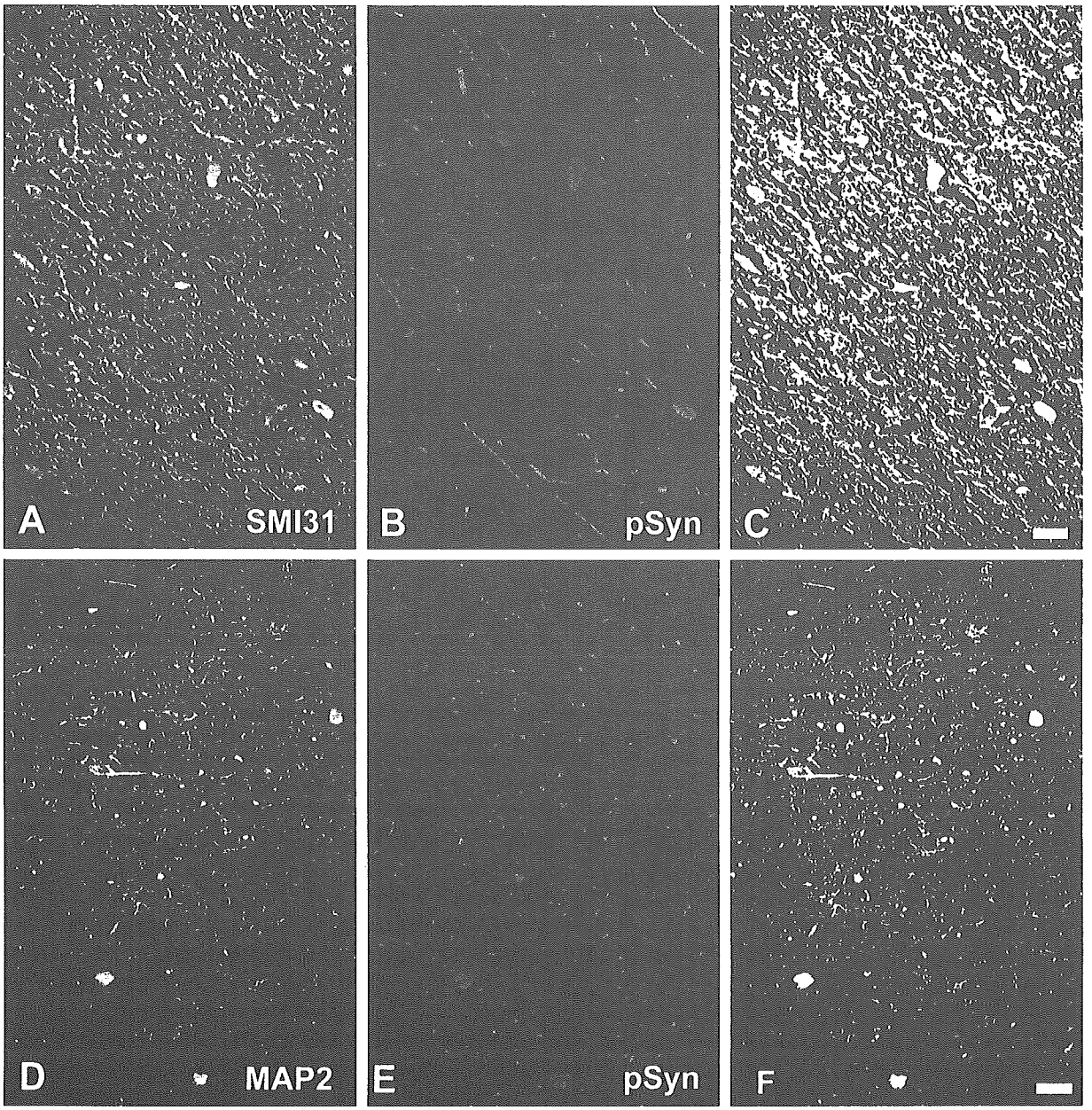

Fig. 4. Axonal and somatodendritic localization of phosphorylated α-synuclein. Sections of amygdala were double immunostained with polyclonal anti-phosphorylated -synuclein antibody (anti-PSer129) and with anti-phosphorylated neurofilament (SMI 31) (**A–C**) or anti-MAP2 (HM-2) (**D–F**). The primary antibodies were visualized with anti-rabbit Alexa 568 Fluor™ (red) and anti-mouse IgG Alexa 488™ (green) (Case 2). **A–C:** Confocal image of the white matter of the amygdala (the same area as Fig. 2F). The epitope of anti-Pser129 (red) is almost exclusively colocalized with that of SMI 31 (green). Anti-Pser129-immunoreactive axonal swellings are scattered. **D–F:** In the amygdala proper, some anti-Pser129-immunoreactive dots and threads (red) are colocalized with the epitope of anti-MAP2 antibody (green). **A, D:** Alexa 488 (green) for phosphorylated neurofilament (**A**) or MAP2 (**D**). **B, E:** Alexa 568 (red) for polyclonal phosphorylated α-syncuclein, anti-Pser129. **C, F:** Merged views for (**A/B**) and (**D/E**). Scale bar = 20 μm.

including the occipital cortex (Fig. 3G). In the sections double-immunostained for both psyn#64 and anti-Aβ 11–28, almost all senile plaques contained anti-psyn-immunoreactive dystrophic neurites in the areas where the immunoreactivity with anti-psyn was abundant. Although very small in number, some amyloid cores appeared to contained the epitope of psyn.

Electron microscopic observation of the molecular layer of the anterior subiculum confirmed the presence of intraneuritic Lewy bodies. Ultrastructurally, the Lewy

TABLE 1
Early Lewy Body-Related Pathology Detected with Anti-Phosphorylated α-Synuclein Immunohistochemistry

Age (yr)	Sex	Neurological diagnosis	Pathological diagnosis	Distribution and extent					Original staging of Lewy body
				DMNX	RF	LC	SN	Amy	
Stage 0.5									
93	F	Dementia	DG	+−	−	+−	−	−	0
69	M	Unremarkable	Unremarkable	−	+−	−	+−	−	0
98	F	Dementia	DG	+−	+−	−	+−	+−	0
76	M	Unremarkable	Unremarkable	+−	+−	+−	+−	+−	0
86	M	AD	AD	−	−	−	−	+−	0
Stage I									
73	F	CVD	CVD	+	−	−	−	−	0
86	M	Unremarkable	Unremarkable	+	+	+−	−	+−	0
88	F	Hepatic coma	Unremarkable	+	+	−	+	+−	0
78	F	Dementia	DG/ NFTD	+	+	−	+−	+−	0
94	F	CVD s/o	CVD	+−	+	++	−	++	0
72	M	Unremarkable	CNG	−	+−	−	−	+	0
70	M	Unremarkable	Unremarkable	+−	+	++	+	+−	I
69	M	CVD, dementia	VD	++	+	++	+	+−	I
84	F	Unremarkable	Unremarkable	++	++	++	++	++	I
82	M	Unremarkable	CVD	++	++	++	++	+	I
78	M	Unremarkable	Unremarkable	+	+	++	+	+	I
80	M	Unremarkable	Unremarkable	++	+	+	+	++	I
78	M	Dementia	DG/ CVD	+	++	−	+	++	I
98	M	AD	AD	+−	−	−	+−	+	I
78	M	AD	AD	+−	+−	−	−	+	I

