Analysis of the Adrenal Gland Is Useful for Evaluating Pathology of the Peripheral Autonomic Nervous System in Lewy Body Disease

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Abstract

Lewy body disease is defined as Lewy body-related neuronal degeneration involving the nigrostriatal system, limbic-neocortical system, and peripheral autonomic nervous system (PANS). We investigated whether the adrenal gland, which is evolutionarily related to sympathetic ganglia and is routinely examined in general autopsy, could be used to assess pathology of the PANS in Lewy body disease. Brains, spinal cords, and adrenal glands from 783 consecutive autopsy cases from a general geriatric hospital were examined immunohistochemically with antiphosphorylated α-synuclein antibodies and routine staining. Parkinson disease (PD) with dementia and dementia with Lewy bodies (DLB) were defined using 1996 Consensus Guidelines for DLB and the secondary Lewy body-related α-synucleinopathy or amygdala variants using previously established criteria. Lewy body-related α-synucleinopathy was found in 207 (26.4%) of 783 cases, with 1 case solely in the adrenal gland. In all 18 PD cases with or without dementia and in 33 of 38 DLB cases, the adrenal gland was involved, but it was spared in all cases of amygdala variants. Our results indicate that the adrenal gland can provide useful information for evaluation of the PANS in Lewy body disease.

Key Words: Alzheimer disease, Amygdala variant, Autonomic failure, Dementia with Lewy bodies, Parkinson disease, Sympathetic ganglion, α-Synucleinopathy

INTRODUCTION

Lewy body disease was originally defined pathologically as degeneration of the central nervous system associated with Lewy bodies (1, 2) and includes Parkinson disease (PD) and dementia with Lewy bodies (DLB). Subsequently, clinical and pathologic studies indicated that progressive autonomic failure of the Lewy body type presented with Lewy body-related pathology in the peripheral autonomic nervous system, as well as in the central nervous system (3). Clinical and pathologic studies confirmed that DLB always accompanies Lewy body-related pathology in the peripheral autonomic nervous system (4). Thus, it is more practical to use the term “Lewy body disease” to designate disorders involving both the central nervous system and the peripheral autonomic nervous system, which clinically present with various combinations of parkinsonism, cognitive decline, or autonomic failure (5).

Clinical evaluation of the involvement of the peripheral autonomic nervous system in Lewy body disease has been improved by the adoption of [123I]metaiodobenzylguanidine (MIBG) cardiac scintigraphy (6), which shows low uptake of [123I] in PD and progressive autonomic failure (7, 8). Histologically, this low uptake corresponds to a decrease in the number of tyrosine hydroxylase-immunoreactive axons (9) associated with α-synucleinopathy in the epicardium of the anterior wall of the left ventricles of the heart (10) seen on postmortem examination. MIBG cardiac scintigraphy reportedly has 100% specificity and sensitivity for the differential diagnosis of DLB and Alzheimer disease (AD) (11). Thus, evaluation of the peripheral autonomic nervous system is now a standard for confirmation of the pathologic diagnosis of Lewy body disease.
The sympathetic ganglia are the most widely used specimen for the evaluation of the peripheral autonomic nervous system in Lewy body disease (12). However, these ganglia and the epicardium of the anterior wall of the left ventricle of the heart are not a routine site for investigation in general autopsy. In contrast, the adrenal gland is always included in routine autopsy examinations and is a good candidate for examination of the peripheral autonomic nervous system because it is evolutionarily related to sympathetic ganglia and includes autonomic nerves and ganglia in the capsular fatty tissue. Several previous studies indicated that the adrenal gland might be involved in PD (13). However, the exact incidence of adrenal gland involvement in Lewy body disease is not well established.

We recently reported a staging paradigm for Lewy body-related α-synucleinopathy (LBAS) in consecutive autopsy cases roughly representing a general cohort of the elderly (14, 15). Employing the same strategy in the present study, we provide evidence that evaluation of the peripheral autonomic nervous system in Lewy body disease is possible through the examination of archival paraffin blocks of adrenal glands. Our studies also suggest that adrenal involvement may be associated with orthostatic hypotension in Lewy body disease.

