Multiple Organ Involvement by Alpha-Synuclein Pathology in Lewy Body Disorders

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Abstract: Lewy body (LB) diseases are characterized by alpha-synuclein (AS) aggregates in the central nervous system (CNS). Involvement of the peripheral autonomic nervous system (pANS) is increasingly recognized, although less studied. The aim of this study was to systematically analyze the distribution and severity of AS pathology in the CNS and pANS. Detailed postmortem histopathological study of brain and peripheral tissues from 28 brain bank donors (10 with Parkinson’s disease [PD], 5 with dementia with LB [DLB], and 13 with non-LB diseases including atypical parkinsonism and non-LB dementia). AS aggregates were found in the pANS of all 15 LB disease cases (PD, DLB) in stellate and sympathetic ganglia (100%), vagus nerve (86.7%), gastrointestinal tract (86.7%), adrenal gland and/or surrounding fat (53.3%), heart (100%), and genitourinary tract (13.3%), as well as in 1 case of incidental Lewy body disease (iLBD). A craniocaudal gradient of AS burden in sympathetic chain and gastrointestinal tract was observed. DLB cases showed higher amounts of CNS AS aggregates than PD cases, but this was not the case in the pANS. No pANS AS aggregates were detected in Alzheimer’s disease (AD) cases with or without CNS AS aggregates. All pathologically confirmed LB disease cases including 1 case of iLBD had AS aggregates in the pANS with a craniocaudal gradient of pathology burden in sympathetic chain and gastrointestinal tract. AS was not detected in the pANS of any AD case. These findings may help in the search of peripheral AS aggregates in vivo for the early diagnosis of PD. © 2014 International Parkinson and Movement Disorder Society

Key Words: Parkinson’s disease; dementia with Lewy bodies; Parkinson’s disease with dementia; autonomic diseases; alpha-synuclein

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Abnormal aggregation of alpha-synuclein (AS) in the perikaryon, axons, and dendrites of neurons of different brain regions represents the molecular and pathological hallmark of Parkinson’s disease (PD) and other Lewy body (LB) diseases. These aggregates have been increasingly found in tissues outside the central nervous system (CNS), mainly in the peripheral autonomic nervous system (pANS).1-5 These observations have changed our understanding of PD from the conception of a disorder with selective involvement of nigrostriatal dopaminergic neurons to a much broader multisystem LB disorder, also characterized as “Parkinson complex”6 or Lewy-complex.7

It is still unclear whether peripheral pathology precedes or occurs concomitantly to the central pathology and whether it progresses in a predictable way as has been proposed for Lewy pathology in the CNS.7 It is a relatively frequent finding in subjects who have LB pathology at autopsy but have not shown parkinsonism during life (so-called incidental Lewy body disease [iLBD]) and in some such cases, postmortem studies have reported AS aggregates in pANS even without CNS involvement8 (see Supporting Table 1), suggesting that pANS involvement might be an early, pre-CNS event in PD pathogenesis. A detailed revision of the peripheral autonomic involvement in neurodegenerative disorders in general and alpha-synucleinopathies in particular, has been published recently.9

In the present study we aimed to systematically assess the distribution and severity of AS pathology in central and peripheral structures of the nervous system in postmortem tissue of donors with clinical diagnosis of PD and dementia with Lewy bodies (DLB) and other non-LB related neurodegenerative diseases.

Materials and Methods

Consecutive brain donors of the Neurological Tissue Bank of the Biobank-Hospital Clinic-IDIBAPS with the diagnosis of PD, DLB, and other non-LB disorders (atypical parkinsonisms and non-LB dementia) were included in the study. Written informed consent was obtained from donors and/or next of kin and the study was approved by our Institutional Ethics Committee.

The following tissues were prospectively collected: brain and spinal cord, pituitary gland, vagus nerve at brainstem and thoracic level, stellate ganglion and paravertebral sympathetic ganglia of both sides, mesenteric sympathetic ganglion, adrenal glands and surrounding fat tissue, distal esophagus, stomach (cardias, corpus, and fundus), ileum, colon (transverse and descending), rectum, periprostatic plexus or uterus, urinary bladder, heart, abdominal skin, and psoas muscle.

