Confluence of α-Synuclein, Tau, and β-Amyloid Pathologies in Dementia With Lewy Bodies

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Abstract

Dementia with Lewy bodies (DLB) is pathologically characterized by α-synuclein aggregates in the brain. Most patients with DLB also show cerebral Alzheimer disease-type pathology (i.e. β-amyloid plaques and hyperphosphorylated tau deposits). It is unclear whether this overlap is coincidental or driven by specific regional or cellular interactions. The aims of this study were to investigate the regional convergence of α-synuclein, tau, and β-amyloid and to identify patterns of cellular co-occurrence of tau and α-synuclein in DLB. The study group consisted of 22 patients who met clinical and neuropathologic criteria for DLB. Protein aggregates were assessed semiquantitatively in 17 brain areas. APOE and MAPT genotypes were determined. Cellular co-occurrence of tau and α-synuclein was evaluated by double immunofluorescence. We found that total β-amyloid pathology scores correlated positively with total α-synuclein pathology scores (r = 0.692, p = 0.001). The factors that correlated best with the amount of α-synuclein pathology were the severity of β-amyloid pathology and presence of the MAPT H1 haplotype. Tau and α-synuclein frequently colocalized in limbic areas, but no correlation between total pathology scores was observed. This study confirms and extends the pathology of τ and the α-synuclein H1 haplotype, which are known to be associated with DLB. The coexistence of different pathologies in these neurodegenerative diseases occurs both at the regional and cellular levels. Tau and α-synuclein have been found to coaggregate in the same neuronal populations in the brain of patients with DLB (11–14), PD, PD dementia (PDD) (13, 15–17), and AD (18–20).

Whether the regional overlap between α-synuclein and AD pathology leads to synergistic effects in these neurodegenerative diseases is controversial. From a clinical point of view, the presence of cerebral β-amyloid accumulation in DLB and PD (detected by immunohistochemistry in postmortem studies [21] or by amyloid imaging using the Pittsburgh compound B [22]) may influence the development of cognitive impairment and dementia. However, other studies have attributed the appearance of dementia in PD to cortical Lewy pathology (23, 24) or to the combination of Lewy and AD-type pathologies (25). Although some studies have found a positive correlation between the cerebral β-amyloid and α-synuclein burden in DLB, PD, and PDD (25–30) and between tau and α-synuclein in PD, PDD (28, 31), and AD with amygdala LBs (19), other studies have not identified any relationship between these pathologies in some of these disorders (32).

The possible interaction between tau, α-synuclein, and β-amyloid has also been investigated in cellular and animal models of neurodegenerative diseases. Findings indicate that tau, α-synuclein, and β-amyloid can seed, interact, and promote the aggregation of each other (16, 33–36), although the specific underlying mechanisms remain unknown. Genetic studies also support a link between tau and α-synuclein. A common genomic inversion containing the microtubule-associated protein tau gene (MAPT, H1 haplotype) has consistently shown (LBs) and Lewy neurites (LNs), aggregates formed mainly of α-synuclein (1). Although LBs and LNs are the core pathologic hallmarks of DLB, up to 80% of patients show coexistent Alzheimer disease (AD) pathology in the form of extracellular β-amyloid plaques and intracellular aggregates of the microtubule-associated protein tau in neurofibrillary tangles (NFTs) and neuropil threads (NTs) (2). Alzheimer disease pathology also coexists in other disorders with primary deposition of α-synuclein, such as Parkinson disease (PD), where it is present in approximately 40% of cases (3). Alpha-synuclein deposition has also been identified in disorders characterized by prominent tau pathology, such as familial and sporadic AD (4, 5), Down syndrome (6), progressive supranuclear palsy (7), parkinsonism dementia complex of Guam (8), and familial frontotemporal dementia (9, 10). The coexistence of different pathologies in these neurodegenerative diseases occurs both at the regional and cellular levels. Tau and α-synuclein have been found to coaggregate in the same neuronal populations in the brain of patients with DLB (11–14), PD, PD dementia (PDD) (13, 15–17), and AD (18–20).