**MATERIALS AND METHODS**

**Tissue Source**

For the present study, we used 783 consecutive autopsy brains, spinal cords, and adrenal glands obtained from the Tokyo Metropolitan Geriatric Hospital (TMGH). This hospital provides community-based medical service to the aged population 24 hours/day in cooperation with local general practitioners. The number of patients requiring emergency admission to the hospital reaches almost 5,000 per year. The hospital holds 711 beds in its ward and is directly run by the Tokyo Metropolitan Government to promote the health and welfare of an aged population of nearly 1 million residents of the Tokyo metropolitan area. In the present study, 452 of the 783 examined cases overlapped cases used in a previous study (15). The patient ages ranged from 48 to 104 years (80.68 ± 8.8 years, mean ± SD) at the time of death, and the male to female ratio was 455:328. The postmortem interval ranged from 52 minutes to 88 hours (13.16 ± 6:36 hours). Tissue samples were collected after informed consent was obtained from relatives of the deceased according to the Article 18 of the Cadavers Autopsy and Preservation Act in Japan.

**Neuropathology**

**Routine Staining**

All brains and spinal cords were examined as described previously (15). Briefly, 6-μm-thick sections of the representative anatomical areas were stained with hematoxylin and eosin using the Klüver-Barrera method and further examined by means of modified methenamine (16) and Gallyas-Braak silver (17) staining to detect senile changes, Congo red staining to detect amyloid deposition, and elastic Masson trichrome staining to detect vascular changes. In addition, the bilateral adrenal glands were fixed in 10% buffered formalin and embedded in paraffin and then 3-μm-thick serial sections were obtained for hematoxylin and eosin staining.

**Immunohistochemistry**

A Ventana NX20 automated immunostainer (Ventana, Tucson, AZ) was used (18) with the following antibodies: anti-phosphorylated tau (ptau) (AT8, monoclonal; Innogenetics, Temse, Belgium), anti-β amyloid (11–28, 12B2, monoclonal; IBL, Maebashi, Japan), anti-phosphorylated α-synuclein (psyn#64 [14] and Pser129 polyclonal [19]), anti-α-synuclein (LB509, amino acids 115–122 [20], monoclonal), anti-ubiquitin (polyclonal, Sigma-Aldrich, St. Louis, MO), anti-phosphorylated neurofilament (SMI31, monoclonal; Sternberger Immunoreagents, Bethesda, MA) and anti-tyrosine hydroxylase (anti-TH, monoclonal; Calbiochem-Novabiochem Corporation, Darmstadt, Germany).

**TABLE 1.** Lewy Body (LB) Stages in the Central Nervous System (14, 15)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Substantia Nigra and Locus Ceruleus: Loss of Pigmentation</th>
<th>LB</th>
<th>Dementia</th>
<th>Parkinsonism</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0-10</td>
</tr>
<tr>
<td>III</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0-10</td>
</tr>
<tr>
<td>IV</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3-6</td>
</tr>
<tr>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>7-10</td>
</tr>
</tbody>
</table>

*, No dementia or parkinsonism associated with Lewy body-related α-synucleinopathy.
†, Differential diagnosis of PDD and DLB was based on the “1-year rule” according to the Consensus Guidelines (21).

LB, Lewy body; DLB, dementia with Lewy bodies, with a Lewy body score corresponding to the value for the neocortical form; DLBT, dementia with Lewy bodies, with a Lewy body score corresponding to the value for the transitional form; PDDN, Parkinson disease with dementia, with a Lewy body score corresponding to the value for the neocortical form; PDDT, Parkinson disease with dementia, with a Lewy body score corresponding to the value for the transitional form.
Lewy Body-Related Pathology
Central Nervous System