Processing of Samples

Brain

The left brain hemisphere and hemicerebellum were sliced in the fresh state and frozen at −80°C. The right hemisphere, hemicerebellum, and alternate sections of brainstem and spinal cord were fixed in 10% buffered formaldehyde and multiple brain areas were embedded in paraffin for histopathological evaluation.

Peripheral Tissues

One half of the sampled tissues was fresh-frozen and stored at −80°C, the other half was fixed in 10% buffered formaldehyde solution, embedded in paraffin and cut into 5-μm-thick sections for histopathological assessment.

Brain and spinal cord sections were stained with hematoxylin and eosin (H&E) and by immunohistochemistry using an automated immunostainer (Dako Autostainer plus, Glostrup, Denmark) with the following monoclonal and polyclonal (pc) primary antibodies: anti-alpha-synuclein (Novocastra, Newcastle, UK; clone KM51), anti-phospho-alpha-synuclein (Wako Pure Chemical Industries LTD, Japan; phosphorylated at Ser 129), anti-bA4-amyloid (DAKO, Glostrup, Denmark, clone 6F/3D), anti-phosphorylated tau (Thermo Scientific, Rockford, IL, USA; clone AT8), anti-ubiquitin (DAKO, pc), anti-TDP-43 (Abnova, Taipei, Taiwan; clone 2E2-D3), anti-RD3 (Millipore, Temecula, CA, USA; clone 8E6/C11), anti-RD4 (Millipore, clone 1E1/A6), anti-p62 (BD Transduction Laboratories, NJ, USA; clone 3/p62 lck ligand).

Peripheral tissues were stained with H&E and by immunohistochemistry for detection of alpha-synuclein (clone KM51) and tyrosine-hydroxylase (TH) (Sigma-Aldrich, St. Louis, MO, USA; clone TH-16). In selected cases and areas, anti-phospho-alpha-synuclein (pAS), considered to be a more specific and sensitive marker of LB-related pathology, anti-TDP43, anti-hpTau, and anti-betaA4 antibodies were also applied in the same conditions as in the CNS.

Semiquantitative Evaluation

The presence of LBs in H&E-stained sections was evaluated as present or absent. The density of AS-immunoreactive LBs and neurites (using the KM51

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Age</th>
<th>Gender Male (%)</th>
<th>PMI (h)</th>
<th>Disease Duration (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD (4)</td>
<td>79.75 (8.1)</td>
<td>25</td>
<td>9.58 (6)</td>
<td>11.5 (9)</td>
</tr>
<tr>
<td>PDD (6)</td>
<td>79.67 (6.1)</td>
<td>50</td>
<td>9.97 (5)</td>
<td>18 (8.8)</td>
</tr>
<tr>
<td>DLB (5)</td>
<td>80 (3.7)</td>
<td>20</td>
<td>14.23 (4.3)</td>
<td>4.6 (2.6)</td>
</tr>
<tr>
<td>AD (8)</td>
<td>86.13 (5.1)</td>
<td>25</td>
<td>10.67 (6.6)</td>
<td>8.75 (4.1)</td>
</tr>
<tr>
<td>Others (5)</td>
<td>78.4 (10.2)</td>
<td>40</td>
<td>10.78 (5.2)</td>
<td>9.4 (6.4)</td>
</tr>
</tbody>
</table>

Values are mean (SD) except where indicated.

*Others: Progressive supranuclear palsy (1), vascular dementia (1), vascular dementia and Alzheimer-type pathology (1), frontotemporal lobar degeneration (1), hepatic encephalopathy + mild AD-related changes (1).

PMI, postmortem interval; PD, Parkinson’s disease; PDD, Parkinson’s disease-dementia; DLB, dementia with Lewy bodies; AD, Alzheimer’s disease.

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antibody) was semiquantitatively assessed according to McKeith et al.\textsuperscript{10} as: 0, absent; 1, isolated or mild (Fig. 3N); 2, moderate (Fig. 3F); and 3, abundant (Fig. 3B). Density of TH-immunoreactive nerve fibers and neurons was also semiquantitatively assessed in selected areas as: 3, normal (Fig. 3H); 2, moderately reduced (Fig. 3G); 1, severely reduced (Fig. 3K); and 0, absent. In selected cases, stellate ganglion was analyzed for the presence of AT8, betaA4, TDP43-immunoreactive structures and was indicated as present (1) or absent (0).

