase ≥2, AGD with Saito stage ≥II and/or presence of TDP-43 pathology.

case had all 4 assessed pathologies. Individual data for all cases can be found in Supplementary Data S1.

Clinicopathologic Associations of α-Synuclein

The groups with dementia (PDD and DLB) showed higher levels and wider distribution of α-synuclein deposition than PD (Table). DLB cases had higher LB Braak stages than those with PDD.

Regarding the regional distribution of α-synuclein aggregates, the amygdala was the most affected area in all groups, with less involvement of cortical areas (Fig. 3A). Nevertheless, the differences in α-synuclein load between PD and dementia groups were greatest in cortical areas. The only region that showed differences in α-synuclein load between PDD and DLB cases was the striatum (both caudate nucleus and putamen, DLB > PDD). The total α-synuclein score positively correlated with that of β-amyloid in all LBD groups (PD: $r = 0.720$, $p = 0.002$; PDD: $r = 0.705$, $p < 0.001$; DLB: $r = 0.775$, $p < 0.001$). The presence of *MAPT* H1 haplotype was associated with higher α-synuclein total scores in DLB ($p = 0.004$), as previously reported (36), but this association was not found in PDD or in PD groups.

Regarding associations with clinical variables other than dementia, there was a positive correlation between α-synuclein scores and higher age at death in PD cases ($r = 0.574$, $p = 0.02$).

Clinicopathologic Associations of pTau

Braak NFT stage ≥III was found in 31.3% of PD, 53.6% of PDD, and 89.5% of DLB cases.

As shown in the Table, the patients with dementia (PDD and DLB groups) showed higher NFT Braak stages then those without (PD group). DLB cases also had higher NFT Braak stages than PDD cases.

Possible or definite PART was found in 62.5% of PD compared with only 17.9% and 15.8% of PDD and DLB cases, respectively, due to the higher β-amyloid burden in those cases (see below). However, only 2 PD and 2 DLB cases met criteria for definite PART, while the other cases met criteria for possible PART or for AD (Table). AGD stages II or III were present in 18.5% of LBD cases but no differences were found in the frequency of AGD among the LBD subgroups (Table).

Limbic regions were the most affected by pTau pathology in all 3 LBD subgroups (Fig. 3C). A significant increase of pTau score was observed in DLB compared with PD (but not PDD) in brainstem, limbic, subcortical and cortical areas. Comparing PD and PDD, pTau scores were found only significantly increased in PDD in the CA1 sector of the hippocampus and in the frontal and occipital cortices. As expected, the level of pTau aggregates positively correlated with those of β-amyloid in PDD ($r = 0.559$, $p = 0.006$) and DLB ($r = 0.675$, $p = 0.002$) cases. Total pTau pathology scores or the presence of AGD were not associated with the levels of α-synuclein or TDP-43, and were not influenced by the *APOE* allele or *MAPT* haplotype.

Regarding associations with clinical variables other than dementia, DLB cases with AGD stages II or III had an older age at death ($p = 0.012$). The 2 DLB patients with "definite PART" and the only DLB case with "possible PART" all had a younger age at death than the rest of DLB cases ($p = 0.047$).

Clinicopathologic Associations of Aβ

Aβ Thal phase ≥2 was found in 56.6% of PD, 96.4% of PDD, and 78.9% of DLB cases. The groups with dementia (PDD and DLB) showed higher Thal Aβ-phases and CERAD plaque scores than PD indicating a wider distribution of β-amyloid deposits (Table). No differences in Aβ-phases were found between DLB and PDD, although the DLB group had nearly twice as much plaque score "C" cases than the PDD group. CAA was common in all LBD groups with no significant differences between groups.

Both PDD and DLB showed more β-amyloid deposits (diffuse, primitive, and cored plaques) than PD in all limbic, subcortical and cortical areas (Fig. 3B). In general, the DLB group had more β-amyloid plaques than the PDD group but this difference was only significant in the entorhinal and the temporo-occipital cortices.