INTRODUCTION

Dementia with Lewy bodies (DLB) is a progressive neurodegenerative disease characterized by Lewy bodies (LBs) and Lewy neurites (LNs), aggregates formed mainly of α-synuclein (1). Although LBs and LNs are the core pathologic hallmarks of DLB, up to 80% of patients show coexistent Alzheimer disease (AD) pathology in the form of extracellular β-amyloid plaques and intracellular aggregates of the microtubule-associated protein tau in neurofibrillary tangles (NFTs) and neuropil threads (NTs) (2). Alzheimer disease pathology also coexists in other disorders with primary deposition of α-synuclein, such as Parkinson disease (PD), where it is present in approximately 40% of cases (3). Alpha-synuclein deposition has also been identified in disorders characterized by prominent tau pathology, such as familial and sporadic AD (4, 5), Down syndrome (6), progressive supranuclear palsy (7), parkinsonism dementia complex of Guam (8), and familial frontotemporal dementia (9, 10). The coexistence of different pathologies in these neurodegenerative diseases occurs both at the regional and cellular levels. Tau and α-synuclein have been found to coaggregate in the same neuronal populations in the brain of patients with DLB (11–14), PD, PD dementia (PDD) (13, 15–17), and AD (18–20).

Whether the regional overlap between α-synuclein and AD pathology leads to synergistic effects in these neurodegenerative diseases is controversial. From a clinical point of view, the presence of cerebral β-amyloid accumulation in DLB and PD (detected by immunohistochemistry in postmortem studies [21] or by amyloid imaging using the Pittsburgh compound B [22]) may influence the development of cognitive impairment and dementia. However, other studies have attributed the appearance of dementia in PD to cortical Lewy pathology (23, 24) or to the combination of Lewy and AD-type pathologies (25). Although some studies have found a positive correlation between the cerebral β-amyloid and α-synuclein burden in DLB, PD, and PDD (25–30) and between tau and α-synuclein in PD, PDD (28, 31), and AD with amygdala LBs (19), other studies have not identified any relationship between these pathologies in some of these disorders (32).

The possible interaction between tau, α-synuclein, and β-amyloid has also been investigated in cellular and animal models of neurodegenerative diseases. Findings indicate that tau, α-synuclein, and β-amyloid can seed, interact, and promote the aggregation of each other (16, 33–36), although the specific underlying mechanisms remain unknown. Genetic studies also support a link between tau and α-synuclein. A common genomic inversion containing the microtubule-associated protein tau gene (MAPT, H1 haplotype) has consistently shown
to be associated with the risk of PD in all groups except Asians (37) and to be an independent predictor of dementia in PD patients (38, 39). In addition, we recently described higher cerebral α-synuclein deposition in DBL patients with the MAPT H1 haplotype, thereby providing a possible mechanistic explanation (40).

The frequent overlap between α-synuclein, tau, and β-amyloid pathologies in DBL provides a suitable environment to investigate the possible relationship between these common pathologic protein aggregates. The present work aimed to study aggregates frequently observed in DBL and their interaction with 2 genetic risk factors implicated in β-amyloid and α-synuclein pathologies (APOE e4 and MAPT haplotype). We also investigated the patterns of cellular co-occurrence of tau and α-synuclein using confocal microscopy techniques.

**MATERIALS AND METHODS**

**Standard Protocol Approval and Patient Consent**

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 at Hospital de Sant Pau, Barcelona, Spain.

**Human Brain Samples**

Human brain samples were obtained from the Neurological Tissue Bank at the Biobanc-Hospital Clinic-IDIBAPS, Barcelona. Brain sampling and processing protocols were applied as previously described (41) and as internationally recommended (42). The study group consisted of 22 patients who fulfilled clinical and neuropathologic criteria for DBL (43). Clinical data were retrospectively obtained from the clinical records at the Neurological Tissue Bank. Some clinical and neuropathologic data of these patients have been previously published (40). There were 3 amygdala-only cases fulfilling clinical criteria of DLB that were excluded from the study because they could represent a separate clinicopathologic entity (20).

**Neuropathologic Assessment**

We assessed formalin-fixed and paraffin-embedded tissue blocks from 17 brain regions: 6 cortical areas (frontal, occipital, parietal, insular, cingulate and temporoparietal cortices), 5 subcortical areas (caudate nucleus, putamen, hippocampus cornu ammonis [CA] 1, amygdala, and entorhinal region), and 6 brainstem areas (substantia nigra, periaqueductal gray matter, dorsal raphe, locus coeruleus, dorsal nucleus of the vagus, and intermediate reticular zone).