Other, non-LB related pathologies in the brain were evaluated according to their respective diagnostic criteria.

**Comparative Studies and Statistical Analyses**

Comparisons between central and peripheral pathology and clinical variables among the different disease groups were performed using Kruskal Wallis or Spearman correlation tests when appropriate.

**Results**

**Patients**

Twenty-eight adult donors were included between April 2009 and January 2010. Thirty-two percent were male. Mean age was 81.36 years (range, 62–93). The mean postmortem delay was 11.02 hours (range, 4.25–21.35).

Clinicopathological diagnoses comprised PD (n = 10; 6 with dementia), DLB (n = 5), AD (n = 8), progressive supranuclear palsy (PSP) (n = 1), vascular encephalopathy with small vessel disease (n = 2, one with associated AD neuropathological changes), and frontotemporal lobar degeneration with motor neuron disease and TDP43+ inclusions (n = 1). One case with cognitive impairment showed iLBD/AS pathology in brain stem nuclei and intermediolateral column of spinal cord (n = 1) (Table 1), along with mild AD neuropathological changes, and was considered an iLBD case.

Mean disease duration from the time of diagnosis to death was 10.5 years (range 1–30). Patients with LB pathology (PD and DLB, n = 15) had a mean age at the time of death of 79.8 years (range, 68–86) and a mean disease duration of 11.8 years (range, 1–30). DLB patients presented a shorter disease duration compared to PD (4.6 vs 15.4 years; Mann-Whitney U, P = 0.012), without differences in age at the time of death (80 vs 79.7 years).

**Distribution of AS Aggregates in the CNS**

All cases with the diagnosis of an LB disorder (n = 15) presented AS aggregates in the intermediolateral column of spinal cord, brainstem, limbic region, and variably in cortical areas. PD cases had a median Braak stage of 5 (range, 4–5)\textsuperscript{7} and DLB cases were all classified as neocortical stage.\textsuperscript{10} Data from a semiquantitative assessment are shown in Supporting Figure 1 and in Table 2. The highest AS density was found in the olfactory and limbic regions in both LB conditions, while there was less severe cortical involvement in PD than in DLB (Supporting Fig. 1). Overall, DLB patients showed a higher amount of CNS AS aggregates compared to PD (mean 2.24 vs 1.89; Mann-Whitney U, P = 0.017) (Fig. 1A). This difference was mainly due to a greater involvement of limbic and cortical structures (limbic 2.41 vs 2.0, Mann-Whitney U, P = 0.022; cortical 1.8 vs 1.23, Mann-Whitney U, P = 0.022). A correlation study showed a weak, nonsignificant negative relationship between mean AS load and disease duration.

In 4 AD cases, mild to moderate concomitant AS immunoreactive (AS+) LBs and Lewy neurites (LNs) were observed in amygdala and/or olfactory bulb but not in other brain regions. In the 1 iLBD case, infrequent LN were observed in brainstem nuclei such as dorsal nucleus of the vagal nerve, formatio reticularis, locus coeruleus, and in the intermediolateral column of spinal cord, corresponding to Braak stage 2.

**Distribution of AS/pAS Aggregates in Peripheral Organs**

LB and AS/pAS aggregates in pANS were found in the 15 LB disorders and the single iLBD case in different grades of severity in the following distribution (see also Figs. 2 and 3, Supporting Fig. 2, and Table 2). No AS aggregates were detected in the pANS of non-LB diseases.

**Paravertebral Sympathetic Ganglia**

Sympathetic paravertebral ganglia were affected in all LB disease cases and were the structures with the highest AS/pAS burden. Affected ganglia showed some vacuolated neurons and variable loss of pigmented and nonpigmented neurons with increased cellularity and formation of Nageotte nodules (Figs. 2A, 3A). Abundant LB and LN-like structures were observed in H&E-stained sections as large, pale, or bright eosinophilic amorphous structures, in neuronal processes and in the perikaryon of sympathetic neurons (Fig. 2A). The highest density of LB and LN occurred at the periphery of the ganglia. LB and LN were immunoreactive for AS (Fig. 3B), pAS (not shown), and ubiquitin (Fig. 2I) and showed a peripheral rim stained by anti-neurofilaments (Fig. 2H). They were also faintly and irregularly stained by anti-hyperphosphorylated Tau (Fig. 2D) and nonspecifically by anti-TDP43 antibodies (Fig. 2F, arrows). No beta-A4 deposits were identified in any ganglion.