As previously mentioned, Aβ scores correlated with both α-synuclein and pTau scores. As expected, patients carrying an *APOE* ϵ4 allele had higher total β-amyloid pathology in both PDD and DLB cases ($p = 0.04$ in PDD, $p = 0.002$ in DLB). Moreover, *APOE* ϵ4 carriers among the PDD group also had higher α-synuclein load ($p = 0.024$).

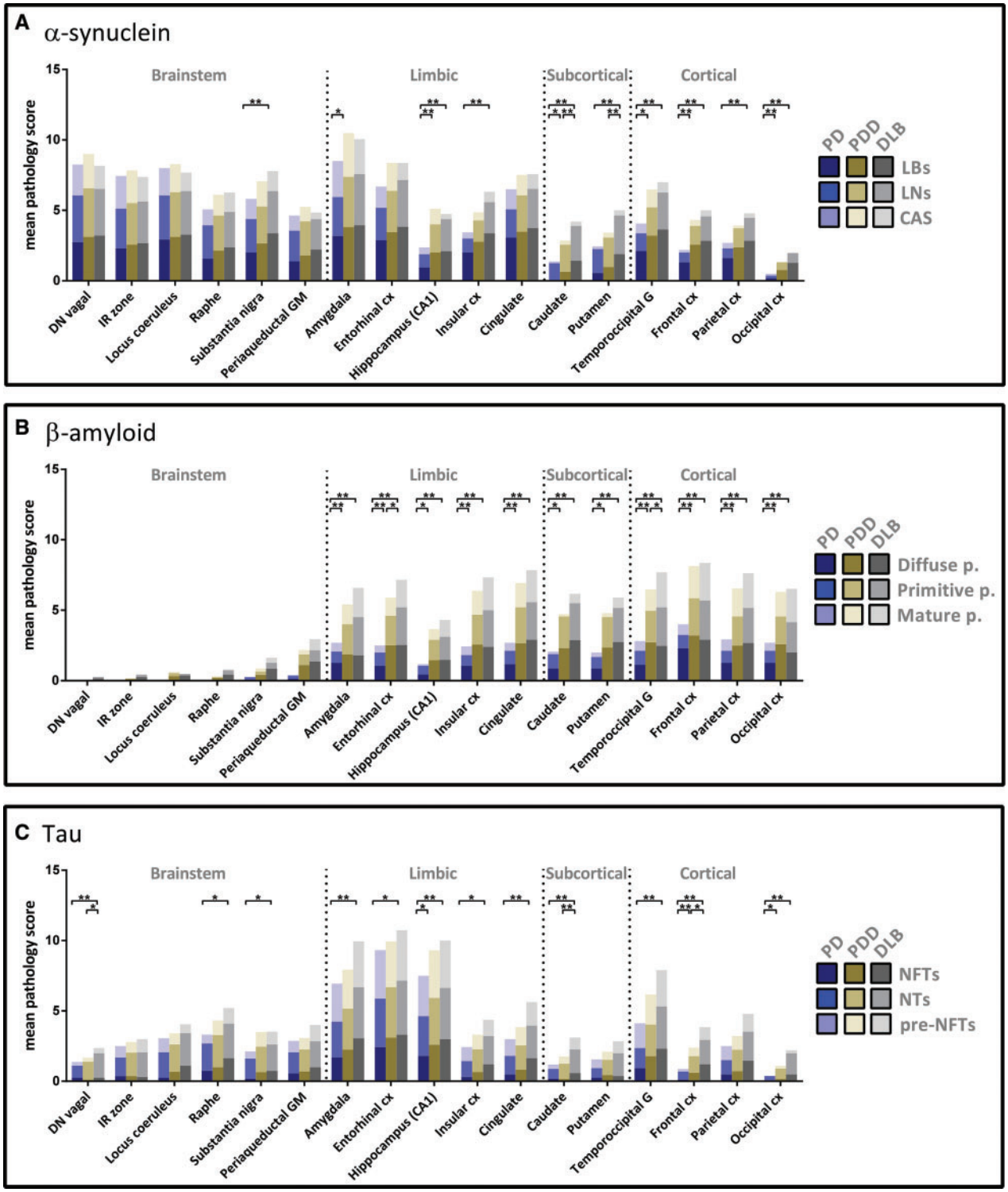

FIGURE 3. Regional distribution of pathologies among PD, PDD and DLB groups. **(A–C)** Mean pathological scores are shown for each pathological entity across 17 brain regions as follows: α-synuclein **(A)**, β-amyloid **(B)**, and pTau **(C)**. Horizontal lines show significant differences among groups in total scores (*$p < 0.05$; **$p < 0.01$). PD cases are represented in blue bars, PDD cases in brown and DLB cases in grey. The color grading from darker to lighter represents the type of assessed aggregate (as illustrated in Fig. 1). LBs, Lewy bodies; LNs, Lewy neurites; CAS, diffuse and punctate cytoplasmic α-synuclein staining; NFTs, neurofibrillary tangles, NTs, neuropil threads; DN vagal, Dorsal nucleus of the vagal nerve; IR, intermediate reticular zone; GM grey matter; CA1, cornu ammonis sector 1; cx, cortex.

Regarding clinical variables, a shorter time to dementia from the onset of motor symptoms in PDD was associated with higher scores of β-amyloid pathology ($r = -0.475$, $p = 0.046$).

Clinicopathologic Associations of TDP-43

The deposition of TDP-43 in limbic areas was frequently found in groups with dementia (PDD and DLB) and was not observed in PD cases (Table). The presence of abnormal TDP-43 protein aggregates was not related to higher α-synuclein scores in the LBD spectrum. However, PDD and DLB cases harboring TDP-43 pathology showed a trend to have higher loads of the 3 pathological aggregates (data not shown). No association was found between the presence of TDP-43 aggregates and any of the assessed clinical variables.