The medulla oblongata at the level of the dorsal motor nucleus of the vagus, the upper pons at the level of the locus ceruleus, and the midbrain (including the substantia nigra, the amygdala, and the anterior hippocampus from all cases) were immunohistochemically stained with anti-phosphorylated α-synuclein antibodies. When positive results were obtained in any case, the anterior cingulate gyrus, the entorhinal cortex, the second frontal and temporal gyri, and the supramarginal gyrus were immunohistochemically examined using anti-ubiquitin antibody to provide Lewy body scores (21), and the results were confirmed using anti-α-synuclein and anti-phosphorylated α-synuclein antibodies. The basal nucleus of Meynert (22), CA2-3 of the posterior hippocampus (23), and several (at least upper, middle, and lower) levels of the thoracic spinal cord were also examined with the anti-phosphorylated α-synuclein antibodies. The Lewy body stage (Table 1) was determined for all the cases examined, as reported previously (14, 15). In this study, we added Stage 0.5 as Lewy neurites alone, or diffuse or fine granular cytoplasmic staining lacking any focal aggregates, in sections immunohistochemically stained with anti-phosphorylated α-synuclein antibodies, following the revised Consensus Guidelines for DLB (22). PD with dementia was differentiated from DLB using the definition in the Consensus Guidelines: “dementia appears more than 12 months after the onset of parkinsonism” (21). In this study, we subcategorized our Stages I and II into primary and secondary α-synucleinopathy, based on our previous results (14, 15). Primary α-synucleinopathy (24) showed accentuation in the brainstem and spread to the spinal cord and was further subdivided into brainstem, transitional, and neocortical forms, according to the Lewy body score (21). Secondary α-synucleinopathy preferentially involved the amygdala and was termed the amygdala variant (25) in both Stage I (IA) and Stage II (IIA) (26).

The Adrenal Glands

The adrenal glands from all 783 cases were studied with hematoxylin and eosin staining and immunohistochemistry using monoclonal and polyclonal anti-phosphorylated α-synuclein antibodies. The immunoreactive structures were screened in the parenchyma as well as in the autonomic nerves or ganglia in the capsular fatty tissue.

Evaluation of Pathology Related to Other Disorders Presenting With Dementia or Parkinsonism

All 783 cases were evaluated with modified methenamine (16) and Gallyas-Braak silver (17) stainings as well as immunohistochemically using anti-phosphorylated tau

### Table 2. Lewy Body-Related α-Synucleinopathy in the Central Nervous System and Adrenal Glands

<table>
<thead>
<tr>
<th>LB Stage*</th>
<th>Type of Distribution/Diagnosis</th>
<th>PA</th>
<th>Dementia</th>
<th>Number of Cases</th>
<th>LBAS in the Adrenal Gland</th>
<th>Ratio (%)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td>577</td>
<td>1</td>
<td>0.2</td>
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<tr>
<td>0.5</td>
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<td></td>
<td></td>
<td>36</td>
<td>1</td>
<td>2.8</td>
</tr>
<tr>
<td>I</td>
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<td></td>
<td></td>
<td>85</td>
<td>14</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td></td>
<td></td>
<td>41</td>
<td>6</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>T</td>
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<td></td>
<td>35</td>
<td>8</td>
<td>22.9</td>
</tr>
<tr>
<td></td>
<td>A</td>
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<td></td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
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<td></td>
<td>29</td>
<td>20</td>
<td>69</td>
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<td></td>
<td>5</td>
<td>4</td>
<td>80</td>
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<td></td>
<td>T</td>
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<td></td>
<td>19</td>
<td>14</td>
<td>73.7</td>
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<td>A</td>
<td></td>
<td></td>
<td>3</td>
<td>0</td>
<td>0</td>
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<tr>
<td>III</td>
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<td>+</td>
<td></td>
<td>4</td>
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<td></td>
<td>27</td>
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<td>92.6</td>
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<tr>
<td></td>
<td>PDDT</td>
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<td>10</td>
<td>10</td>
<td>100</td>
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<tr>
<td></td>
<td>DLBT</td>
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<td></td>
<td>17</td>
<td>15</td>
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<tr>
<td>V</td>
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<td>100</td>
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<td></td>
<td>14</td>
<td>11</td>
<td>78.6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>783</td>
<td>87</td>
<td>11.1</td>
</tr>
</tbody>
</table>