Immunohistochemistry was performed on 5-μm-thick sections on an automated stainer (DAKO Autostainer Plus; DAKO, Glostrup, Denmark) using the following primary antibodies: anti–amyloid β (clone 6F/3D, dilution 1:400; DAKO), anti–phosphorylated tau (clone AT8, dilution 1:2000; Thermo Scientific, Waltham, MA), anti–α-synuclein (clone KM51, dilution 1:500; Novoceastra, Newcastle, UK), and anti–TAR DNA–binding protein 43 ((TDP-43) clone 2E2-D3, dilution 1:500; Abnova, Taipei, Taiwan). Reaction was visualized by the EnVision+ system peroxidase procedure (DAKO).

The densities of pathologic aggregates were assessed by counting the number of immunoreactive structures in each microscopic field (magnification, 100×). The densities of AT8-immunoreactive structures (NFTs, NTs, and pre-tangles) were assessed separately and semi-quantitatively as follows: 0, absent; +, isolated (NTs) or 1 to 2 aggregates; ++, moderate (NTs) or 3 to 6 aggregates; ++++, severe (NTs) or more than 10 aggregates. Neurofibrillary pathology was staged according to Braak criteria (44, 45). The densities of α-synuclein–immunoreactive structures (LBs defined as intracytoplasmic round α-synuclein–reactive aggregates, LNs, and diffuse and punctate cytoplasmic α-synuclein staining) were assessed separately and semi-quantitatively as follows: 0, absent; 1, isolated (LNs) or 1 to 2 aggregates; 2, mild (LNs) or 3 to 6 aggregates; 3, moderate (LNs) or 7 to 10 aggregates; 4, severe (LNs) or more than 10 aggregates. Assignments of LB type and DBL likelihood were performed according to McKKeith criteria (43).

The densities of β-amyloid–immunoreactive structures (mature, primitive, and diffuse plaques) were assessed separately and semi-quantitatively as follows: 0, absent; 1 to 3 plaques, isolated; 3 to 5 plaques, mild; 6 to 30 plaques, moderate; more than 30 plaques, severe. Plaques were considered mature when a dense central core with a less compact peripheral halo was observed; they were considered primitive or immature when uniform spherical deposits without a dense core were seen; and they were considered diffuse when β-amyloid deposits showed more irregular contours than primitive plaques and frequently encompassed normal-appearing neurons and glia (46). Neuritic plaque score (combining β-amyloid and tau immunohistochemistry) was assessed according to the Consortium to Establish a Registry for Alzheimer Disease criteria (47). Beta-amyloid phases were evaluated according to Thal criteria (48) and included the cerebellum. The National Institute on Aging-Alzheimer’s Association Guidelines for neuropathologic assessment of AD were also applied (42).

For tau, α-synuclein, and β-amyloid pathology, each subject was given a total pathology score for the total 17 brain areas. Scores ranged from 0 to 68 for each of the 3 types of tau, α-synuclein, or β-amyloid aggregates and from 0 to 204 for total tau, α-synuclein, or β-amyloid pathology (representing the sum of all types of aggregates).

Concomitant TDP-43 protein aggregates were assessed in frontotemporal and limbic regions as present or absent. Neuro-pathologic evaluation was carried out by 2 of the authors (Marti Colom-Cadena, Ellen Gelpi) on a multiheaded microscope.

