Lewy Bodies in the Amygdala

Increase of α-Synuclein Aggregates in Neurodegenerative Diseases With Tau-Based Inclusions

Anca Popescu, MD; Carol F. Lippa, MD; Virginia M.-Y. Lee, PhD; John Q. Trojanowski, MD, PhD

Background: Increased attention has been given to α-synuclein aggregation in nonsynucleinopathies because α-synuclein–containing Lewy bodies (LBs) influence symptoms. However, the spectrum of disorders in which secondary inclusions are likely to occur has not been defined. Amygdala neurons commonly develop large numbers of secondary LBs, making it a practical region for studying this phenomenon.

Objective: To characterize the spectrum of diseases associated with LB formation in the amygdala of neurodegenerative disease and control cases.

Design: An autopsy series of 101 neurodegenerative disease and 34 aged control cases. Using immunohistochemistry studies, we examined the amygdala for α-synuclein aggregates.

Results: Lewy bodies were often abundant in classic Pick disease, argyrophilic grain disease, Alzheimer disease, and dementia with LBs but not in cases with amygdala degeneration lacking tau-based inclusions, control cases, preclinical disease carriers, or degenerative diseases lacking pathologic involvement of the amygdala. The exposed α-synuclein epitopes were similar in all cases containing LBs.

Conclusions: Abnormal α-synuclein aggregation in the amygdala is disease selective, but not restricted to disorders of α-synuclein and β-amyloid. Our data are compatible with the notion that tau aggregates predispose neurons to develop secondary LBs.

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AGGREGATION OF α-SYNUCLEIN has a primary pathogenic role in sporadic and familial autosomal dominant Parkinson disease, multiple system atrophy (MSA), and dementia with Lewy bodies (DLB). In the past few years, increased α-synuclein aggregation in the form of Lewy bodies (LBs) has been reported in neurodegenerative diseases that are not synucleinopathies. In this context, LBs may be considered a secondary phenomenon that reflects the fibrillation of α-synuclein induced directly by the formation of fibrillary tau lesions or indirectly by cell stress resulting from the formation of these tau inclusions. Regulatory factors involved in α-synuclein expression and the biological changes leading to primary or secondary α-synuclein aggregation in neurodegenerative diseases are not well understood.

Secondary LBs are numerous in the amygdala and adjacent entorhinal cortex in cases of Alzheimer disease (AD), including sporadic and familial AD, and Down syndrome (DS). They occur in at least half of these subjects. It is unknown whether this curious finding is restricted to AD, or whether it is a more universal phenomenon. In the present study, we compared α-synuclein immunoreactivity in the amygdala in a variety of diseases, including disorders of tau, α-synuclein, and β-amyloid, and in control cases to determine how widespread this phenomenon is. We also examined epitope exposure in LBs to screen for obvious differences in LB conformation in different diseases.

METHODS

We examined 135 amygdala specimens from pathologically confirmed cases meeting consensus pathological criteria for DLB (n=9; LBs were common in regions outside the amygdala), sporadic AD (n=20; lacking LBs outside the amygdala), DS (n=9; with or without evidence of cognitive and/or functional decline), early-onset familial AD with presenilin-1 mutations (n=6), preclinical AD with a presenilin-1 mutation (n=1), classic Pick disease (n=6; frontotemporal dementia with numerous Pick bodies; not meeting con-
clonal antibodies (SNL-4, 204, LB509, 211, and 202) described active for LB509 were then immunostained using a panel of monoclonal antibodies. 

graded 1 and 2. When grade 3 changes were present, the highest number of LBs occurred in DLB (2.9±1.1; 8 of 9 cases), followed by familial AD (1.7±1.9; 4 of 7 cases), ADG (1.5±2.1; 1 of 2 cases), DS (1.3±1.6; 4 of 9 cases), PiD (1.3±1.6; 3 of 6 cases), sporadic AD (0.7±1.1; 7 of 20 cases), and DLDH (0.08±0.4; 2 of 40 cases). Lewy bodies were also almost never observed in the amygdalae of aged, cognitively normal control cases (0.1±0.1; 1 of 34 cases). When present in AD, DLB, AGD, and PiD, Lewy bodies were almost always graded 3 or 4. When grade 3 changes were present, the superficial regions (central and medial nuclei) tended to be more heavily involved. However, the atrophy and gliosis were too severe to allow us to distinguish exact borders between subregions, so we did not attempt to compare the subregions. In cases where grade 4 changes were present, LBs occurred throughout the amygdala. Lewy body density was low in DLDH, and the 2 cases with LBs were graded 1 and 2.