*Lewy body stage (14, 15). LB, Lewy body; PA, parkinsonism; LBAS, Lewy body-related α-synucleinopathy; B, brainstem; T, transitional; N, neocortical; A, amygdala variant; PD, Parkinson disease without dementia; PDDT, Parkinson disease with dementia, with a Lewy body score corresponding to the value for the transitional form; DLBT, dementia with Lewy bodies, with a Lewy body score corresponding to the value for the transitional form; PDDN, Parkinson disease with dementia, with a Lewy body score corresponding to the value for the neocortical form; DLBN, dementia with Lewy bodies, with a Lewy body score corresponding to the value for the neocortical form.
(AT8) and anti-β amyloid antibodies. Neurofibrillary tangles were classified into 7 stages as defined by Braak and Braak (27). Senile plaques were also stratified according to Braak and Braak (27) because this Braak stage was the only available stage for parenchymal deposition of β amyloid. Argyrophilic grains were classified into 4 stages as we have previously described (28).

A neurofibrillary tangle stage equal to or greater than IV and senile plaque stage C were adopted for the diagnosis of AD, as previously reported (29). Diagnoses of “dementia with grains” and the “neurofibrillary tangle-predominant form of dementia” were based on Jellinger’s definitions (30, 31). A diagnosis of vascular dementia was based on the National Institute of Neurological Disorders and Stroke (NINDS)-Association Internationale pour la Recherche et l’Enseignement en Neurosciences (AIREN) criteria (32). A diagnosis of progressive supranuclear palsy was based on the NINDS diagnostic criteria (33), skipping the clinical inclusion scheme.

**Clinical Information**

Clinical information, including the presence or absence of parkinsonism and autonomic failure, as well as an assessment of the patient’s cognitive state, was obtained from medical charts. The entire collection of medical records, including neuroimages (magnetic resonance imaging, computed tomography, single photon emission computed tomography, and positron emission tomography) of the patients on whom an autopsy was performed, was stored in the TMGH’s database. When the previous medical history of another hospital was available, the medical records from that hospital were also obtained with written informed consent from the patient’s relatives. Scores from the Mini-Mental State Examination (34) or the Hasegawa Dementia Scale (35) or its revised version (36) and Instrumental Activities of Daily Living scale (37) were used to evaluate cognitive function.

**TABLE 3. Distribution of Lewy Body-Related α-Synucleinopathy in the Adrenal Gland Specimens**

<table>
<thead>
<tr>
<th>Region</th>
<th>Lewy Body-Related Pathology (Number)</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganglia in the adrenal medulla</td>
<td>58</td>
<td>66.7</td>
</tr>
<tr>
<td>Nerve fascicles in the adrenal cortex</td>
<td>23</td>
<td>26.4</td>
</tr>
<tr>
<td>Ganglia in the periadrenal fatty tissue</td>
<td>37 (of 50 cases*)</td>
<td>74.0</td>
</tr>
<tr>
<td>Nerve fascicles in the periadrenal fatty tissue</td>
<td>81</td>
<td>93.1</td>
</tr>
</tbody>
</table>

* Ganglia in the periadrenal fatty tissue were identified in 50 of the 87 cases.
The Clinical Dementia Rating Scale (38) was retrospectively determined by 2 independent board-certified neurologists. If the resulting Clinical Dementia Rating Scale scores were in agreement, the score was accepted. If not, the neurologists reconciled their differences after interviews with the patient’s attending physicians and caregivers. Locomotor activity was evaluated using the Barthel Index of Activity of Daily Living (39). Information about parkinsonism, tremor (resting), rigidity (cogwheel), bradykinesia, and postural instability was extracted from the records of neurologic examinations, and the presence of more than 2 of these symptoms was interpreted as positive for parkinsonism. To assess autonomic failure, documentation of orthostatic hypotension was retrieved from the charts. There were limitations in clinical assessment in this retrospective manner, compared with prospective clinical studies, but we made efforts to decrease the gap, using the merit of community-based settings. The majority of the cases had long-term follow-up (up to more than 40 years) and both cognitive and motor function parameters were routinely evaluated at each admission to TMGH. The majority of the relatives who approved the autopsy were also medically followed by the TMGH, and we tried to have direct interviews with them to confirm descriptions in clinical charts.