DISCUSSION

In this study, we performed a detailed mapping of 5 common neurodegeneration-related proteinopathies in the LBD clinical spectrum. Our results highlight that the presence of different neurodegenerative pathologies is common in LBD and that the degree of Aβ pathology correlates with that of α-synuclein and may influence the onset of dementia in patients with PD.

Common and Widespread Confluence of Pathologies in LBD

Most of the LBD cases assessed in this study showed some degree of AD-related pathology, including CAA, and a relevant number of cases also showed TDP-43 and AGD pathologies. These findings are consistent with a number of studies suggesting that only a small fraction of cases can be explained by a single neuropathological entity (8, 15, 53–57).

These copathologies may be related to aging, as in the case for PART (45), (i.e. 62.5% of PD cases in the present study), or may be part of a neurodegenerative cascade (58). In any scenario, the "secondary" pathologies may interact with the "primary" pathology. Our finding of a positive correlation between Aβ and α-synuclein in LBD cases (and particularly in PD) support an active role of β-amyloid pathology. In postmortem studies of LBD, a positive correlation between β-amyloid and α-synuclein has been described (4,8, 23–27), although this has not been confirmed by others (10, 22, 28, 29). In cellular and animal models, the relationship between β-amyloid and α-synuclein has been also investigated, resulting in a number of possible pathogenetic mechanisms from increased mutual aggregation (20, 59–64) to inhibition of β-amyloid formation by α-synuclein (65). The mechanisms of interaction are still debated; it is possible that different forms or strains of α-synuclein (66, 67) may explain some of the divergent results obtained in mechanistic disease models and in human studies in LBD.