**Double Immunofluorescence**

Formalin-fixed and paraffin-embedded tissue blocks from the amygdala, entorhinal cortex, and frontal cortex of 10 representative cases were stained for double immunofluorescence colocalization analysis. Five-micrometer-thick sections were dewaxed, pretreated with citrate buffer pH 6 and high temperature, and stained for 1 hour at room temperature using the following primary antibodies: anti–phosphorylated tau (clone AT8, dilution 1:2000) and polyclonal rabbit anti–α-synuclein (AB5038, dilution 1:1000; Millipore, Billerica, MA). After washing in PBS, sections were incubated with Alexa Fluor 488 or 555–labeled secondary antibodies (dilution 1:1000; Invitrogen, Carlsbad, CA) for 1 hour at room temperature. After PBS washes,
TABLE 1. Demographic, Clinical, and Genetic Data of Dementia With Lewy Bodies Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (N = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (female/male)</td>
<td>6/16</td>
</tr>
<tr>
<td>Mean age at onset ± SD, years</td>
<td>66.80 ± 6.91</td>
</tr>
<tr>
<td>Mean age at death ± SD, years</td>
<td>76.32 ± 5.36</td>
</tr>
<tr>
<td>Mean duration of disease ± SD, years</td>
<td>9.15 ± 4.44</td>
</tr>
<tr>
<td>APOE e4 allele carriers, n (%)</td>
<td>8 (36.7)</td>
</tr>
<tr>
<td>MAPT H1/H1 carriers, n (%)</td>
<td>12 (54.5)</td>
</tr>
</tbody>
</table>

Sections were stained with a saturated solution of Sudan black B (Merck, Whitehouse Station, NJ) at 0.1% in ethanol 70% for 20 minutes, then washed with PBS and PBS-Tween 0.1% and stained with Hoescht 33258 (dilution 1:1000; Invitrogen) for 7 minutes for visualization of nuclei. Finally, after the last PBS wash, Fluoromount mounting medium (Invitrogen) was added and coverslips were mounted.

Image Acquisition and Analysis

Images were taken using a Leica TCD SP5-AOBS inverted fluorescent confocal microscope (Leica Microsystems GmbH, Wetzlar, Germany) with a 63 × 1.4 NA oil objective and a 3 × zoom. Alexa Fluor 488 or 555 were sequentially excited with the 488- and 561-nm laser lines and imaged in 495–535-nm and 575–650-nm spectral windows, respectively. A pulsed 405-nm laser was used for Hoescht visualization collecting images in a spectral range of 450 to 460 nm. Sections without antibodies or with secondary antibodies only were imaged to ensure specific and independent fluorophore visualization.

For each case, at least 10 images per area were acquired. To capture representative sections of the aggregates, pictures were taken in 3 to 8 z planes with a 0.7-μm pinhole. Maximal intensity projection of each type of aggregate was used for visualization. For colocalization purposes, images were acquired, avoiding saturated pixels. Saturation was only minimally applied for figure visualization.

Protein colocalization was evaluated using FIJI imaging software (49). All images were treated equally following a semiautomated in-house macro procedure. Briefly, for each channel, the lowest intensity signals within a z-stack were removed to avoid minimal background, and the region of interest was delimited. A threshold was then estimated using the Otsu algorithm to create binary images (50). Aggregates with a diameter or length smaller than 10 μm were not included in the analysis. This restrictive method of evaluating co-occurrence prevented us from including the diffuse and punctate forms of tau and α-synuclein deposits (pretangles and cytoplasmic α-synuclein staining). A total of 311 aggregates were included in the analysis. To quantify co-occurrence independently of signal proportionality, the Manders’ colocalization coefficient was calculated for each channel (51, 52).

APoE and MAPT Genotyping

MAPT haplotypes and APoE genotypes were determined using DNA extracted from frozen brain tissue as previously described (53, 54).

Statistical Analyses

Nonparametric Spearman rho correlation coefficient was used to analyze correlations between the total scores of each pathologic aggregate and between clinical variables. A stepwise-selection model-building procedure was used to develop a linear regression model to examine the observed associations. Statistical significance was set at 5% (α = 0.05). All data were analyzed using the Statistical Package for the Social Sciences version 19.0 (SPSS Inc., Chicago, IL).

RESULTS

Demographic, Clinical, Genetic, and Neuropathologic Data of DLB Cases

There was a positive correlation between age of onset and age of death (ρ = 0.74, p < 0.001), and disease duration correlated inversely with the age of onset (ρ = −0.541, p = 0.014) (Table 1). Table 2 provides detailed neuropathologic information of the individual cases. All 22 cases showed sufficient degrees of α-synuclein deposits in neocortical areas to be classified as diffuse neocortical type of DLB (43). All cases had some degree of AD-related pathology; the median NFT Braak stage in the cohort was IV and the median Thal β-amyloid phase was 4. Two cases (9.1%) did not exhibit β-amyloid immunoreactivity but had NFT Braak stage II or III. According to McKeith criteria (DLB likelihood [43], 55% (12 of 22) of cases had a high likelihood of pathology explaining the DLB clinical syndrome and the remaining 45% (10 of 22) had an intermediate likelihood. Pathologic TDP-43 aggregates in frontotemporal and limbic regions were found in 27% of cases (6 of 22).