Statistical Analysis
Statistical analysis was performed using the chi-square test or the Fisher exact test for comparisons of categorical data. Statistical significance was set at \( p < 0.05 \).

RESULTS
Incidence and Distribution of Lewy Body-Related \( \alpha \)-Synucleinopathy in the Adrenal Glands

LBAS was found in 207 (26.4\%) of 783 cases examined. Among them, 87 cases (11.1\%) (Table 2) showed LBAS in the following areas of sections of the adrenal glands: 1) sympathetic ganglion cells in the adrenal medulla (Fig. 1); 2) sympathetic nerve fascicles in the interstitial tissue of the adrenal cortex (Fig. 2); 3) sympathetic ganglia in the fatty tissue surrounding the adrenal capsule (Fig. 3); and 4) nerve fascicles in the fatty tissue surrounding the adrenal capsule (Fig. 4). The above 4 structures were immunoreactive for anti-TH antibody, a marker of the sympathetic nervous system (Figs. 1C and 3C). The regional distribution of LBAS is summarized in Table 3.

So-called “adrenal bodies” (40) were always negative for anti-phosphorylated \( \alpha \)-synuclein antibodies (data not shown). SMI31 stained preserved unmyelinated fibers of TH-immunoreactive nerve fascicles in all of the cases with adrenal LBAS, including PD cases, in contrast to a marked decrease in TH-immunoreactive unmyelinated fibers in the pericardium in these cases (data not shown) (9, 10).

Comparison With the Lewy Body Stage in the Central Nervous System

The correlation between the Lewy body stage in the central nervous system and the presence or absence of \( \alpha \)-synucleinopathy in the adrenal gland is summarized in Table 2. Lewy bodies were found in one Lewy body Stage 0 case and in one Lewy body Stage 0.5 case. All of the PD cases with or without dementia had LBAS in the adrenal gland.

To elucidate the initial stage of LBAS, the percentage of cases with positive anti-phosphorylated \( \alpha \)-synuclein immunoreactivity in the adrenal glands was estimated in each subgroup of Lewy body Stage I and Stage II (Table 2). None of the amygdala variants exhibited anti-phosphorylated \( \alpha \)-synuclein immunoreactivity in the adrenal glands. In contrast, nearly 20\% of Stage I and approximately 80\% of Stage II cases of the primary \( \alpha \)-synucleinopathy presented with LBAS in the adrenal glands.

We further analyzed Lewy body Stage IV and Stage V cases (n = 5) that did not present with Lewy body-related pathology in the adrenal glands. These cases had no clinical description of Parkinsonism or orthostatic hypotension. Four of these cases were complicated by AD pathology (changes in senile plaque stage C and an neurofibrillary tangle stage...
equal to or greater than Stage III), and the fifth case was complicated by argyrophilic grain Stage III. All 14 DLB cases with a clinical description of parkinsonism and all 8 DLB cases with no such description but with other mild senile changes presented with adrenal LBAS. However, the 7 DLB cases with similar Alzheimer pathology and the 3 DLB cases with argyrophilic grain Stage III contained adrenal Lewy body pathology and did not show easily detectable morphologic differences from the above mentioned 5 cases without the adrenal Lewy body pathology.