Abbreviations: DMNX, dorsal motor nucleus of vagus nerve; RF, reticular formation in the medulla oblongata; LC, locus ceruleus; SN, substantia nigra; Amy, amygdala; DG, dementia with grains; AD, Alzheimer disease; CVD, cerebrovascular disease; NFTD, neurofibrillary tangle-predominant form of dementia; CNG: cognitively normal case with grains (14); and VD, vascular dementia. Extent of antiphosphorylated α-synuclein immunoreactive structures are as follows: −, none; +−, dots and threads only; +, intraneuronal (perikaryal) presence of the epitope; ++, dense aggregates of the epitope in the neuronal cytoplasm.

bodies presented as large spherical amorphous cores of high electron density surrounded by meshworks of granulofilamentous profiles (not illustrated).

DISCUSSION

The present study revealed 4 new findings: 1) phosphorylation of α-synuclein (psyn) occurred in about one fourth of the aged population; 2) anti-psyn immunocytochemistry allowed novel visualization of pre-LBs as well as Lewy threads, dots, and axons; 3) α-synucleinopathy begins in the medulla oblongata if it is an independent disease process, or in the amygdala if associated with AD; and 4) severe neocortical involvement by psyn is present in the medial temporal lobe in Parkinson disease, extends to the frontal lobe in DLB transitional form, and additionally involves the parietal and occipital lobes in DLB neocortical form.

We believe that our autopsy series is reasonably representative of the general older population in Japan. The 157 brains we examined are from a serial autopsy series at TMGH. This is one of the oldest and largest public geriatric hospitals in Japan, with 900 outpatients daily and a 700-bed ward. Every effort is made to secure postmortem neuropathological examinations on all patients dying at TMGH, whether or not a neurological disease was diagnosed antemortem. The autopsy rate was 32% during the period of this study.

Anti-psyn immunohistochemistry resulted in better visualization of previously reported LB-related pathology, except in a few anatomical loci such as the inferior olivary nucleus and occipital cortex. Using this more specific and sensitive marker, we found that 25 percent of the cases in our serial autopsy series had LB-related pathology. The limbic pathology specifically linked to DLB was detected in apparently presymptomatic cases, suggesting that phosphorylation of α-synuclein at Ser129 represents a pathological change that precedes LB-related neuronal degeneration. The pre-LBs, Lewy threads, and Lewy dots detected with anti-psyn immunohistochemistry are morphologically analogous to the pretangles, neuropil threads, and argyrophilic grains (18) detected with anti-phosphorylated tau immunohistochemistry. The Lewy threads were thinner and shorter than the Lewy neurites detected with anti-ubiquitin or anti-α-synuclein

TABLE 2A
Clinical Profiles of the Cases Showing Lewy Body-Related Neuronal Degeneration

Case	Age (yr)	Sex	Neurological diagnosis	CDR	PA	Dementia	Pathological diagnosis
1	85	M	PD+AD	3	10 yr	2 yr	DLBN
2	83	F	DLB	1	(–)	>2 yr	DLBN
3	92	F	Senile dementia	3	(–)	>2 yr	DLBN
4	77	M	DLB	3	2 yr	6 yr	DLBN
5	88	M	PD+dementia	1	11 yr	4 yr	DLBT
6	85	F	PD+dementia	1	19 yr	5 yr	DLBT
7	85	M	Drug-induced PA	1	>1 yr	n.d.	DLBT
8	86	F	PD+dementia	2	20 yr	1.75 yr	DLBT
9	79	F	PD+dementia	2	8 yr	>1 yr	DLBT/Fahr
10	79	M	PD	0	>20 yr	(–)	PD
11	77	M	PD	0	>1 yr	(–)	PD
12	84	M	MCI	0.5	(–)	2 yr	Early DLBT?
13	80	F	Unremarkable	0	(–)	(–)	CVD
14	90	F	Senile dementia	1	(–)	0.5 yr	AD/ CS
15	86	F	Unremarkable	0	(–)	(–)	Unremarkable
16	90	F	Senile dementia	1	(–)	>2 yr	DG
17	79	F	FTD	2	(–)	14 yr	AD
18	80	F	Dysphagia	0	(–)	(–)	Lewy body dysphagia
19	86	M	CBD	3	3 yr	n.d.	PSP
20	48	M	Unremarkable	0	(–)	(–)	Unremarkable

Abbreviations: CDR, clinical dementia rating (26) before suffering from terminal illness; PA, duration of Parkinsonism; Dementia, duration of dementia; yr, year; n.d., duration not determined due to unclear onset; PD, Parkinson disease; AD, Alzheimer disease; DLB, dementia with Lewy bodies; DLBN, DLB neocortical form (diffuse Lewy body disease); DLBT, DLB transitional form (limbic form); FTD, fronto-temporal dementia; MCI, mild cognitive impairment; CBD, corticobasal degeneration; CVD, cerebrovascular disease; N/A, not available; NFTD, neurofibrillary tangle-predominant form of dementia; CS, cervical spondylotic myelopathy; CVD, cerebrovascular disease; DG, dementia with grains; PSP, progressive supranuclear palsy; VD, vascular dementia of Binswanger type.

immunohistochemistry. Lewy dots outnumbered Lewy threads, making it unlikely that Lewy dots were simply cross sections of Lewy threads. The functional significance of these psyn-positive structures is open to speculation. Lewy dots and threads accompanied the cortical spongiosis associated with DLB, raising the possibility that they may disrupt synaptic transmission. The anti-psyn-immunoreactive axons in the affected limbic system suggest a possible disruption of axonal transport in LB-related cognitive decline. The presence of psyn-positive structures in the molecular layer of the occipital cortex in DLB neocortical form suggests a cause for the decreased uptake noted in occipital cortex with single photon emission computed tomography (SPECT) and fluordeoxy-glucose positron emission tomography (PET) studies of DLB (19, 20).