Regional Distribution of Tau, β-Amyloid, and α-Synuclein Protein Aggregates

The topographic distribution and semiquantitative assessment of α-synuclein, tau, and β-amyloid pathology in all DLB cases are shown in Figure 1. Alpha-synuclein immunoreactivity was abundant in all studied areas, although some regional differences were detected (Fig. 1A). Overall, limbic areas showed the highest α-synuclein aggregate scores, whereas neocortical areas were comparatively less involved. Brainstem areas exhibited the highest scores for all 3 types of α-synuclein aggregates, whereas in neocortical areas, LBs predominated with fewer LNs and cytoplasmic α-synuclein staining. Tau pathology was found in all studied areas, although the highest scores were mainly observed in limbic regions, where it was common to observe the full spectrum of pathologic tau deposits (Fig. 1B). Tau deposits were less abundant in brainstem regions than α-synuclein aggregates. Finally, β-amyloid deposits covered large neocortical regions, their numbers decreased in limbic and subcortical areas, and they were occasionally present in brainstem areas (Fig. 1C). Diffuse, primitive, and mature β-amyloid plaques were similarly distributed in all studied areas. On analyzing all regions together, limbic regions (in particular, the amygdala and the entorhinal cortex) were markedly affected by all types of protein aggregates. In contrast, brainstem involvement differed between α-synuclein, tau, and β-amyloid pathologies.
Correlation Between Tau, β-Amyloid, and α-Synuclein Pathologies

We next analyzed the possibility of a correlation between the occurrence of total β-amyloid, tau, and α-synuclein aggregates. Although total tau pathology scores did not correlate with total α-synuclein pathology scores, total β-amyloid pathology scores correlated positively with total α-synuclein pathology scores (ρ = 0.692, p = 0.001). The positive correlation was found in both cortical (ρ = 0.619, p = 0.003) and subcortical (ρ = 0.694, p > 0.001) areas but not in brainstem regions. Alpha-synuclein pathology correlated better with the total score of mature and primitive β-amyloid plaques (ρ = 0.579, p = 0.006 and ρ = 0.573, p = 0.007, respectively) than with the total score of diffuse plaques (ρ = 0.458, p = 0.037). As expected, there was a correlation between tau and β-amyloid pathology scores (ρ = 0.579, p = 0.007).

Because we previously reported that the MAPT H1 haplotype enhanced α-synuclein deposition in DLB (40), we generated a stepwise selection linear regression model to explore which other factors could explain the variability of α-synuclein deposition in our sample (Table 3). The model that included the MAPT haplotype and β-amyloid pathology explained 60.5% of the variance observed in total α-synuclein pathology (ρ < 0.001). In this model, neither APOE genotype nor the degree of total tau pathology contributed to explain the α-synuclein variance in our sample.

Cellular Co-occurrence of Tau and α-Synuclein Aggregates

After studying the regional co-occurrence of tau, α-synuclein, and β-amyloid deposits, we investigated the co-occurrence of the intracellular aggregates of tau and α-synuclein in DLB cases. We selected 3 areas commonly affected by tau and α-synuclein pathologies: amygdala, entorhinal cortex, and frontal cortex (Fig. 1). We performed double immunofluorescence on sections from 10 cases and included 311 aggregates for morphologic evaluation after selecting aggregates with a diameter or length more than 10 μm. Protein deposits were classified according to the main protein aggregate and its morphology as follows: LBs or LNs with or without tau immunoreactivity (‘‘tau-positive’’ or ‘‘tau-negative’’ LBs and LNs) and NFTs or NTs with or without α-synuclein immunoreactivity (‘‘α-synuclein-positive’’ or ‘‘α-synuclein-negative’’ NFTs and NTs). The restrictive method of co-occurrence evaluation explained 60.5% of the variance observed in total α-synuclein pathology (ρ < 0.001). In this model, neither APOE genotype nor the degree of total tau pathology contributed to explain the α-synuclein variance in our sample.