A gradient of severity of pathology was observed with the highest AS burden present in the stellate
ganglion and in the cervical sympathetic ganglia followed by a progressive reduction in AS density from upper thoracic to the lower thoracic and lumbar sympathetic ganglia. A reduction of TH immunoreactivity in some neuronal cell bodies and nerve fibers was also observed (Fig. 2B, 2G, and Fig. 3C).

These alterations (cell loss and AS aggregates) and the rostrocaudal gradient were seen in both, PD and DLB cases, but were more prominent in DLB cases.

Moreover, other sympathetic ganglia such as the mesenteric ganglion were also affected in PD and DLB cases and showed a similarly high density of AS aggregates as in the cervical sympathetic ganglia.

**Heart**

Multiple areas were sampled to include the regions more likely to contain AS aggregates. The most frequently involved areas were the anterior and lateral wall of the left ventricle. AS/pAS aggregates were detected as LN of variable thickness in the myocardium, in the intramyocardial nerve fibers (Fig. 3J) and also at perivascular location in 2 cases. Most frequently, however, AS/pAS+ LN and LB-like structures were identified in epicardial fat tissue in small nerves and small autonomic ganglia, both in neuronal processes and cell bodies (Fig. 3F). These changes were observed in all PD and DLB cases. They were also frequently observed in nerve branches around coronary arteries. Immunostaining for TH showed a reduction of the density of TH-positive fibers in AS+ cases compared to cases without AS aggregates between myocytes (Fig. 3K vs 3L) and in epicardial fat tissue fibers (Fig. 3G vs 3H), suggesting some degree of denervation, although these data were not quantified. A positive correlation between TH fiber density and density of AS aggregates, ie, less AS-aggregates in cases with less TH innervation, was observed (data not shown).

**Vagus Nerve**

AS aggregates were observed along nerve fibers most frequently at the brainstem level. At the thoracic level isolated positive fibers were seen in several LB disease cases.

**Gastrointestinal System**

We assessed distal esophagus, stomach (cardias, corpus, and fundus), ileum, colon (transverse and descending), and rectum. Delicate AS/pAS-immunoreactive neuronal processes were detected in the autonomic ganglia of the myenteric plexus in some cases

![FIG. 1. AS density in CNS and pANS. Comparison of semiquantitatively assessed AS density in grouped CNS areas (A) and pANS (B) between PD and DLB. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]](image_url)
Table 2. AS aggregates in the CNS and pANS

<table>
<thead>
<tr>
<th>CNS region</th>
<th>PD (n = 10)</th>
<th>DLB (n = 5)</th>
<th>AD (n = 8)</th>
<th>Others (n = 5)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olfactory</td>
<td>10/10, 100%</td>
<td>5/5, 100%</td>
<td>4/8, 50%</td>
<td>0/5, 0%</td>
</tr>
<tr>
<td>Brainstem</td>
<td>10/10, 100%</td>
<td>5/5, 100%</td>
<td>1/8, 12.5%</td>
<td>1/5, 20%</td>
</tr>
<tr>
<td>Limbic</td>
<td>10/10, 100%</td>
<td>5/5, 100%</td>
<td>3/8, 37.5%</td>
<td>0/5, 0%</td>
</tr>
<tr>
<td>Cortical</td>
<td>9/10, 90%</td>
<td>5/5, 100%</td>
<td>0/8, 0%</td>
<td>0/5, 0%</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>10/10, 100%</td>
<td>5/5, 100%</td>
<td>0/8, 0%</td>
<td>1/5, 20%</td>
</tr>
<tr>
<td>pANS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stellate ganglion</td>
<td>10/10, 100%</td>
<td>5/5, 100%</td>
<td>0/8, 0%</td>
<td>1/5, 20%</td>
</tr>
<tr>
<td>Sympathetic chain</td>
<td>10/10, 100%</td>
<td>5/5, 100%</td>
<td>0/8, 0%</td>
<td>1/5, 20%</td>
</tr>
<tr>
<td>Abdominal ganglia</td>
<td>8/10, 80%</td>
<td>5/5, 100%</td>
<td>0/8, 0%</td>
<td>1/5, 20%</td>
</tr>
<tr>
<td>Vagus nerve</td>
<td>9/10, 90%</td>
<td>4/5, 80%</td>
<td>0/8, 0%</td>
<td>1/5, 20%</td>
</tr>
<tr>
<td>GI</td>
<td>8/10, 80%</td>
<td>5/5, 100%</td>
<td>0/8, 0%</td>
<td>1/5, 20%</td>
</tr>
<tr>
<td>Cardiac</td>
<td>10/10, 100%</td>
<td>5/5, 100%</td>
<td>0/8, 0%</td>
<td>1/5, 20%</td>
</tr>
<tr>
<td>GU</td>
<td>0/10, 0%</td>
<td>2/5, 40%</td>
<td>0/8, 0%</td>
<td>0/5, 0%</td>
</tr>
<tr>
<td>Adrenal</td>
<td>6/10, 60%</td>
<td>2/5, 40%</td>
<td>0/6, 0%</td>
<td>0/5, 0%</td>
</tr>
<tr>
<td>Psoas</td>
<td>6/10, 60%</td>
<td>0/5, 0%</td>
<td>0/8, 0%</td>
<td>0/5, 0%</td>
</tr>
<tr>
<td>Skin</td>
<td>0/10, 0%</td>
<td>0/5, 0%</td>
<td>0/8, 0%</td>
<td>0/5, 0%</td>
</tr>
</tbody>
</table>