Table. Summary Table Showing the Incidence of LBs and the Average Severity of LB Pathology Across Diseases

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Cases</th>
<th>No. (%) of Cases With LBs</th>
<th>Mean LB Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLB</td>
<td>9</td>
<td>8 (89)</td>
<td>2.9</td>
</tr>
<tr>
<td>PS-1 AD</td>
<td>7</td>
<td>4 (57)</td>
<td>1.7</td>
</tr>
<tr>
<td>PiD</td>
<td>6</td>
<td>3 (50)</td>
<td>1.3</td>
</tr>
<tr>
<td>DS</td>
<td>9</td>
<td>4 (44)</td>
<td>1.3</td>
</tr>
<tr>
<td>AGD</td>
<td>2</td>
<td>1 (50)</td>
<td>1.5</td>
</tr>
<tr>
<td>SAD</td>
<td>20</td>
<td>7 (35)</td>
<td>0.7</td>
</tr>
<tr>
<td>DLDH</td>
<td>40</td>
<td>2 (5)</td>
<td>0.1</td>
</tr>
<tr>
<td>Aged normal controls*</td>
<td>34</td>
<td>1 (3)</td>
<td>0.1</td>
</tr>
<tr>
<td>ALS</td>
<td>4</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>MSA</td>
<td>3</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>PSP</td>
<td>1</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; AGD, argyrophilic grain disease; ALS, amyotrophic lateral sclerosis; DLB, dementia with LBs; DLDH, dementia lacking distinctive histopathological features; DS, Down syndrome; LB, Lewy body; MSA, multiple system atrophy; PiD, Pick disease; PS-1 AD, AD related to presenilin-1 mutations; PSP, progressive supranuclear palsy; SAD, sporadic AD.

*Indicates elderly subjects with no significant neurological disease.

current criteria for AD,31 dementia lacking distinctive histopathological features (DLDH), amyotrophic lateral sclerosis (ALS), argyrophilic grain disease (AGD), and Lewy bodies were absent in cases with amyloid plaques but no neurofibrillary tangles (the younger patients with DS and our patient with a clinical presenilin-1 mutation). In cases where LBs are present, the LB density is typically higher than that described in other brain regions.31 Our semiquantitative grading (reported as mean±SD indicate that the highest number of LBs occurred in DLB (2.9±1.1; 8 of 9 cases), followed by familial AD (1.7±1.9; 4 of 7 cases), ADG (1.5±2.1; 1 of 2 cases), DS (1.3±1.6; 4 of 9 cases), PiD (1.3±1.6; 3 of 6 cases), sporadic AD (0.7±1.1; 7 of 20 cases), and DLDH (0.08±0.4; 2 of 40 cases). Lewy bodies were also almost never observed in the amygdalae of aged, cognitively normal control cases (0.1±0.1; 1 of 34 cases). When present in AD, DLB, AGD, and PiD, Lewy bodies were almost always graded 3 or 4. When grade 3 changes were present, the superficial regions (central and medial nuclei) tended to be more heavily involved. However, the atrophy and gliosis were too severe to allow us to distinguish exact borders between subregions, so we did not attempt to compare the subregions. In cases where grade 4 changes were present, LBs occurred throughout the amygdala. Lewy body density was low in DLDH, and the 2 cases with LBs were graded 1 and 2.

We usually found α-synuclein–positive threads in cases with an LB burden of 2 or greater. They were most common in DLB (mean±SD grade, 1.3±0.9), but were also present in familial AD (1±1.3), DS (0.8±1.3), PiD (0.4±0.9), AGD (0.5±0.7), and sporadic AD (0.18±0.38). Lewy threads were not present in cases lacking LBs.

Because LBs in amyloidopathies are often restricted to the amygdala, we characterized the distribution of LBs in the PiD cases by screening other brain regions containing Pick bodies, including the frontal cortex (middle frontal and cingulate gyri), temporal lobe (entorhinal cortex and hippocampus), and brainstem (locus coeruleus, midbrain, and medulla). In PiD cases where amygdala LBs were abundant, we observed occasional LBs in the periamygdaloid entorhinal cortex. One PiD case lacking LBs in the amygdala had an isolated LB in the locus coeruleus. Lewy bodies were absent in other areas.