Effect of Copathologies on Dementia: β-Amyloid Pathology

The frequency and impact of AD-related pathology on the risk and onset of dementia in LBD has been widely discussed but it is still a matter of debate (1, 3, 68). We found that accumulations of α-synuclein, pTau, and β-amyloid were higher in most areas in DLB than in PDD, which in turn were higher than in PD. Our data point to a possible major role of β-amyloid pathology in the appearance of dementia in patients with LBD. Higher levels of β-amyloid pathology were found in all limbic, subcortical, and cortical areas in cases with dementia, and its presence lowered the age of onset of dementia in PD. These results are in line with a recent quantitative study where β-amyloid load was found to be increased in LBD cases with dementia (15). However, pTau may also be indirectly involved in our findings because pTau pathology is closely related to β-amyloid deposits and many molecular links have been proposed (17, 19, 69–72). Despite the possible role of β-amyloid and pTau on dementia onset, it might not be an essential condition because we and others (2, 15) found demented cases with minimal or even absent pTau and β-amyloid loads.

Regarding other postmortem studies assessing the impact of copathologies on dementia in LBD, some groups described a major role of cortical, striatal or limbic regional aggregation of α-synuclein on the development of dementia (4–7), while others suggest an effect of the combination of α-synuclein and pTau and/or β-amyloid (8–14).

The differences between these previous studies include regions assessed, staining methods used, and statistical tests applied, which makes comparisons of results difficult. Taken together, our results and the previous findings support the idea of a disease-modulating role of coexisting pathologies. In turn, the distribution of pathologies reproduced the staging systems proposed for each individual pathology, likely reflecting a disease-independent and pathology-specific topographical pattern rather than disease-specific copathology.

Effect of Other Copathologies: TDP-43 and AGD

We observed a higher presence of TDP-43 pathology in cases with dementia (PDD and DLB groups), which is in general agreement with previous reports (30, 33, 73). Moreover, in agreement with others (10, 32, 74), AGD pathology was not associated with dementia in LBD, which suggests that AGD may be involved in other clinical manifestations (75). AGD has been reported to affect elderly patients (76), and indeed our DLB patients with AGD were older at death.

The Influence of Genetic Variants

Finally, we assessed the effect of common genetic variants on the presence and severity of α-synuclein, β-amyloid, and pTau pathologies in LBD. As previously reported, we found an overrepresentation of the *APOE* ϵ4 allele in DLB cases compared with PD cases (1) and *APOE* ϵ4 carriers showed higher β-amyloid load.

The *MAPT* H1 haplotype has been identified as a risk factor in genome-wide association studies in PD (77, 78) and in neuropathologically confirmed DLB and PDD cases (79, 80). *MAPT* H1 has been also associated with an increased risk of dementia in PD in prospective studies (81). We have previously described an increase of LB load in *MAPT* H1H1 car-

riers in DLB (36), but we could not extend this observation to PDD or PD cases in the present study. It might be possible that this association is only detectable in those cases with higher levels of α-synuclein pathology, such as DLB. Nonetheless, a recent study found more abundant LB aggregates in neocortical areas in PD patients carrying the *MAPT* H1 haplotype (37). In contrast, another study found no association between *MAPT* H1H1 carriers and α-synuclein pathology in PD, PDD, and DLB (14). Taken together, our findings along with previous studies indicate that the *MAPT* H1H1 haplotype may favor α-synuclein aggregation at least in some subtypes of LBD, although this needs to be confirmed in larger neuropathological series.

Although our study is based on a relatively small sample size with the inherent limitations of retrospective postmortem series (particularly the lack of reliable clinical data on cognitive measures), the detailed assessment of multiple pathologies in several brain regions and the analyses of some clinical variables and common genetic risk factors strengthen our results.

Concluding Remarks

In summary, our results highlight the frequent confluence of α-synuclein, β-amyloid, pTau, TDP-43, and AGD pathologies in LBD and particularly emphasize the role of AD pathology on the appearance of dementia in PD. Moreover, β-amyloid pathology seems to be intimately related with α-synuclein. These common and interrelated pathologies may influence the clinical phenotypes in LBD and be used to refine biomarker-based diagnostics and develop better therapeutic strategies for LBD.