TABLE 2. Neuropathologic Data of Dementia With Lewy Bodies Cases

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>McKeith Lewy Body Type Pathology</th>
<th>NFT Braak Stage</th>
<th>CERAD Neuritic Plaque Stage</th>
<th>DLB Likelihood</th>
<th>Thal Aβ Phase</th>
<th>NIA-AA</th>
<th>TDP-43 Limbic Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>Diffuse neocortical</td>
<td>V</td>
<td>Frequent</td>
<td>Intermediate</td>
<td>4</td>
<td>A3, B3, C3 high</td>
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</tr>
<tr>
<td>2</td>
<td>M</td>
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<td>A3, B3, C2 intermediate</td>
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</tr>
<tr>
<td>3</td>
<td>F</td>
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<td>A3, B3, C3 high</td>
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<tr>
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</tr>
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</tr>
</tbody>
</table>

AB, amyloid-β; CERAD, Consortium to Establish a Registry for Alzheimer’s Disease; DLB, dementia with Lewy Bodies; F, female; M, male; NFT, Neurofibrillary tangle; NIA-AA, National Institute of Aging–Alzheimer’s Association guidelines 2012; “A,” amyloid phases; “B,” Braak neurofibrillary stage; “C,” CERAD plaque score (all from 0 to 3); TDP-43, TAR-DNA binding protein 43.
FIGURE 1. Topographic distribution and semiquantitative scores of total α-synuclein, tau, and β-amyloid pathologies classified by type of aggregate. Semiquantitative scores of total α-synuclein (A), tau (B), and β-amyloid (C) pathologies according to region and type of aggregate are plotted. One case was excluded because not all areas were available for analysis. Pseudo-colored brain diagrams representing the sum of all pathology scores are shown on the right. Colors represent semiquantitative scores (white = 0 to dark blue = 250). Note that limbic areas are severely affected by all 3 pathologies, whereas the brainstem is generally affected by α-synuclein and tau pathologies. CAS, diffuse and punctate cytoplasmic α-synuclein staining; LBs, Lewy bodies; LNs, Lewy neurites; pTs, pretangles; NFTs, neurofibrillary tangles; NTs, neuropil threads; p., β-amyloid plaques; s. nigra, substantia nigra; periaqueductal g.m., periaqueductal gray matter; reticular F., reticular formation; L. coeruleus, locus coeruleus.

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TABLE 3. Stepwise Selection Linear Regression Model to Predict Total α-Synuclein Pathology

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>SE</th>
<th>β</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>76.006</td>
<td>6.160</td>
<td>12.339</td>
<td>-0.001</td>
<td></td>
</tr>
<tr>
<td>Total β-amyloid path</td>
<td>0.244</td>
<td>0.060</td>
<td>0.603</td>
<td>4.052</td>
<td>0.001</td>
</tr>
<tr>
<td>MAPT haplotype</td>
<td>16.372</td>
<td>4.456</td>
<td>0.547</td>
<td>3.674</td>
<td>0.002</td>
</tr>
</tbody>
</table>

prevented us from including the diffuse cytoplasmic and punctate forms of tau and α-synuclein deposits (pretangles and cytoplasmic α-synuclein staining) in the colocalization study.

Intracellular co-occurrence was found in both α-synuclein and tau-predominant aggregates and in all areas evaluated, following some characteristic morphologic patterns. In τ-positive LBs, τ immunoreactivity was mainly found in the periphery of α-synuclein deposits, showing a pattern different from the fibrillar tau immunoreactivity observed in NFTs. In most cases, τ immunoreactivity in LBs was limited to small focal deposits (Fig. 2A–C). However, in some instances, LBs were completely surrounded by a rim of τ immunoreactivity (Fig. 2D–F). This pattern was mainly found in the amygdala and entorhinal cortex, and it was not observed in the frontal cortex. In τ-positive LN (as in τ-positive LBs), τ immunoreactivity also surrounded α-synuclein deposits (Fig. 2G–I); this pattern was observed in all assessed areas. In α-synuclein-positive NFTs, fibrillar α-synuclein immunoreactivity was found irregularly filling the innermost part of the tau aggregate (Fig. 2J–L). These patterns are similar to those described by Ishizawa et al (11) (Type 1, Type 4, and Type 2, respectively) and others (13, 55) in brainstem areas using immunohistochemistry and confocal microscopy. Finally, in NTs, α-synuclein immunoreactivity was rarely observed, and colocalization was mainly caused by LN crossing close to the NTs (Fig. 2M–O).