Because focal LBs are not commonly described in high densities in tauopathies, we further examined the PiD cases using double-label immunohistochemistry studies to determine how frequently LBs coexisted with Pick bodies. Double-label studies with α-synuclein and tau proteins with DLB and AD. Moreover, we made the novel observation that LBs were also often present in the amygdala of PiD cases. Three of the 6 PiD cases studied showed numerous LBs in the amygdala (Figure). Rare diffuse amyloid plaques were present in the amygdala of 1 PiD case; the other 2 cases lacked plaques. Lewy bodies were also numerous in 1 case of AGD, another tau disorder involving the amygdala. Lewy bodies were rare in neurodegenerative diseases where the amygdala is not heavily involved by the disease process (ALS and PSP). In MSA, occasional glial cytoplasmic inclusions in the surrounding white matter were immunoreactive for α-synuclein, but intraneuronal aggregates were not seen. Lewy bodies and Lewy threads were present in only 2 of 40 cases of DLDH, although severe neuronal loss was seen in most amygdalae. Amygdala LBs were also absent in cases with amyloid plaques but no neurofibrillary tangles (the younger patients with DS and our patient with a clinical presenilin-1 mutation).

Using the LB509 antibody, we confirmed our previous finding that LBs are common in the amygdala of pa-
demonstrated that LBs in PiD usually colocalized with tau-positive Pick bodies. Here, both proteins could be commixed, although sometimes the inclusions remained discrete, with a Pick body adjacent to the LB. When we obtained counts in a band of adjacent fields across the medial-to-lateral amygdala in PiD cases, tau-positive immunoreactivity was present in 82% of neurons containing α-synuclein aggregates. Only 18% of PiD neurons with LBs lacked clear-cut tau aggregates in the same section. This is similar to our AD and DS cases where LBs typically colocalize with tau-positive neurofibrillary tangles.8,9

To address the question of whether secondary α-synuclein exposure was similar in all diseases, we examined our cases containing LBs with the additional epitope-specific α-synuclein antibodies described in the “Methods” section. When we compared the epitope-mapping properties of LBs in PiD with those in familial and sporadic AD, DS, and DLB, we found epitope mapping to be identical with strong exposure of all epitopes. In contrast, our MSA cases showed nonuniform epitope mapping in glial aggregates, with amino-terminal epitopes showing less consistent immunoreactivity. This finding is consistent with epitope mapping in other MSA studies.13

**COMMENT**

We report for the first time, to our knowledge, the presence of intraneuronal α-synuclein aggregates in the PiD amygdala, a region heavily affected with Pick bodies. Despite the small PiD sample size, it was clear that LBs were abundant when present, sometimes reaching densities of greater than 20 per high-power field. They usually colocalized with Pick bodies. In our AD cases, LBs colocalized with tau-positive neurofibrillary tangles. One of our AGD cases also had amygdala LBs, supporting the notion that tau is important in the process of LB formation. Because AGD is a 4-repeat (4R) tau disorder, PiD is a 3R tau disorder, and AD has equal tau ratios, our data suggest that amygdala LBs occur regardless of the 3R:4R tau ratio. Progressive supranuclear palsy, a tauopathy lacking amygdala LBs, also lacked tau aggregates in the amygdala. Secondary LBs developed less commonly in con-
control cases, asymptomatic carriers (those with early DS and the preclinical case with the presenilin-1 mutation), and degenerative disease cases lacking tau aggregates (DLDH). Preexisting amygdala pathology appears to be an important factor in LB formation. The frequent colocalization of tau and α-synuclein in the same neuron in diseases with tau aggregates and the rare occurrence of LBs in diseases without tau pathology (normal controls and DLDH) speak for an association between tau and α-synuclein aggregation that could directly or indirectly be mediated by tau inclusions that promote the fibrillation of α-synuclein to form LBs in regions with abundant fibrillary tau lesions.

Immunoreactivity of α-synuclein has previously been reported in tauopathies. Although nigral LB counts did not differ between control subjects and those with PSP, Tsuboi and colleagues14 found LBs and Lewy neurites in 18% of PSP cases, with amygdala LBs being one of the most involved regions. Using epitope specific antibodies, Takeda et al15 found that a C-terminus fragment α-synuclein antibody stained tau-positive aggregates in AD, PSP, corticobasal degeneration, and PiD. These interesting findings support the notion that α-synuclein fragments commonly co-occur with pathologic tau inclusions. Evidence of an interaction between tau and α-synuclein at a biochemical level has also been found. Microtubule-associated, soluble axonal tau protein binds to the C-terminus of α-synuclein, which in turn modulates tau phosphorylation.16

It could be argued that α-synuclein aggregates are not true LBs if they do not have a fibrillary nature. A previous study from our group demonstrated the fibrillary structure of amygdala LBs by means of electron microscopy.8 Thioflavine stains have also demonstrated the fibrillary nature of these lesions.