ACKNOWLEDGMENTS

We are indebted to the Neurological Tissue Bank of the Biobanc-Hospital Clinic-IDIBAPS for data and sample procurement and to Sara Charif and Veronica Santiago for excellent technical support. We deeply acknowledge brain donors and their families for generous brain donation for research. The authors also thank Olivia Belbin for editing support.

REFERENCES

1. Halliday GM, Leverenz JB, Schneider JS, et al. The neurobiological basis of cognitive impairment in Parkinson's disease. Mov Disord 2014;29:634–50
2. Walker Z, Possin KL, Boeve BF, et al. Lewy body dementias. Lancet 2015;386:1683–97
3. Irwin DJ, Lee VM, Trojanowski JQ. Parkinson's disease dementia: Convergence of α-synuclein, tau and amyloid-β pathologies. Nat Rev Neurosci 2013;14:626–36
4. Apaydin H, Ahlskog JE, Parisi JE, et al. Parkinson disease neuropathology. Arch Neurol 2002;59:102
5. Hall H, Reyes S, Landeck N, et al. Hippocampal Lewy pathology and cholinergic dysfunction are associated with dementia in Parkinson's disease. Brain 2014;137:2493–508
6. Hurtig HI, Trojanowski JQ, Galvin J, et al. Alpha-synuclein cortical Lewy bodies correlate with dementia in Parkinson's disease. Neurology 2000;54:1916–21
7. Horvath J, Herrmann FR, Burkhard PR, et al. Neuropathology of dementia in a large cohort of patients with Parkinson's disease. Park Relat Disord 2013;19:864–8
8. Compta Y, Parkkinen L, O'Sullivan SS, et al. Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: Which is more important? Brain 2011;134:1493–505
9. Kempster PA, O'Sullivan SS, Holton JL, et al. Relationships between age and late progression of Parkinson's disease: A clinico-pathological study. Brain 2010;133:1755–62
10. Irwin DJ, White MT, Toledo JB, et al. Neuropathologic substrates of Parkinson disease dementia. Ann Neurol 2012;72:587–98
11. Kalaitzakis ME, Walls AJ, Pearce RKB, et al. Striatal Aβ peptide deposition mirrors dementia and differentiates DLB and PDD from other Parkinsonian syndromes. Neurobiol Dis 2011;41:377–84
12. Kalaitzakis ME, Graeber MB, Gentleman SM, et al. Striatal β-amyloid deposition in Parkinson disease with dementia. J Neuropathol Exp Neurol 2008;67:155–61.
13. Halliday GM, Song YJC, Harding AJ. Striatal β-amyloid in dementia with Lewy bodies but not Parkinson's disease. J Neural Transm 2011;118:713–9
14. Ruffmann C, Calboli FCF, Bravi I, et al. Cortical Lewy bodies and Aβ burden are associated with prevalence and timing of dementia in Lewy body diseases. Neuropathol Appl Neurobiol 2016;42:436–50
15. Hepp DH, Vergoossen DLE, Huisman E, et al. Distribution and load of amyloid-β pathology in Parkinson disease and dementia with Lewy Bodies. J Neuropathol Exp Neurol 2016;75:936–45
16. Giasson BI. Initiation and synergistic fibrillization of tau and α-synuclein. Science 2003;300:636–40