Figure 3 shows the quantification of the total number of aggregates analyzed by confocal microscopy. Aggregates were classified as “pure” when no colocalization was found and as “mixed” if colocalization was observed. Colocalization was found to occur in approximately half the limbic aggregates (amygdala and entorhinal cortex regions) but was less frequent (~25%) in frontal cortex regions (Fig. 3). This observation was similar for all types of assessed aggregates. Colocalization in limbic regions was independent of the NFT Braak stage.

**DISCUSSION**

In the present study, we found that the MAPT H1 haplotype and the density of β-amyloid deposits were associated with the amount of α-synuclein deposits in the brains of patients with DLB. We also observed that, at the cellular level, tau and α-synuclein frequently coaggregate in specific neuronal populations following a discrete set of patterns.

After assessing the associations between total protein aggregates, we confirmed a positive correlation in DLB between β-amyloid (but not tau) and α-synuclein pathologies (26, 29). In agreement with previous studies (13, 56–58), all our DLB cases showed different degrees of AD-related pathology and 27% also showed TDP-43 aggregates. Beta-amyloid deposition has also been associated with α-synuclein pathologies in postmortem PD and PDD cases, suggesting that this association may be independent of the clinical syndrome (27, 30, 59). Other studies, however, have found that the degree of α-synuclein pathology correlated better with tau pathology in PDD (31), or with both tau and β-amyloid pathologies in PD and PDD (28). It is important to note that methodological differences (e.g. sample size, areas analyzed, types of pathologies assessed, or method of quantification) across studies may limit the possibility of comparisons among them (60).

Whether the regional overlap between α-synuclein and β-amyloid pathologies in DLB is coincidental or may influence the clinical expression of the disease is controversial. In the present study, the correlation between β-amyloid and α-synuclein deposits depended on the number of primitive and mature β-amyloid plaques in cortical regions. Because mature plaques are recognized as the type of plaque most prone to induce neuronal and dendritic abnormalities (60), our results suggest that β-amyloid plays an active role in the disease process in DLB. This observation is supported by many studies that link β-amyloid and α-synuclein in different ways. Alpha-synuclein directly interacts with the β-amyloid peptide in vitro (61, 62), and incubation of α-synuclein with the 42-amino acid β-amyloid peptide (Aβ42) induces the formation of high-molecular-weight α-synuclein oligomers (63). The relationship between Aβ42 and α-synuclein is also supported by the observation that many families with autosomal-dominant AD show LB pathology. These families carry mutations in the amyloid precursor protein or presenilin genes that lead to overproduction of Aβ42 (64, 65). Although the interactions between β-amyloid and α-synuclein have been well documented, the exact mechanisms underlying this association remain uncertain. Possible mechanisms include 1) a direct effect of β-amyloid on α-synuclein because both proteins have been shown to coimmunoprecipitate in brain samples from LB disease and AD patients (61) and 2) an indirect effect through either τ pathology, which in turn may lead to increased α-synuclein phosphorylation (66) or through impaired degradation by the autophagy pathway (2).

In addition to studying the effect of concomitant pathologies in DLB, we also investigated whether the MAPT H1 haplotype, a well-known risk factor for PD and PDD (67), had an effect on the neuropathologic variables. Our group recently observed that the MAPT H1 haplotype enhanced α-synuclein deposition in patients with DLB (40). Here we extend these data and describe for the first time the combined effect of β-amyloid pathology and MAPT H1 haplotype on LB pathology in DLB. It is of note that, in prospective studies, MAPT H1 haplotype was shown to be the strongest independent predictor of dementia in PD (38, 68). The lack of correlation between the MAPT H1 haplotype and tau pathology in DLB is not unexpected because the MAPT H1 haplotype has been shown to increase 4-repeat tau transcripts in LB disease cases without increasing total tau expression (38). Our present results highlight the importance of the MAPT H1 haplotype in DLB and add to our knowledge about the genetic-pathologic interactions in synucleinopathies.