Clinicopathologic Correlation With Lewy Body Pathology in the Adrenal Glands

Orthostatic hypotension was clinically described in the medical records for 6 of the 783 cases. Five of these cases showed LBAS in the adrenal glands: one case of PD without clinical description of dementia, one case of PD with dementia with the Lewy score of the transitional form, one case of PD with dementia with the Lewy score of the neocortical form, and 2 cases with DLB transitional form. Of the 2 cases in which Lewy bodies were restricted to the adrenal glands, one case with Lewy body Stage 0.5 clinically presented with syncope-like attack, but there was no definite evidence of orthostatic hypotension.

DISCUSSION

Our studies represent the first demonstration in the literature of the following. 1) LBAS always involved the adrenal gland in PD, with or without dementia. 2) Adrenal glands were always free of LBAS in cases with the amygdala variant. 3) DLB cases that lacked LBAS in the adrenal glands were always complicated by the presence of moderate to severe Alzheimer pathology or argyrophilic grain disease and had no clinical description of parkinsonism. 4) LBAS in the adrenal glands can occur independently of LBAS in the central nervous system. Thus, the immunohistochemical evaluation of adrenal glands with anti-phosphorylated α-synuclein antibodies can be used to evaluate Lewy body pathology involving the peripheral autonomic nervous system.

Lewy bodies and their related structures are present in the adrenal glands of patients with PD or DLB (13, 41). However, the detection ratio was only approximately 30% (41), which differed from the ratio in the sympathetic ganglia (42), in which Lewy bodies were always present in patients with PD or DLB. In the present study we were able to detect Lewy body-related pathology immunohistochemically in adrenal glands or their associated sympathetic tissues with anti-phosphorylated α-synuclein antibodies in

**FIGURE 3.** Lewy body-related α-synucleinopathy in a sympathetic ganglion from the fatty tissue surrounding the adrenal capsule. (A) Lewy bodies (arrow) are visible in a hematoxylin and eosin-stained section. (B) Anti-phosphorylated α-synuclein (Pser129) immunostaining visualizes the abundant Lewy body-related α-synucleinopathy (arrow). This image represents a serial section of that shown in (A). (C) Anti-tyrosine hydroxylase staining in the neuronal cytoplasm, neurites, and Lewy bodies (arrow). This image is a serial section of that shown in (B). (D) Anti-phosphorylated neurofilament antibody (SMI31) reveals axons (arrowheads) and some neuronal perikarya (double arrows). The periphery of some Lewy bodies (arrow) is intensely stained by the antibody. Scale bars = (A–D) 25 μm.)
all cases of PD. Because adrenal glands are routine sites of investigation in general autopsy, our results indicate that evaluation of the peripheral autonomic nervous system in Lewy body disease is possible through the examination of archival paraffin blocks of adrenal glands. Pathologic examination in TMGH requires strict removal of fatty tissue from adrenal glands to evaluate their exact weight. When such removal is not done, the detection rate of periadrenal paraganglia was almost 100% (Dr. K. Kawabata, Director, Department of Pathology, Akashi City Hospital, personal communication, 2006). Because the periadrenal retroperitoneal space contains abundant paraganglia and associated sympathetic ganglia and nerves, even the very thin surrounding tissue of the adrenal glands in our series always included useful peripheral sympathetic nervous tissue.

Adrenal glands are frequently affected by autolysis, inflammation, or metastasis, but our study suggests that the organs and the surrounding sympathetic ganglia and nerves are nevertheless useful for assessing morphologic changes in the peripheral autonomic nervous system in PD or DLB.

The amygdala variant of α-synucleinopathy is complicated by either a severe burden of tangles and plaques or by argyrophilic grains in the amygdala. This type of α-synucleinopathy is associated with AD and Down syndrome (25, 26, 43, 44), as well as with other tauopathies (45). We termed this type “secondary” (14, 15). The present study clearly shows that immunopathologic studies of the adrenal glands can distinguish secondary α-synucleinopathy from PD.