17. Badiola N, de Oliveira RM, Herrera F, et al. Tau enhances α-synuclein aggregation and toxicity in cellular models of synucleinopathy. PLoS One 2011;6:e26609
18. Uversky VN. Neuropathology, biochemistry, and biophysics of α-synuclein aggregation. J Neurochem 2007;103:17–37
19. Ciaccioli G, Martins A, Rodrigues C, et al. A powerful yeast model to investigate the synergistic interaction of α-synuclein and tau in neurodegeneration. PLoS One 2013;8:e55848
20. Marsh SE, Blurton-Jones M. Examining the mechanisms that link β-amyloid and α-synuclein pathologies. Alzheimers Res Ther 2012;304:11
21. Clinton LK, Blurton-Jones M, Myczek K, et al. Synergistic Interactions between Abeta, tau, and alpha-synuclein: Acceleration of neuropathology and cognitive decline. J Neurosci 2010;30:7281–9
22. Jellinger KA, Attems J. Prevalence and impact of vascular and Alzheimer pathologies in Lewy body disease. Acta Neuropathol 2008;115:427–36
23. Mattila PM, Rinne JO, Helenius H, et al. Alpha-synuclein-immunoreactive cortical Lewy bodies are associated with cognitive impairment in Parkinson's disease. Acta Neuropathol 2000;100:285–90
24. Dickson DW, Fujishiro H, Orr C, et al. Neuropathology of non-motor features of Parkinson disease. Park Relat Disord 2009;15:S1–5
25. Tsuboi Y, Uchikado H, Dickson DW. Neuropathology of Parkinson's disease dementia and dementia with Lewy bodies with reference to striatal pathology. Park Relat Disord 2007;13:S221–4
26. Lashley T, Holton JL, Gray E, et al. Cortical α-synuclein load is associated with amyloid-β plaque burden in a subset of Parkinson's disease patients. Acta Neuropathol 2008;115:417–25
27. Pletnikova O, West N, Lee MK, et al. Aβ deposition is associated with enhanced cortical α-synuclein lesions in Lewy body diseases. Neurobiol Aging 2005;26:1183–92
28. Aarsland D, Perry R, Brown A, et al. Neuropathology of dementia in Parkinson's disease: A prospective, community-based study. Ann Neurol 2005;58:773–6
29. Attems J, Thomas A, Jellinger K. Correlations between cortical and subcortical tau pathology. Neuropathol Appl Neurobiol 2012;38:582–90
30. Cykowski MD, Takei H, Van Eldik LJ, et al. Hippocampal sclerosis but not normal aging or Alzheimer disease is associated with TDP-43 pathology in the basal forebrain of aged persons. J Neuropathol Exp Neurol 2016;75:397–407
31. Yokota O, Davidson Y, Arai T, et al. Effect of topographical distribution of α-synuclein pathology on TDP-43 accumulation in Lewy body disease. Acta Neuropathol 2010;120:789–801
32. Sabbagh MN, Sandhu SS, Farlow MR, et al. Correlation of clinical features with argyrophilic grains at autopsy. Alzheimer Dis Assoc Disord 2009;23:229–33
33. McAleese KE, Walker L, Erskine D, et al. TDP-43 pathology in Alzheimer's disease, dementia with Lewy Bodies, and ageing. Brain Pathol 2016;1–8