Our study confirmed that α-synucleinopathy may start in the medulla oblongata, as previously reported by Del Tredici (9). However, in 5 of 15 AD cases, the process appeared to start in the amygdala at Stage 0.5 and then progressed to mildly involve the brainstem in Stage II. These findings suggest that there are 2 types of LB-related α-synucleinopathy: a primary type that starts in the medulla oblongata, and a secondary type that starts in the amygdala. The primary type appears to progress into Parkinson disease and then DLB transitional form. The secondary type is associated with AD and possibly other tauopathies (21, 22), as was suggested by the association of one of our cases with PSP. The relation between DLB neocortical form and these primary and secondary types of α-synucleinopathy remains to be clarified.

Neostriatal pathology was reported with allosteric form-specific anti-α-synuclein antibody (23) and was confirmed by our anti-psyn immunocytochemistry. We observed numerous Lewy dots in a ventrolateral to dorsomedial gradient, consistent with the progression of Parkinson disease reported in a dopamine PET and biochemical study (24). The epitope of psyn was colocalized within plaques in dystrophic neurites or cores exclusively in some cases above Stage II. The possible association of Aβ and psyn deserves further study.

Our study showed that the neuropil pathology visualized with anti-psyn antibody was more widespread than reported previously. The observation that the presence of LBs was always accompanied by abundant neuropil pathology in the background supports the validity of the diagnostic criteria of DLB based on the LB score adopted

TABLE 2B
Neuropathological Summary of the Cases with Phosphorylated α-Synuclein-Related Neuronal Degeneration

Case	Stage	Score	tENT	Ci	F	T	P	O	NFT	SP
1	V	10 (10)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	1	III	C
2	V	10 (9)	2 (2)	2 (2)	2 (2)	2 (2)	2 (1)	1	III	C
3	V	10 (8)	2 (2)	2 (2)	2 (1)	2 (2)	2 (1)	1	II	B
4	V	10 (9)	2 (2)	2 (2)	2 (2)	2 (2)	2 (1)	1	I	B
5	IV	10 (7)	2 (2)	2 (2)	2 (1)	2 (2)	2 (0)	1	I	B
6	IV	10 (7)	2 (2)	2 (2)	2 (1)	2 (2)	2 (0)	2	IV	B
7	IV	10 (7)	2 (2)	2 (2)	2 (1)	2 (2)	2 (0)	1	I	B
8	IV	10 (6)	2 (2)	2 (2)	2 (0)	2 (2)	2 (0)	1	I	A
9	IV	10 (6)	2 (2)	2 (2)	2 (0)	2 (2)	2 (0)	1	I	A
10	III	10 (6)	2 (2)	2 (2)	2 (0)	2 (2)	2 (0)	0.5	III	0
11	III	10 (6)	2 (2)	2 (2)	2 (0)	2 (2)	2 (0)	0.5	I	0
12	IIN	8 (6)	2 (2)	2 (2)	1 (0)	2 (2)	1 (0)	0.5	I	B
13	IIN	10 (8)	2 (2)	2 (2)	2 (0)	2 (2)	2 (1)	1	I	C
14	IIN	9 (5)	2 (2)	2 (1)	1 (1)	2 (0)	2 (1)	0.5	V	C
15	IIN	8 (5)	2 (2)	2 (2)	1 (0)	2 (1)	1 (0)	0	I	B
16	IIN	9 (6)	2 (2)	2 (1)	1 (0)	2 (2)	2 (1)	0.5	III	B
17	IIL	6 (2)	2 (0)	2 (1)	0 (0)	2 (1)	0 (0)	0	V	C
18	IIL	5 (3)	2 (1)	1 (1)	0 (0)	2 (1)	0 (0)	0	III	B
19	IIL	5 (0)	2 (0)	1 (0)	0 (0)	2 (0)	0 (0)	0	III	0
20	IIL	4 (0)	2 (0)	1 (0)	0 (0)	1 (0)	0 (0)	0	I	0

Scoring for Lewy bodies (score, tENT, Ci, F, T, and P) was assessed in sections immunostained for phosphorylated α-synuclein (psyn), following the consensus guidelines for dementia with LBs (6). The score in parentheses was determined by H&E staining and ubiquitin immunostaining. The score for Lewy bodies in the occipital cortex was determined by immunostaining for phosphorylated α-synuclein. Scores 0, 1, and 2 followed the consensus guidelines for dementia with LBs (6); the score 0.5 indicated Lewy threads and dots without intraneuronal perikaryal inclusions.

Abbreviations: NFT, neurofibrillary tangles, Braak staging (27); SP, senile plaque, Braak staging (27); Stage, Lewy body stage; Score, Lewy body score following consensus guidelines for dementia with LBs (6); tENT, transentorhinal area; Ci, cingulate gyrus; F, frontal lobe; T, temporal lobe; P, parietal lobe; O, occipital lobe.

by the consensus guidelines (6). The presence of widespread neuropil pathology is also consistent with previous reports that a decline of choline acetyl transferase (ChAT) is linearly correlated with the number of cortical LBs in the temporal neocortex (25).

Anti-psyn immunocytochemistry was too sensitive for assessment of Lewy scores using traditional criteria, making it necessary for us to adopt revised criteria. In our study, neocortical involvement was definitely present in the medial temporal lobe in Parkinson disease, spread to the frontal lobe in DLB transitional form, and additionally involved the parietal and occipital lobes in DLB neocortical form. This progressive involvement of the neocortex suggests that a revision of the diagnostic guidelines for LB-related cognitive decline may be warranted.

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Lewy Body-Related α-Synucleinopathy in Aging

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Abstract. To clarify the significance of Lewy body (LB)-related α-synucleinopathy in aging, we investigated the incidence of LBs in 1,241 consecutive autopsy cases (663 males and 578 females). LB pathology was identified histologically in sections stained with hematoxylin and eosin and with anti-ubiquitin and anti-α-synuclein antibodies. Cases without LBs were classified as LB stage 0 (987 cases). Cases with LBs were classified as follows: LB stage I = incidental LBs (149 cases); LB stage II = LB-related degeneration without attributable clinical symptoms (47 cases); LB stage III = Parkinson disease without dementia (10 cases); LB stage IV = dementia with Lewy bodies (DLB) transitional (limbic) form (25 cases); and LB stage V = DLB neocortical form (23 cases). The average age at death was greater for those cases with LBs. There were no gender differences in the LB pathology. G842A polymorphism in the paraoxonase 1 gene was associated with men in LB stage II or above and suggests a gender-specific risk factor. LB stage V had higher stages of neurofibrillary tangle and senile plaque involvement and also had a higher frequency of apolipoprotein E ε4. Our findings indicate that LBs are associated with cognitive decline, either independently or synergistically with neurofibrillary tangles and senile plaques.

Key Words: Alzheimer disease; Apolipoprotein E; Dementia with Lewy body; Neurofibrillary tangle; Paraoxonase 1; Parkinson disease; Senile plaque.