Values are absolute numbers (percentage).
*aOthers: Progressive supranuclear palsy (1), vascular dementia (1), vascular dementia and Alzheimer-type pathology (1), frontotemporal lobar degeneration (1), hepatic encephalopathy = mild AD-related changes (1).

AS, alpha-synuclein; CNS, central nervous system; pANS, peripheral autonomic nervous system; PD, Parkinson's disease; DLB, dementia with Lewy bodies; AD, Alzheimer's disease; GI, gastrointestinal system; Cardiac, heart, cardiac plexus and epicardial fat; GU, genitourinary system; Adrenal, adrenal gland.

(Fig. 3N), whereas in others, coarse LB-type inclusions were seen. AS/pAS aggregates were observed in distal esophagus, stomach, and colon, in all DLB cases and 80% of PD. We observed a gradient of severity of pathology with the highest AS burden present in distal esophagus and stomach, and the lowest in rectum. No AS/pAS aggregates were observed in lamina propria mucosae. The presence of AS aggregates was not related to the presence of inflammatory infiltrates.

Adrenal Glands

Isolated LB and/or LN were seen in the medulla of adrenal gland but were more frequently found in the autonomic ganglia embedded in the periadrenal fat tissue. TH immunoreactivity was very intense in adrenal medulla in both non-LB and LB disorders.

Vesicoprostatic/Vesicouterine Plexus

AS/pAS aggregates were not detected in any PD case and only in 2 DLB cases.

Psoas Muscle

Fine AS+ neurites were identified in 6 cases in nerve fibers crossing the muscle, but not in muscle cells themselves.

Abdominal Skin

No AS aggregates were detected in abdominal skin in any of the cases studied.

In PD and DLB cases there were significantly more aggregates in the CNS than in the pANS (Wilcoxon test, \( P = 0.005 \) and \( P = 0.043 \), respectively). No significant differences in the amount of peripheral AS between DLB and PD patients were detected (mean 1.11 vs 1.08, \( P = \text{nonsignificant} \)) (Fig. 1B). There was a weak, but nonsignificant relationship between AS density in the pANS and disease duration. No relationship between TH density and disease duration was found.

Discussion

In this study, all pathologically confirmed LB disorders had pANS involvement with AS/pAS aggregates, irrespective of the clinical diagnosis, including 1 iLBD case. These results are in accordance with previous studies showing that extensive AS pathology occurs in the pANS in PD, DLB, and iLBD.

As none of our cases with AD with or without associated olfactory and/or amygdala LB had AS aggregates in pANS, it might be suggested that CNS accumulation of AS in these cases may possibly represent a consequence of a more local effect of abnormal brain proteins such as beta-amyloid and tau, promoting AS fibrillation and its aggregation, rather than a “primary” phenomenon as observed in PD.