34. Kobayashi S, Tateno M, Park TW, et al. Apolipoprotein E4 frequencies in a Japanese population with Alzheimer's disease and dementia with Lewy bodies. PLoS One 2011;6:e18569
35. Polvikoski T, Sulkava R, Haltia M, et al. Apolipoprotein E, dementia, and cortical deposition of beta-amyloid protein. N Engl J Med 1995;333: 1242–7
36. Colom-Cadena M, Gelpi E, Martí MJ, et al. MAPT H1 haplotype is associated with enhanced α-synuclein deposition in dementia with Lewy bodies. Neurobiol Aging 2013;34:936–42
37. Robakis D, Cortes E, Clark LN, et al. The effect of MAPT haplotype on neocortical Lewy body pathology in Parkinson disease. J Neural Trans 2016;583–8
38. Wakabayashi K, Kakita A, Hayashi S, et al. Apolipoprotein E epsilon4 allele and progression of cortical Lewy body pathology in Parkinson's disease. Acta Neuropathol 1998;95:450–4
39. Colom-Cadena M, Gelpi E, Charif S, et al. Confluence of alpha-synuclein, tau, and beta-amyloid pathologies in dementia with Lewy bodies. J Neuropathol Exp Neurol 2013;72:1203–12
40. Balasa M, Gelpi E, Antonell A, et al. Clinical features and APOE genotype of pathologically proven early-onset Alzheimer disease. Neurology 2011;76:1720–5
41. Emre M, Aarsland D, Brown R, et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. Mov Disord 2007;1689–707
42. McKeith IG, Dickson DW, Lowe J, et al. Diagnosis and management of dementia with Lewy bodies: Third report of the DLB consortium. Neurology 2005;1863–72
43. Alafuzoff I, Pikkarainen M, Al-Sarraj S, et al. Interlaboratory comparison of assessments of Alzheimer disease-related lesions: A study of the BrainNet Europe Consortium. J Neuropathol Exp Neurol 2006;65: 740–57
44. Braak H, Alafuzoff I, Arzberger T, et al. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol 2006;112:389–404
45. Crary JF, Trojanowski JQ, Schneider JA, et al. Primary age-related tauopathy (PART): A common pathology associated with human aging. Acta Neuropathol 2014;128:755–66
46. Saito Y, Ruberu NN, Sawabe M, et al. Staging of argyrophilic grains: An age-associated tauopathy. J Neuropathol Exp Neurol 2004;63:911–8
47. Braak H, Del Tredici K, Rüb U, et al. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003;24:197–211
48. Mirra SS, Heyman A, McKeel D, et al. the Consortium to Establish a Registry for Alzheimers Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimers disease. Neurology 1991; 41:479–86
49. Thal DR, Rüb U, Orantes M, et al. Phases of A beta-deposition in the human brain and its relevance for the development of AD. Neurology 2002; 58:1791–800
50. Montine TJ, Phelps CH, Beach TG, et al. National Institute on Aging-Alzheimer's association guidelines for the neuropathologic assessment of Alzheimer's disease: A practical approach. Acta Neuropathol 2012; 123:1–11
51. Vonsattel JP, Myers RH, Hedley-Whyte ET, et al. Cerebral amyloid angiopathy without and with cerebral hemorrhages: a comparative histological study. Ann Neurol 1991;30:637–49
52. Clarimón J, Molina-Porcel L, Gómez-Isla T, et al. Early-onset familial Lewy body dementia with extensive tauopathy: A clinical, genetic, and neuropathological study. J Neuropathol Exp Neurol 2009;68:73–82
53. Halliday GM, Holton JL, Revesz T, et al. Neuropathology underlying clinical variability in patients with synucleinopathies. Acta Neuropathol 2011;122:187–204
54. Deramecourt V, Bombois S, Maurage CC, et al. Biochemical staging of synucleinopathy and amyloid deposition in dementia with Lewy bodies. J Neuropathol Exp Neurol 2006;65:278–88
55. Higashi S, Iseki E, Yamamoto R, et al. Concurrence of TDP-43, tau and α-synuclein pathology in brains of Alzheimer's disease and dementia with Lewy bodies. Brain Res 2007;1184:284–94
56. Kovacs GG, Molnár K, László L, et al. A peculiar constellation of tau pathology defines a subset of dementia in the elderly. Acta Neuropathol 2011;122:205–22
57. James BD. Dementia from Alzheimer disease and mixed pathologies in the oldest old. JAMA 2012;307:1798