INTRODUCTION

Lewy body (LB)-related α-synucleinopathy is one of the most important post-translationally modified protein accumulations in the aging human brain. However, unlike senile plaques (SPs) or neurofibrillary tangles (NFTs), only limited studies are available on the incidence and biological significance of LBs in age-related motor and cognitive decline (1).

Tokyo Metropolitan Geriatric Hospital (TMGH) serves as a community-based care facility for the elderly in the Tokyo metropolitan area and performs postmortem examinations on a relatively high percentage of hospital cases, irrespective of their clinical symptoms and cause of death. The brains from these cases are ideal for evaluating the incidence of pathological processes in the aging population. As a routine procedure at TMGH, the brain is bisected at the time of autopsy, one hemisphere is deep-frozen and other hemisphere is sampled for light and electron microscopic examination. In this study, we investigated the incidence of LB changes, their contribution to parkinsonism and dementia, and their association with apolipoprotein E (ApoE) and paraoxonase 1 (PON1) genotypes in the most recent 1,241 autopsy cases at TMGH. Our findings indicate that LBs may independently or synergistically contribute to cognitive decline.

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MATERIALS AND METHODS

Tissue Source

One thousand two hundred forty-one consecutive autopsy brains at TMGH over the past 5 years were the basis of the present work. The patients' ages ranged from 48 to 104 years, with a mean age of 80.6 ± 8.9 years, and a male to female ratio of 663:578.

Clinical Information

Clinical information, including parkinsonism and cognitive state, was obtained from medical charts and interviews with the patients' personal physicians and caregivers. The Mini-Mental State Examination (MMSE) (2) or the Hasegawa dementia scale (3) was employed for evaluation of cognitive function, and a clinical dementia rating (CDR) (4) was used for grading of dementia. Almost all cases of suspected degenerative dementia received a clinical diagnosis of "senile dementia" based on the recognition that the final diagnosis should be made after postmortem examination of the brain.

Neuropathology

Formalin-fixed (20% neutral buffered formalin), paraffin-embedded sections of representative areas of the brain were examined, following the recommendations of the Consortium to Establish a Registry for Alzheimer Disease (CERAD) (5) and the consensus guidelines for the diagnosis of dementia with Lewy bodies (DLB) (6). Areas examined included frontal pole, cingulate gyrus, amygdala, temporal neocortex, anterior and posterior hippocampus with entorhinal and transentorhinal cortex, motor cortex, parietal lobe including the intraparietal sulcus, visual cortex, basal ganglia and hypothalamus at the level of the mamillary body, subthalamic nucleus, thalamus at the

TABLE 1
Correlation Between Lewy Body Stage, Lewy Body Score and Clinical Symptoms

	LB score	Parkinsonism	Dementia	Total cases
Stage I	0–1	NA*	NA*	149
Stage II	0–10	NA*	NA*	47
Stage III	NA**	10 cases	0 cases	10
Stage IV	3–6†	10 cases	25 cases	25
Stage V	≥7††	10 cases	23 cases	23

LB: Lewy body; LB score: Lewy body score by consensus guidelines (6); NA: not applicable.

* By definition, stage I and stage II cases have neither parkinsonism nor dementia attributable to LB-related neuronal degeneration.

** LB scoring was originally developed for dementia with Lewy bodies (DLB) and not for Parkinson disease without dementia. However, if our LB stage III cases (PD without dementia) were scored, their LB score would be 3 to 6.

† Lewy body score of 3 to 6 or greater than 6 with at least 1 neocortical score of zero.

†† Lewy body score of 7 or greater and no neocortical score of zero.

TABLE 2
Relationship of Lewy Body Stages to Parkinson Disease Without Dementia (PD), Parkinson Disease With Dementia (PDD), and Dementia With Lewy Bodies (DLB), Following the Nomenclature of the 1996 Consensus Guidelines for Dementia With Lewy Bodies (6)

	PD	PDD	DLB
LB Stage III	10 cases		
LB Stage IV		8 cases	17 cases
LB Stage V		5 cases	18 cases
Average age (years)	77.2*‡	82.3*	85.1‡

The average age at death in PD without dementia is significantly younger than that in PDD or DLB.

* p = 0.031.

‡ p = 0.0014.

level of the red nucleus, midbrain, upper and middle pons, medulla oblongata, cerebellar vermis, dentate nucleus, and the cervical, thoracic, and lumbar spinal cord.

Six-μm-thick sections were routinely stained with hematoxylin and eosin (H&E) and the Klüver-Barrera method. Selected sections were stained with the modified methenamine silver (7) and Gallyas-Braak silver methods (8) for senile changes, with Congo red for amyloid deposition, and with elastica Masson trichrome for vascular changes.