Braak et al (46) proposed a staging system for α-synucleinopathy in the brains of a nondemented general cohort and in cases of PD. Our series, with a cohort having a mean age of approximately 80 years, included a high percentage of dementia, as was expected. The differential diagnosis between DLB and PD with dementia is often difficult in such aged cohorts. Thus, Braak et al’s staging paradigm could not be applied effectively to our group (15). We always examined the spinal cord, a structure not included in Braak et al’s staging, to evaluate the preganglionic sympathetic neurons. Our results show that some DLB cases, whose α-synucleinopathy definitely involved sympathetic preganglionic neurons, did not present with α-synucleinopathy in the adrenal or periadrenal tissues. All of these cases met the morphologic criteria for AD from the elderly cohort (47) or for dementia with grains (28) and lacked a clinical description of parkinsonism. However, many other cases of DLB, complicated by similar changes in AD or dementia with grains and lacking a clinical description of parkinsonism, presented with α-synucleinopathy involving the adrenal or periadrenal tissues. Although

![FIGURE 4. Lewy body-related α-synucleinopathy in a nerve fascicle from the fatty tissue surrounding the adrenal capsule. (A) An axonal pale body (arrowheads) is detectable with hematoxylin and eosin staining. (B) Anti-phosphorylated α-synuclein antibody (psyn#64) stains the pale body (arrowheads). Lewy dots are also visualized by the antibody. This image is a serial section of that shown in (A). (C) Anti-tyrosine hydroxylase antibody clearly visualizes the pale body (arrowheads) as well as adjacent axons in the fascicle. This image is a serial section of that shown in (B). (D) Anti-phosphorylated neurofilament antibody (SMI31) stains the periphery of the pale body (arrowheads) as well as axons of the nerve fascicle. This image is a serial section of that shown in (C). Scale bars = (A-D) 25 μm.](http://jnen.oxfordjournals.org/)
morphologic differences in the Lewy body pathology in the central nervous system at the final stage of the illness may become unclear, the pathologic examination of LBAS in the peripheral autonomic nervous system could delineate those DLB cases complicated by other senile changes and presenting with a limbic-neocortical-dominant distribution of Lewy body pathology, lesser involvement of the brainstem and spinal cord, and a lack of adrenal and periadrenal Lewy body pathology from other DLB cases with a pathology more common to PD (with or without dementia), which always presents with adrenal or periadrenal Lewy body pathology.

Although the number of cases was small, 2 cases presented with Lewy bodies only in the adrenal glands and lacked Lewy bodies in the central nervous system. These cases could possibly represent the earliest stage of Lewy body-related progressive autonomic failure. From the points of disease pathogenesis and hierarchy of the Lewy body disorders, this result indicates that the adrenal gland could be the initial and primary target for these progressive disorders.

In the present study, we retrospectively investigated the correlation between clinical and pathologic presentations of adrenal glands. Although there were definite limitations in our study based on the review of medical records, the observed Lewy body-related pathology involving adrenal tissues did not correspond to any symptomatology of adrenal insufficiency, except for orthostatic hypotension. Also, our studies did not indicate how to detect this adrenal or periadrenal pathology clinically. Reduced uptake of MIBG in cardiac scintigraphy in Lewy body disease corresponds to decreased TH-immunoreactivity as well as to α-synucleinopathy in unmyelinated fibers from the epicardial fatty tissue of the anterior wall of the left ventricle of the heart (8–10). However, because the TH-immunoreactive unmyelinated fibers in the periadrenal fatty tissue were relatively preserved in this study, MIBG scintigraphy, which is also used for the detection of pheochromocytoma of the adrenal glands, may not be useful for detection of this Lewy body-related pathology in adrenal tissues. Therefore, we are now planning a prospective functional study of cases with Lewy body disease consisting of the tilt test and simultaneous blood sampling to gauge the serum noradrenergic level, as well as the resting adrenalin level, to detect this adrenal pathology clinically.

In conclusion, the immunohistochemical examination of adrenal glands with anti-phosphorylated α-synuclein antibodies can help differentiate the primary and the secondary forms of LBAS, as well as identify where LBAS starts in the human body and how it spreads.

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