58. Duyckaerts C, Braak H, Brion J, et al. PART is part of Alzheimer disease. Acta Neuropathol 2015;129:749–56
59. Winslow AR, Moussaud S, Zhu L, et al. Convergence of pathology in dementia with Lewy bodies and Alzheimer's disease: A role for the novel interaction of alpha-synuclein and presenilin 1 in disease. Brain 2014; 137:1958–70
60. Lin CL, Cheng YS, Li HH, et al. Amyloid-β suppresses AMP-activated protein kinase (AMPK) signaling and contributes to α-synuclein-induced cytotoxicity. Exp Neurol 2016;275:84–98
61. Overk CR, Cartier A, Shaked G, et al. Hippocampal neuronal cells that accumulate α-synuclein fragments are more vulnerable to Aβ oligomer toxicity via mGluR5-implications for dementia with Lewy bodies. Mol Neurodegener 2014;9:18
62. Tsigelny IF, Crews L, Desplats P, et al. Mechanisms of hybrid oligomer formation in the pathogenesis of combined Alzheimer's and Parkinson's diseases. PLoS One 2008;3:e3135
63. Masliah E, Rockenstein E, Veinbergs I, et al. β-Amyloid peptides enhance α-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. Proc Natl Acad Sci USA 2001;98:12245–50
64. Mandal PK, Pettegrew JW, Masliah E, et al. Interaction between Aβ peptide and α-synuclein: Molecular mechanisms in overlapping pathology of Alzheimer's and Parkinson's in dementia with Lewy body disease. Neurochem Res 2006;31:1153–62
65. Bachhuber T, Katzmarski N, McCarter JF, et al. Inhibition of amyloid-β plaque formation by α-synuclein. Nat Med 2015;21:802–7
66. Guo JL, Covell DJ, Daniels JP, et al. Distinct α-synuclein strains differentially promote tau inclusions in neurons. Cell 2013;154:103–17
67. Bousset L, Pieri L, Ruiz-Arlandis G, et al. Structural and functional characterization of two alpha-synuclein strains. Nat Commun 2013;4:2575
68. Gratwicke J, Jahanshahi M, Foltynie T. Parkinson's disease dementia: A neural networks perspective. Brain 2015;138:1454–76
69. Waxman EA, Giasson BI. Induction of intracellular tau aggregation is promoted by α-synuclein seeds and provides novel insights into the hyperphosphorylation of tau. J Neurosci 2011;31:7604–18
70. Kotzbauer PT, Giasson BI, Kravitz AV, et al. Fibrillization of α-synuclein and tau in familial Parkinson's disease caused by the A53T α-synuclein mutation. Exp Neurol 2004;187:279–88
71. Roy B, Jackson GR. Interactions between tau and α-synuclein augment neurotoxicity in a Drosophila model of parkinson's disease. Hum Mol Genet 2014;23:3008–23
72. Galpern WR, Lang AE. Interface between tauopathies and synucleinopathies: A tale of two proteins. Ann Neurol 2006;59:449–58
73. Josephs KA, Murray ME, Whitwell JL, et al. Updated TDP-43 in Alzheimer's disease staging scheme. Acta Neuropathol 2016;131:571–85
74. Braak H, Rüb U, Jansen Steur EN, et al. Cognitive status correlates with neuropathologic stage in Parkinson disease. Neurology 2005;64:1404–10
75. Grau-Rivera O, Gelpi E, Rey MJ, et al. Prominent psychiatric symptoms in patients with Parkinson's disease and concomitant argyrophilic grain disease. J Neurol 2013;260:3002–9
76. Pham CT, de Silva R, Haïk S, et al. Tau-positive grains are constant in centenarians' hippocampus. Neurobiol Aging 2011;32:1296–303
77. Simón-Sánchez J, Schulte C, Bras JJM, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet 2009;41:1308–12
78. Zabetian CP, Hutter CM, Factor SA, et al. Association analysis of MAPT H1 haplotype and subhaplotypes in Parkinson's disease. Ann Neurol 2007;62:137–44
79. Cervera-Carles L, Pagonabarraga J, Pascual-Sedano B, et al. Copy number variation analysis of the 17q21.31 region and its role in neurodegenerative diseases. Am J Med Genet 2016;171:175–80
80. Setó-Salvia N, Clarimón J, Pagonabarraga J, et al. Dementia risk in Parkinson disease: Disentangling the role of MAPT haplotypes. Arch Neurol 2011;68:359–64
81. Goris A, Williams-Gray CH, Clark GR, et al. Tau and α-synuclein in susceptibility to, and dementia in, Parkinson's disease. Ann Neurol 2007;62: 145–53