At a cellular level, colocalization between tau and α-synuclein has been observed in DLB (11–14), in other synucleinopathies (13, 15–17), and in tauopathies (18–20). In this study, we identified morphologic patterns of intracellular...
FIGURE 2. Types of tau and α-synuclein aggregates detected by double immunofluorescence in dementia with Lewy bodies (DLB) cases. Representative images of the morphologic types of α-synuclein (green) and tau (red) coaggregates identified in the amygdala, entorhinal cortex, and frontal cortex of 10 representative DLB cases. (A–F) Tau-positive LB with focal (A–C) or peripheral (D–F) tau immunoreactivity. (G–I) Tau-positive LNs with peripheral tau immunoreactivity. (J–L) Alpha-synuclein-positive NFTs with irregular central α-synuclein immunoreactivity. (M–O) Neuropil threads (NTs) with colocalization caused by crossing of an LN and NT. LB, Lewy body; LN, Lewy neurite; M1, Manders' coefficient of tau overlapping α-synuclein; M2, Manders' coefficient of α-synuclein overlapping tau immunoreactivity; NFT, neurofibrillary tangle. Scale bar = 10 μm.
coaggregation of tau and α-synuclein; 3 of these patterns resembled those described by Ishizawa et al (11). According to these authors, Type 1 (tau-positive LBs) and Type 4 (tau-positive LNs) have a peripheral rim of tau immunoreactivity surrounding LBs (Fig. 2F–I) or LNs (Fig. 2G–I), and Type 2 has α-synuclein immunoreactivity inside NFT-like deposits (Fig. 2J–I). In addition, we found that the intracellular colocalization of tau and α-synuclein was more frequent in the amygdala and entorhinal cortex than in the frontal cortex. The frequency of colocalization was independent of the Braak stage, as previously described (11), suggesting that this neuronal phenomenon is region dependent.

Recent evidence from cellular and animal models of synucleinopathy indicates that seeding and propagation of abnormally folded proteins though neural networks may be a common disease mechanism in neurodegenerative diseases (69). This mechanism involves common pathways of internalization and transmission of proteins along neurons and could explain the hierarchical regional involvement observed in most neurodegenerative disorders. A synergistic relationship between α-synuclein and tau aggregates has been described (33, 34, 36), and a common entrance mechanism for both has been suggested (70). The absence of regional correlation between tau and α-synuclein pathologies in our study despite the frequent cellular co-occurrence suggests a local, rather than a global, synergistic relation modulated by regional and cellular factors. It has recently been shown that distinct "strains" of α-synuclein can differentially promote tau aggregation (71). Different types of α-synuclein aggregates could underlie the variability in the cellular and regional patterns observed among tau–α-synuclein coaggregates.

The strengths of this study are the inclusion of a detailed neuropathologic characterization of all cases; the evaluation of many cortical, subcortical, and brainstem areas; the semiquantitative analysis of the full spectrum of α-synuclein aggregates; and the use of confocal microscopy to assess colocalization. The main limitations are the relatively small sample size and the fact that the degree of optical colocalization between tau and α-synuclein may be influenced by the image processing analyses or antibodies used.

In summary, we found that, despite the frequent cellular co-occurrence between tau and α-synuclein in DLB, regional α-synuclein pathology was mainly influenced by the degree of β-amyloid pathology and the presence of the MAPT H1 haplotype. These results provide further evidence of the complex interplay between tau, α-synuclein, and β-amyloid underlying DLB pathology.

ACKNOWLEDGMENTS

Human brain samples were obtained from the Neurological Tissue Bank of the Biobanc-Hospital Clinic-IDIBAPS. The Neurological Tissue Bank of the Biobanc-Hospital Clinic-IDIBAPS thanks all brain donors and their relatives for their generous donation for research. The authors also thank Rosa Rivera, Abel Muñoz, and Leire Etxarri for technical assistance; Carina Antiga for administrative support; and Ignasi J. Gich for statistical support.

REFERENCES


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