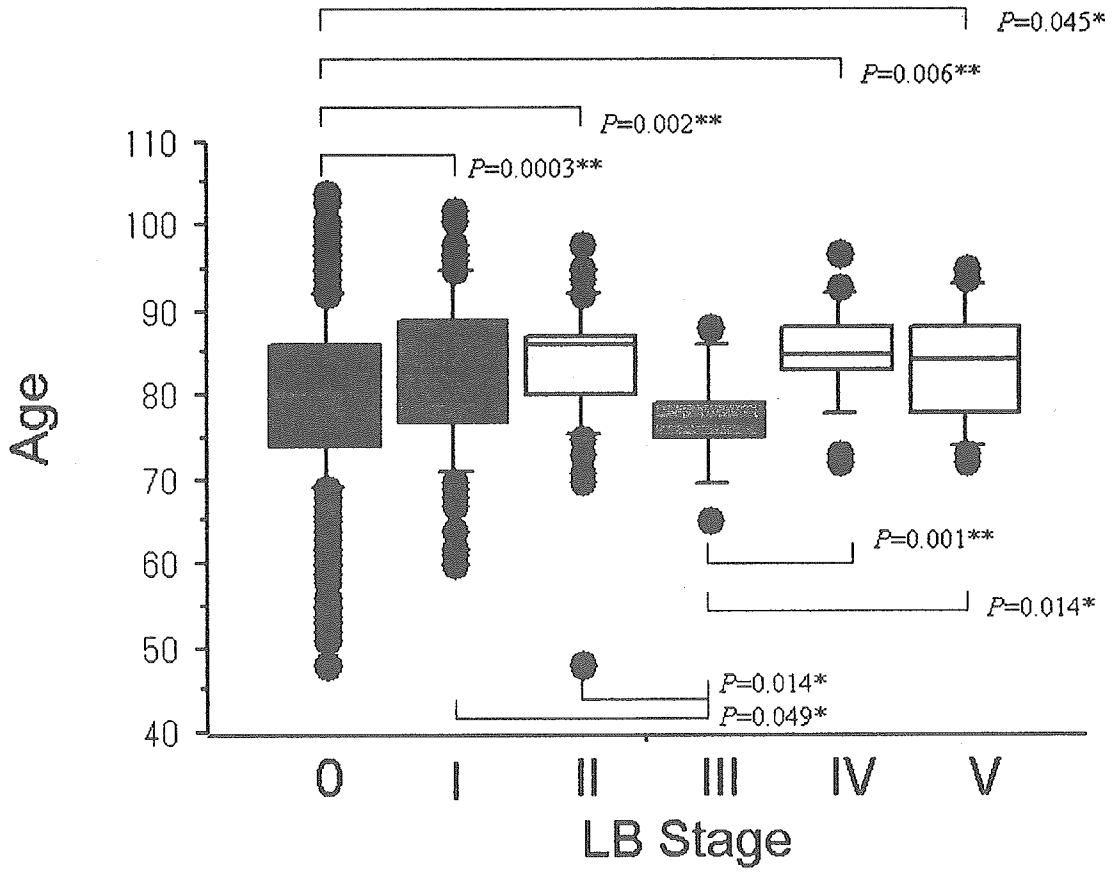

Fig. 1. Age distribution in each stage. The average age at death in Lewy body (LB) stages I, II, IV, and V was significantly greater than in LB stage 0 or LB stage III.

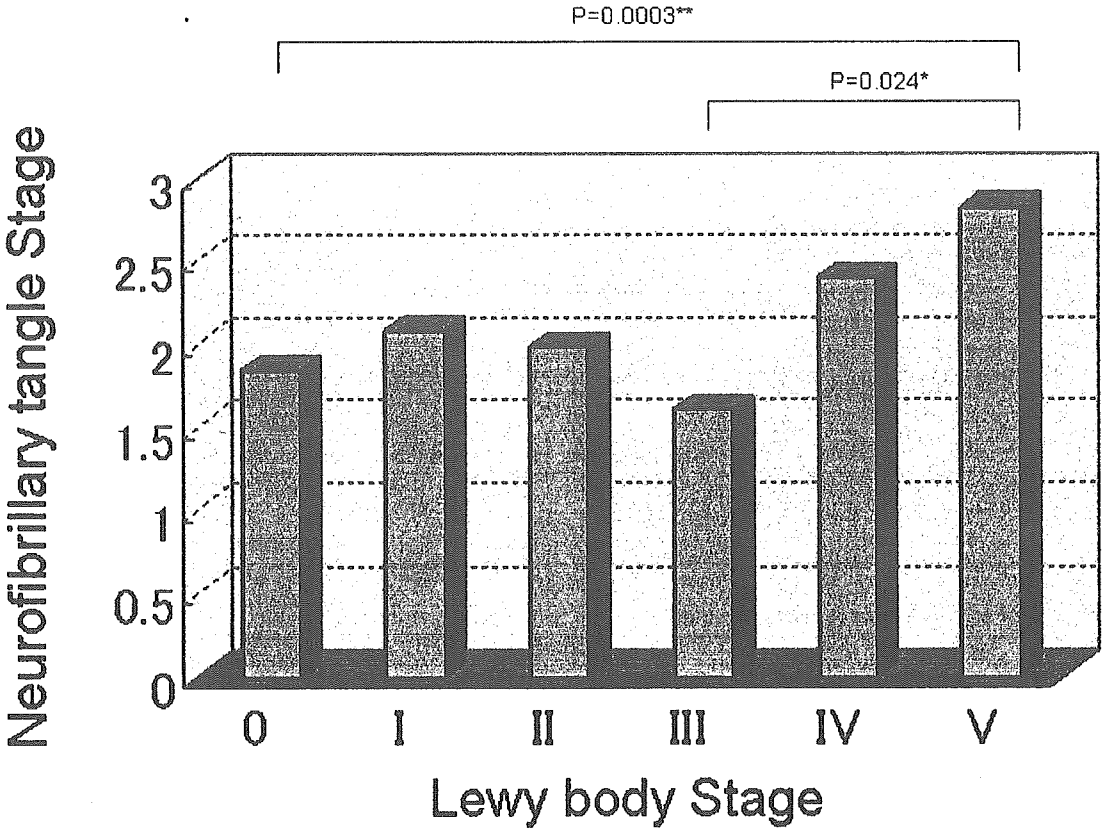

Fig. 2. Lewy body stage versus neurofibrillary tangle (NFT) stage. NFT stage is significantly higher in Lewy body (LB) stage V than in LB stage 0 or LB stage III.

Immunohistochemistry

Six-μm-thick serial paraffin sections were immunohistochemically stained using a Ventana 20NX autostainer (Ventana, Tucson, AZ), as previously described (9). The antibodies employed were as follows: anti-α-synuclein (LB509, monoclonal, kind gift from Dr. T. Iwatsubo); phosphorylated α-synuclein (psyn) [psyn#64 (10) and Pser129 (11)]; phosphorylated tau (ptau) (AT8, monoclonal, Innogenetics, Temse, Belgium); amyloid β (Aβ)11–28 (12B2, monoclonal, IBL, Maebashi, Japan); Aβ1–42 (polyclonal, IBL); ubiquitin (polyclonal, Sigma-Aldrich, St. Louis, MO); glial fibrillary acidic protein (GFAP)(polyclonal, DAKO, Glostrup, Denmark); and HLA-DR (monoclonal, CD68, DAKO). Sections of midbrain and amygdala from all cases were stained with anti-ubiquitin and anti-α-synuclein antibodies. Additionally, in the most recent 600 cases, sections of medulla oblongata at the level of dorsal motor nucleus of vagus, upper pons at the level of locus ceruleus, midbrain, basal ganglia, entorhinal cortex, amygdala, and the anterior cingulate, second frontal, temporal, and supramarginal gyri were stained with anti-α-synuclein and anti-psyn antibodies.

Evaluation of Lewy Body-Related Neuropathology

Histologic sections of brain were initially evaluated for LB pathology with H&E staining and with anti-ubiquitin immunohistochemistry. The presence of LB pathology was confirmed by immunohistochemistry with anti-α-synuclein and anti-psyn antibodies, and the "LB score" for each case was calculated following consensus guidelines (6).

Evaluation of Other Disorders Presenting with Dementia and/or Parkinsonism

Our modification (12) of the NIA-Regan criteria (13) was used for the diagnosis of Alzheimer disease (AD). The diagnoses of "dementia with grains" (DG) and "neurofibrillary tangle-predominant form of dementia" (NFTD) were based on Jellinger's criteria (14, 15). The diagnosis of vascular dementia was based on NINDS-AIREN criteria (16).