Organs invariably harboring AS pathology in our LB disease study subjects were the stellate ganglion and paravertebral sympathetic chain and the heart, with other systems more variably affected.

We observed a craniocaudal gradient of pathology burden involving paravertebral sympathetic chain and gastrointestinal tract; ie, more abundant AS aggregates in cervical and upper thoracic sympathetic ganglia than in lower lumbar ganglia, and more abundant AS
aggregates in esophagus and stomach than in colon. The rostrocaudal gradient within the gastrointestinal system has also been observed by others and may reflect distribution of lesions in the territory of vagal innervation. The rostrocaudal gradient of pathology observed in the sympathetic chain might reflect greater involvement of cardiac than of the gastrointestinal autonomic system. In the heart, we observed a greater involvement with AS of the anterior wall of the left ventricle and of the epicardial autonomic tissue than of the myocardium, and a reduction of the density of AS aggregates with decreasing density of myocardial and epicardial TH-positive fibers, supporting a degeneration of sympathetic fibers as suggested by Orimo and colleagues. Involvement of paravertebral sympathetic ganglia as well as of cardiac and gastrointestinal autonomic nervous system has been reported previously in postmortem studies (see Supporting Table 1) showing results comparable to ours. We did not assess submandibular or salivary glands as recently described, neither endocrine or respiratory organs. In contrast to the frequent involvement of vesicoprostatic plexus described, for example, by Minguez-Castellanos and colleagues in asymptomatic subjects and Beach and colleagues, we did not observe involvement of these structures in our postmortem material. AS/pAS aggregates in adrenal medulla were much less prominent than in periadrenal ganglia. Involvement of adrenal glands has been studied in detail by Fumimura and colleagues and AS aggregates have been detected in about 26% of a series of 783 consecutive autopsies. Although they found involvement in all PD cases and 91% of DLB cases, the adrenal gland was not affected by AS aggregates in amygdala variant cases and LBD cases with concomitant AD pathology. Similarly, in our AD cases with amygdala-only LB we did not detect peripheral AS aggregates. Moreover, we could not detect AS

FIG. 2. Stellate ganglion. Histological images of an affected stellate ganglion in DLB showing some enlarged neurons (A, HE) that lost TH immunoreactivity (B, anti-TH) and are immunoreactive for ubiquitin (C, anti-ubiquitin). Some figures reminiscent of Nageotte nodules were also detected (A, inset). Some of the LB-like structures show a delicate rim of hPTau-immunoreactivity, as frequently seen in the CNS (D, AT8). Frequent CD68 positive microglia/macrophages were seen between neuronal bodies (E, anti-CD68). TDP43 immunohistochemistry showed physiological nuclear stain of sympathetic neurons and some unspecific stain of tortuous Lewy neurites (F, arrows). Some of the LB and LN were immunolabeled by anti-TH antibodies (G, center of the image), as did anti-neurofilaments (H) and anti-ubiquitin (I). Scale bars: 20 μm (A, B, D-I), 50 μm for (C). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
aggregates in abdominal skin, described by Ikemura and colleagues\(^4\) in 24% of LBD autopsy cases. The cause of pANS involvement by LB-type pathology, as is the case for the selective involvement of some central neuronal systems, remains unclear. It has been speculated that peripheral toxins entering the body via the gastrointestinal tract could in part explain the prominent and early pANS involvement\(^{15,16}\) in the LB disorders. The fact that the long parasympathetic neurons in the dorsal nucleus of the vagal nerve and the long sympathetic neurons in the paravertebral sympathetic ganglia are most severely affected supports Braak’s consideration that the length of unmyelinated axons may stand behind this vulnerability.