The common coexistence of LBs and tau aggregates (neurofibrillary tangles, Pick bodies, or 4R grains) could be related to failure of tau function (including microtubule assembly and/or axonal transport) or could be a direct effect of the tau aggregate. Tau hyperphosphorylation may lead to structural changes in α-synuclein that accelerate its aggregation. Another possibility is that impaired axonal transport leads to backup and concentration of α-synuclein in the cytoplasm, which predisposes to fibrillogenesis and aggregation. However, since Aβ grains most commonly occur in dendrites, we do not think the process is triggered exclusively within the axon. Alternatively, the LBs may form as more of a general compensatory response to cell stress or other perturbations resulting from the accumulation of filamentous tau aggregates.17

The reason why the amygdala is susceptible to LB formation is unknown. Tsuchiya et al18 studied 8 autopssied classic PiD cases and found that the amygdala showed the most severe lesions of all basal ganglia regions. In our 3 cases with PiD and LBs in the amygdala, none had LBs in the basal ganglia or brainstem (except for 1 case with LBs in the locus coeruleus). Yamazaki et al19 reported 5 cases of Guam dementia complex with α-synuclein–positive inclusions in the amygdala reminiscent of cortical LBs. These coexisted with tau-positive pretangles and/or neurofibrillary tangles. Overall, the amygdala can be viewed as an area susceptible to primary and secondary α-synuclein inclusions, although the reasons for this remain elusive. Indeed, Marui et al20 recently reported that Lewy pathology in the cerebrum begins in the amygdala in LB disease. This differs from the staging system for PD, where pathology starts in the medulla.21 We examined other brain regions in our cases, including the medulla, and found little subcortical α-synuclein pathology. This is in keeping with the findings of Jellinger,22 who also noted that α-synuclein aggregates in AD do not follow the regional pattern of distribution described by Braak et al23 for PD.

One interesting question is whether the formation of LBs in the amygdala influences the clinical course. Lewy bodies influence symptoms in AD cases, even when they are present in much lower densities.23 Results of retrospective medical chart reviews in cases where history was available showed only occasional cases with classic features of DLB (spontaneous parkinsonism or visual hallucinations). However, PD cases with amygdala LBs have more visual hallucinations than comparable PD cases lacking amygdala LBs.24 Although the preexisting tau pathology and neuronal loss makes it likely that the clinical presentations would have features of the primary disorder, prospective studies are needed to more fully determine to what extent, if any, amygdala LBs have an impact on symptoms. It is possible that these subjects would experience differential involvement of autonomic, olfactory, visceral, endocrine, affective, and mnemonic activities (which are subserved by intra-amygdala circuits).

Disease duration did not correlate strongly with the presence of LBs. The mean duration of illness in PiD cases with LBs in amygdala was 12.7 years (range, 3–17 years), whereas in non–LB-containing PiD cases, it was 17.8 years (range, 14–19 years). The Braak AD stage was VI in almost all of our postmortem symptomatic AD cases, regardless of whether amygdala LBs were present. Our symptomatic cases all had end-stage symptoms at death, requiring assistance for all activities of daily living. This made it impossible to determine whether LB formation is related to symptom severity. However, LBs did not occur in young patients with DS or in the case with preclinical, presenilin-related AD, suggesting that amygdala LBs are not an early finding.

The reason why central nervous system proteins co-precipitate remains fertile ground for future research. A growing body of evidence suggests that one pathologic protein promotes fibrillation of other abnormal proteins.25 Herein, we focused on cases with tau as a primary aggregate. This study does not address mechanisms involved in secondary (tau) inclusion formation in cases where α-synuclein is the primary aggregate, such as the Contursi kindred.27 The present study also does not completely address whether β-amyloid deposition influences amygdala LB formation. However, β-amyloid deposition is not crucial for secondary LB formation in the amygdala in PiD, because our affected PiD cases had little β-amyloid deposition. Future studies are also needed to explain why the 2 types of pathology overlap only in certain brain regions. It is feasible that all 3 pathologic proteins (tau, α-synuclein, and β-amyloid) can promote secondary inclusion formation under different cir-
cumstances. Future studies are needed to better understand mechanisms by which abnormal processing and/or deposition of one central nervous system protein is related to deposition of other central nervous system proteins.

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Correspondence: Carol F. Lippa, MD, Department of Neurology, Drexel University College of Medicine, 3300 Henry Ave, Philadelphia, PA 19129 (carol.lippa@drexel.edu).

Author Contributions: Study concept and design: Popescu, Lippa, Lee, and Trojanowski. Acquisition of data: Popescu and Lippa. Analysis and interpretation of data: Popescu, Lippa, Lee, and Trojanowski. Drafting of the manuscript: Popescu and Lippa. Critical revision of the manuscript for important intellectual content: Popescu, Lippa, Lee, and Trojanowski. Obtained funding: Lippa. Administrative, technical, and material support: Lippa. Study supervision: Popescu, Lippa, Lee, and Trojanowski.

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