Semiquantitative Analysis

LB pathology was classified into 6 LB stages according to our previously published criteria (10). These 6 stages are as follows: LB stage 0 = no LBs; LB stage I = scattered LBs without cell loss; LB stage II = abundant LBs with macroscopic loss of pigmentation in substantia nigra and locus ceruleus and/or gliosis demonstrated by GFAP immunohistochemistry in areas containing LBs but without attributable parkinsonism or dementia; LB stage III = PD without dementia; LB stage IV = DLB, transitional (limbic) form (DLBT); and LB stage V = DLB, neocortical form (diffuse Lewy body disease) (DLBN). Because of controversy surrounding the definition of PD with dementia (PDD), we included PDD as a subgroup in LB stages IV and V.

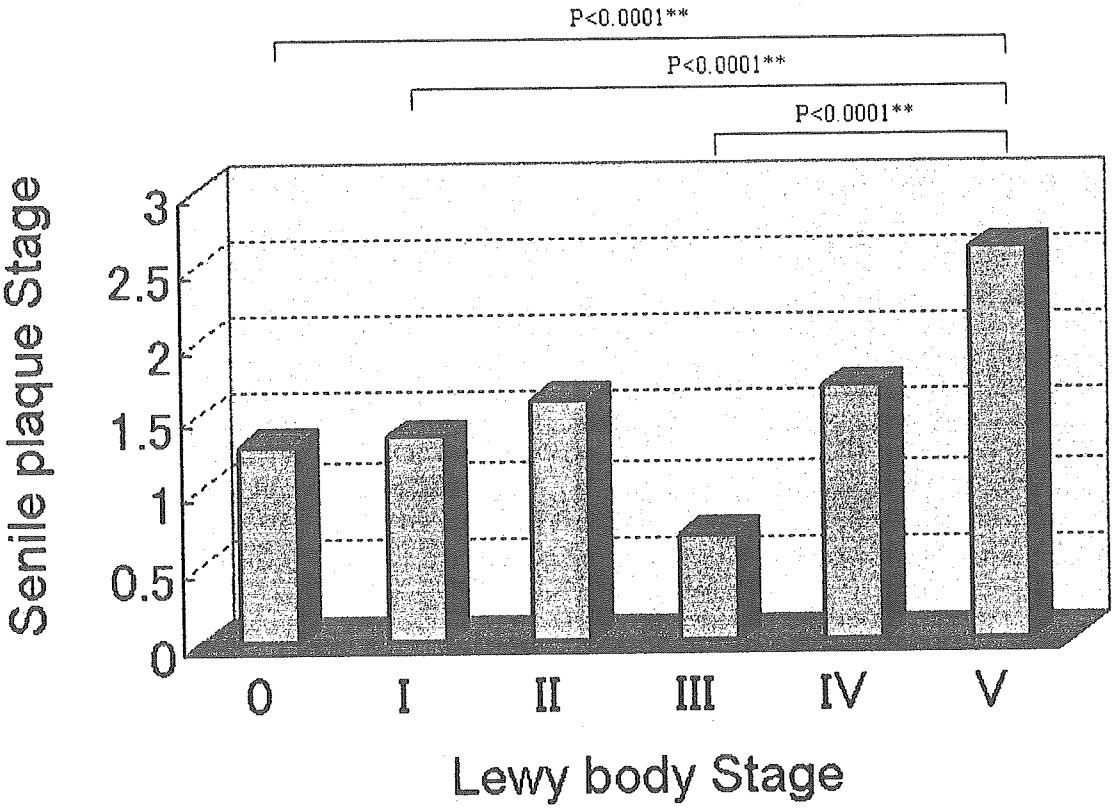

Fig. 3. Lewy body stage versus senile plaque (SP) stage. SP stage is significantly higher in Lewy body (LB) stage V than in LB stage 0, LB stage I or LB stage III.

The presence of NFTs and SPs was evaluated with H&E, Klüver-Barrera, Gallyas-Braak, and modified methenamine silver stains and confirmed immunohistochemically with anti-ptau and Aβ antibodies. NFT pathology was classified into 7 NFT stages, and SP pathology was classified into 4 SP stages, based on the Braak criteria (17).

Molecular Pathology

Genomic DNA was extracted from frozen kidney obtained at autopsy. The genotyping of ApoE was done as previously reported (9) in 1,114 cases from January 1997 to September 2003. The genotyping of the PON1 gene was determined on Q191R, L54M, G(−907)C, G(−824)A, T(−107)C, G(−161)A, and G(−125)C polymorphisms (18–21) in 511 cases from January 1997 to August 2000. The interval of the study of each genotyping was determined separately by the legal committee of Tokyo Metropolitan Institute of Gerontology and TMGH.

Statistic Analysis

Statistical analysis was performed using chi-square test or Fisher exact test for comparisons of categorical data, Student *t*-test for comparison of means for continuous outcomes, Mann-Whitney *U*-test for nonparametric analysis, and Spearman correlation coefficient by rank for correlation of discrete scores. Statistical significance was established at the $p < 0.05$ level.

RESULTS

Clinical Profiles

Parkinsonism was reported in 66 (5.3 %) of 1,241 cases. Clinical dementia ratings were available in 1,105 cases as follows: CDR0 = 436 cases, CDR 0.5 = 190 cases, CDR 1 = 193 cases, CDR 2 = 124 cases, and CDR3 = 162 cases.

Neuropathology

The morphological changes in cases with dementia were as follows: 218 cases had a neurodegenerative etiology, 104 cases had a vascular etiology, and 11 cases had combined neurodegenerative and vascular etiologies. The neurodegenerative dementias included 97 cases of AD, 53 cases of DG, 33 cases with DLB (of which 20 cases were DLBT and 13 cases were DLBN), 13 cases of NFTD, and 8 cases of progressive supranuclear palsy. Dementia cases with both LB pathology and other neurodegenerative pathology included 9 cases of DLBN plus AD, 4 cases of DLBT plus AD, 1 case of DLBT plus DG, and 1 case of DLBN plus progressive supranuclear palsy.

Lewy Body Pathology

LBs were found in 254 (20.5%) of the 1,241 cases. Of these 254 cases, 58 (22.8%) had clinical parkinsonism or

TABLE 3
Dementia With Lewy Bodies (DLB) and Alzheimer-type Senile Changes

DLB, Transitional Form

	SP stage			
	0	A	B	C
NFT stage				
0	**0**	**0**	0	0
I	**3**	**3**	3	1
II	**1**	**4**	0	0
III	0	0	3	2
IV	0	0	1	*0*
V	0	0	0	*3*
VI	0	0	0	1

DLB, Neocortical Form

	SP stage			
	0	A	B	C
NFT stage				
0	**0**	**0**	0	0
I	**0**	**2**	2	1
II	**0**	**0**	3	3
III	0	0	0	3
IV	0	0	0	*6*
V	0	0	0	*3*
VI	0	0	0	*0*

Boldfaced numerals indicate the pure form of DLB or DLB without significant Alzheimer changes. Italicized numerals indicate DLB plus Alzheimer disease.