Burden of AS may vary with disease progression both in the CNS and the peripheral nervous system. In the CNS, cases with shorter survival and more aggressive course show higher LB loads than cases with longer clinical course.\(^{17}\) In our study, higher AS load occurred in DLB brains when compared to PD cases, with or without dementia. DLB cases had shorter disease duration and the higher AS burden could reflect a more rapidly evolving process and, accordingly, a reduced AS turnover and increased deposition. However, differences in AS burden was not observed in the

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**FIG. 3.** Involvement of pANS. Representative images of pANS involvement of different organs in a DLB case and a non-DLB case. (A-C) represents stellate ganglion, (E-G) the epicardial fat tissue, (I-K) myocardial muscle and nerve fibers, and (M-O) myenteric plexus of esophagus in a DLB case compared to the same structures in (D), (H), (L), and (P) in a non-DLB case. A: Abundant LBs are already seen in HE stain between pigmented sympathetic neurons. These are strongly immunoreactive for AS (clone KM51), with a peripheral enhancement (B). Mild loss of TH immunoreactivity is observed in neuronal bodies and fibers between them (C) when compared to the density in an unaffected case (D). E-H: In epicardial fat tissue large autonomic nerves can be seen already in HE-stained sections (E). There, AS aggregates are seen along the nerve fibers as coarse deposits (F; clone KM51). There is a reduced density of TH-immunoreactive fibers (G) when compared to an unaffected case (H). I-L: In the myocardium, some large nerve fibers can be seen in HE-stained sections (I). Also these fibers may show coarse AS-aggregates in cases with shorter disease course (J). In these cases, there is a marked reduction of TH-immunoreactive profiles in the myocardium (K) when compared to an unaffected case (L). M-P: In the gastrointestinal system, autonomic ganglia of the myenteric plexus as seen in HE-stained sections (M) are usually the area where AS-aggregates can be found. These can be seen in some cases as delicate processes surrounding neuronal bodies (N, arrows). TH-immunoreactivity is only seen in few neurons (O), as seen also in nonaffected cases (P). Scale bars: 20 μm (A, F, G, H, K, N); 50 μm (B, C, D, E, I, J, L, M, O, P). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
pANS in these 2 disorders, maybe suggesting a similar peripheral AS turnover.

The results of our report are based on the study of subjects with late, very advanced disease. Distribution and severity of peripheral AS/pAS pathology may not be similar though in early PD subjects. It has been suggested that AS aggregation occurs at the axonal endings of neurons and later in the soma, progressively disappearing from the distal sites at later disease stages. Should such developments take place, one could expect higher levels of AS/pAS in the distal end organs in early than in late disease stages. Postmortem studies in early PD are needed to validate this hypothesis. In inLBD, considered to represent an early, premotor stage of PD, AS aggregates have been described in the myenteric plexus of the esophagus and stomach, submandibular gland, paravertebral sympathetic ganglia, vagus nerve, sciatic nerve, and endocrine system. In our single iLBD case, however, we did not observe higher AS load in peripheral organs.

The observed distribution of AS pathology in our subjects and in previous studies, suggests that the sympathetic cardiac and stellate/paravertebral ganglia would be informative targets for identifying AS aggregates in living subjects, and we have recently studied epicardial fat autonomic tissue obtained during surgery and observed AS/pAS in 8% of subjects without parkinsonism. The obvious difficulties and risks involved in the access of such tissues through biopsy makes them unfeasible for diagnostic purposes. Cardiac sympathetic innervation, however, can be studied by functional imaging, using ligands such as 123I-metaiodobenzylguanidine (MIBG) single-photon emission computed tomography (SPECT), and this imaging tool is being increasingly used for the differential diagnosis of heart disease. In vivo functional studies have early in PD are needed to validate this hypothesis. In inLBD, considered to represent an early, premotor stage of PD, AS aggregates have been described in the myenteric plexus of the esophagus and stomach, submandibular gland, paravertebral sympathetic ganglia, vagus nerve, sciatic nerve, and endocrine system. In our single iLBD case, however, we did not observe higher AS load in peripheral organs.

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Several recent studies have studied colonic and salivary glands and identified AS in most of the tissues biopsied in advanced, early, and even premotor PD. Due to its accessibility the distal gastrointestinal tract has been the focus of several recent studies on the presence of AS pathology in PD although diverging results in these tissues have been reported. Our study suggests that AS aggregates may not always be present in these tissues in late stage of PD. In conclusion, we observed AS/pAS in pANS in all LB disease cases studied, reinforcing the view that pANS involvement is an integral part of these disorders. pANS involvement, on the other hand, did not occur in AD cases despite the frequent presence of concomitant CNS LBs. The extensive involvement of the pANS in LB disorders suggests that tissue-based and functional studies of this system may prove useful in the future for an accurate diagnosis of LB diseases in vivo.

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References


Supporting Data

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