TABLE 4
Apolipoprotein E Genotyping and Lewy Body Stage

	Lewy Body Stage					
	0	I	II	III	IV	V
Genotyping						
23	72	12	1	0	2	1
33	673	103	28	7	18	8
34	133	15	13	1	2	10*
44	13	2	0	0	0	1
Allelic Frequency						
2	72	12	1	0	2	1
3	1,551	233	70	15	40	27
4	159	19	13	1	2	12**

* $p < 0.0001$, compared with LB stage 0.
** $p < 0.001$, compared with LB stage 0.

cognitive decline. The LB staging of these 1,241 cases was as follows: LB stage 0 = 987 cases (male:female = 528:459); LB stage I = 149 cases (male:female = 86:63); LB stage II = 47 cases (male:female = 22:25); LB stage III = 10 cases (male:female = 4:6); LB stage IV = 25 cases (male:female = 10:15); and LB stage V = 23 cases (male:female = 13:10) (Table 1). No significant gender difference was observed in the LB stage, in the frequency of LBs, or in the frequency of LB-related clinical symptoms.

Because our LB staging did not distinguish Parkinson-associated "primary" α-synucleinopathy from AD- or tauopathy-associated "secondary" α-synucleinopathy (10), we categorized the LB stages I and II cases into primary and secondary types. LB stage I contained 144 cases of primary α-synucleinopathy and 5 cases of secondary α-synucleinopathy. LB stage II contained 44 cases of primary α-synucleinopathy and 3 cases of secondary α-synucleinopathy. The cases of primary α-synucleinopathy showed progressive involvement of the brainstem, limbic system, and neocortex, as previously reported (10).

The cases of primary α-synucleinopathy from our LB stages I through V were also staged using the criteria for staging of PD proposed by Braak et al (1). With one exception, all of our LB stage I cases belonged to Braak PD stage 1. The one exception had LBs only in the locus ceruleus. Our LB stage II cases were scored over Braak PD stages 3 to 6. All of our LB stage III cases had involvement of the temporal neocortex to a minor degree and would be classified as Braak PD stage 5. Our LB stage IV cases had involvement of frontal and temporal neocortex and would be classified as Braak PD stage 5. Our LB stage V cases had involvement of parietal and occipital cortex, as well as mild but constant involvement of primary motor and sensory cortex, and would be classified as Braak PD stage 6.

Aging and Lewy Bodies (LBs)

Average age at death in cases with LBs was 83.0 ± 8.3 years and was significantly greater (Student t-test, $p < 0.0001$) than the average age at death in cases without LBs (79.9 ± 8.8 years). The average age at death in each LB stage was as follows (Fig. 1): stage 0 = 80.0 ± 8.9 (years); stage I = 82.8 ± 8.8; stage II = 84.1 ± 8.1; stage III = 77.2 ± 6.1; stage IV = 84.9 ± 5.6; and stage V = 83.7 ± 6.8. The average age at death in LB stages I, II, IV, and V was significantly greater than in LB stage 0 (Student *t*-test, p = 0.0003, 0.002, 0.006, and 0.045, respectively). The average age at death in LB stage III was significantly less than in LB stages I, II, IV, and V (Student *t*-test, p = 0.049, 0.014, 0.001, and 0.014, respectively). The results were the same if LB stages IV and V were subclassified into PDD and DLB, following consensus guidelines (6) (Table 2).

Lewy Body (LB) Stage and Neurofibrillary Tangle (NFT) Stage

The average NFT stage in each LB stage was as follows: LB stage 0 = 1.84; LB stage I = 2.08; LB stage II = 1.98; LB stage III = 1.60; LB stage IV = 2.40; and LB stage V = 2.83. The average NFT stage was significantly higher in LB stage V than in LB stage 0 (Mann-Whitney *U*-test, p = 0.0003) or LB stage III (p = 0.024) (Fig. 2).

TABLE 5
Genotype Distributions of the Paraoxonase 1 (PON1) Polymorphisms

			Men			Women		
Genotypes	n	Frequency	LB stage 0–I (n = 237)	LB stage II≤ (n = 24)	p*	LB stage 0–I (n = 230)	LB stage II≤ (n = 20)	p*
G(-907)C								
GG	122	0.24	56	8	0.2844	57	1	0.1056
GC	263	0.51	122	13		114	14	
CC	126	0.25	61	3		57	5	
G (-824)A								
GG	272	0.53	129	7	0.0246	123	13	0.372
GA	198	0.39	89	12		90	7	
AA	41	0.08	19	5		17	0	
G (-161)A								
GG	416	0.81	192	18	0.7733	188	18	0.5805
GA	70	0.14	31	4		33	2	
AA	25	0.05	12	5		8	0	
G (-125)C								
	41							
GG	9	0.81	195	18	0.8857	188	18	0.5512
GC	67	0.14	29	3		33	2	
CC	25	0.05	14	2		9	0	
T (-107)C								
	21							
TT	4	0.42	102	9	0.5753	92	11	0.1171
	17							
TC	3	0.34	80	7		78	8	
	12							
CC	4	0.24	54	11		58	1	
55pol								
	43							
TT (LL)	2	0.84	201	18	0.2383	195	18	0.8557
TA (LM)	76	0.15	37	6		30	3	
AA (MM)	3	0.01	0	0		3	0	
192pol								
	17							
GG	9	0.35	71	8	0.5074	94	6	0.492
	29							
AG	0	0.57	152	13		114	11	
AA	42	0.08	16	3		20	3	

* Fisher exact probability test, LB stages 0–I versus LB stages II–V.

Lewy Body (LB) Stage and Senile Plaque (SP) Stage

The average SP stage in each LB stage was as follows: LB stage 0 = 1.3; LB stage I = 1.36; LB stage II = 1.6; LB stage III = 0.7; LB stage IV = 1.68; and LB stage V = 2.61. The average SP stage was significantly higher in LB stage V than in LB stage 0 (Mann-Whitney *U*-test, $p < 0.0001$), LB stage I ($p < 0.0001$), and LB stage III ($p < 0.0001$) (Fig. 3).

Senile Changes in LB Stage IV and LB Stage V

Senile changes in LB stage IV (DLBT) and LB stage V (DLBN) were compared. The pure form of DLB (22) (defined as minimal senile changes, such as NFTs in the entorhinal stage and SPs in Braak stages 0 or A) was found in 11 of the 25 cases of DLBT and in 2 of the 23 cases of DLBN. Combined AD pathology was seen in 4 of the 25 cases of DLBT and in 9 of the 23 cases of DLBN. The pure form of DLB was preferentially seen in DLBT, and combined AD pathology was preferentially seen in DLBN (Table 3).

ApoE Genotyping and the Lewy Body (LB) Stages

ApoE genotyping was available in 1,114 of the 1,241 cases. ApoE genotyping and allelic frequency in each LB stage are summarized in Table 4. The incidence of genotype ApoE ε3/ε4 and the allelic frequency of ε4 were

significantly higher in LB stage V than in LB stage 0 (chi-square test, $p < 0.0001$ and $p < 0.001$).

PON1 Gene Polymorphism

The distribution of the PON1 genotypes is listed in Table 5. Statistical analysis was done for PON1 gene polymorphism in each gender and stage. Significance differences in G(−824)A polymorphism were found when male cases in LB stage II or above were compared with male cases less than LB stage II. The proportion of male cases with LB stage II or above was highest in the AA genotype (20.8%), less in the GA genotype (11.9%), and least in the GG genotype (5.1%). This difference in genotypic distribution was significant ($p = 0.024$). The allelic frequencies of A(−824) and G(−824) were also significantly different between male cases in LB stage II or above and male cases in less than LB stage II ($p = 0.007$).

DISCUSSION

Our study of 1,241 consecutive autopsy brains from a geriatrics hospital revealed the following findings: 1) LBs were present in approximately 20% of this elderly population; 2) the incidence of LBs increased with age but was not influenced by gender; 3) Alzheimer-type pathology and ApoE ε4 genotype were associated with the neocortical form of DLB; and 4) PON1 G(−824)A polymorphism was associated with LB pathology in men.

Our series of consecutive autopsy cases reasonably represents the aging general population, as previously reported (10). Cases with LBs were significantly older than cases without LBs, implying that LBs are an age-associated change like NFTs and SPs. Our staging of cases with LB pathology roughly paralleled Braak PD staging (1), but there were a few differences. One of our early cases (LB stage I) had LBs only in locus ceruleus, a finding also reported by others (23, 24). Our staging criteria separated PD with dementia (our LB stage IV) from PD without dementia (our LB stage III), whereas the Braak criteria lump them into one stage (Braak PD stage 5). We believe that the separation of these 2 clinicopathologic entities may be advantageous for the study of LB-related cognitive decline.

The average age at death in cases with LB stage III (PD without dementia) was not significantly different from the age at death in cases without LBs and was less that the average age at death in other stages with LB pathology. It is possible that PD patients without dementia died of causes other than PD before manifesting dementia.

The presence of a pure form of DLB (22) indicates that neither NFTs nor SPs are required for DLB. In our autopsy series, the pure form of DLB was more frequent in the transitional (limbic) form of DLB than in the neocortical form of DLB. There was a significant increase in both the NFT stage and the SP stage in the neocortical form of DLB, but not in the transitional form of DLB, which suggests a synergistic effect of these 3 types of abnormally accumulating, post-translationally modified proteins in the neocortex.

There is controversy over whether ApoE ε4 is a risk factor for DLB (25–27). Our data revealed that ApoE ε4 was associated with DLBN, but that this association may be due to concomitant AD-type senile changes (28).

PON 1 is an esterase associated with a high-density lipoprotein in serum. The esterase has antioxidant properties, but its natural substrate is unknown. There have been no consistent findings of an association between PD and 2 polymorphisms in the coding region of PON1. However, we found that the G(−824)A polymorphism showed a correlation with LB stage II and above in men, raising the possibility that LB-related neuronal degeneration is influenced by PON1 in men.

In conclusion, our study provides evidence that LBs are a form of age-associated neuronal change and contribute to cognitive decline independently, as in the pure form of DLB, or synergistically with SPs and NFTs, as in DLB plus AD. Elucidation of the mechanisms by which these 3 types of abnormally deposited, post-translationally modified proteins cause brain dysfunction may help clarify the relationship among PD, AD, and DLB.

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Short communication

Pathology of the sympathetic nervous system corresponding to the decreased cardiac uptake in ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy in a patient with Parkinson disease

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Abstract

Decreased cardiac uptake in ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy has been adopted as one of the most reliable diagnostic tests for Parkinson disease (PD) in Japan. To investigate the morphological basis for this finding, we performed a detailed neuropathological study of the cardiac sympathetic nervous system of a 71-year-old autopsy-proven PD patient, who presented with a marked decrease in cardiac uptake of MIBG, just 1 year prior to death. We carefully examined the intermediolateral column at several levels of the thoracic spinal cord, the sympathetic trunk and ganglia, and the nerve plexus of the anterior wall of the left ventricle and compared the findings with those of five age-matched controls. We found that the cardiac plexus was more heavily involved than the sympathetic ganglia in this patient with PD. Our study may provide further evidence that the markedly decreased cardiac uptake of MIBG observed in PD cases represents preferential involvement of the cardiac sympathetic nerve plexus in this disorder.

Keywords: Lewy body; α-synuclein; Distal axonopathy

1. Introduction

^{123}I-metaiodobenzylguanidine (MIBG) is an analogue of noradrenaline and is metabolized by noradrenergic neurons. It is therefore used as a tracer in myocardial scintigraphy for the evaluation of cardiac sympathetic innervation. Markedly decreased cardiac uptake of MIBG shown by myocardial scintigraphy is a specific finding in Parkinson disease (PD) or dementia with Lewy bodies (DLB) and is useful for the differential diagnosis of other Parkinsonian syndromes [1–4] or Alzheimer's disease [5]. This decrement has been seen even in PD patients without autonomic symptoms [2–4].

A follow-up MIBG scintigraphy study recently revealed the occurrence of a progressive decrement of MIBG uptake in cases of Yahr Stage I PD (Dr. S. Orimo, abstract of the 45th Annual Meeting of the Japanese Association of Neurology, May 2004, Tokyo) while another report showed that PD patients with normal MIBG scintigraphy have a higher incidence of mutations of the *parkin* gene (Dr. M. Yamamoto, abstract of the 45th Annual Meeting of the Japanese Association of Neurology, May 2004, Tokyo). These observations suggest that the decreased uptake of MIBG is not necessarily a finding invariably observed in patients with levodopa-responsive-Parkinsonism.

Orimo et al. reported markedly decreased tyrosine hydroxylase (TH)-immunoreactive nerve fibers in the heart of a patient with pathologically proven PD, whose

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cardiac uptake of MIBG had been found to be severely decreased 1 year before death [6]. Amino et al. reported that not only TH-immunoreactive but also neurofilament (NF)-immunoreactive nerve fibers were markedly decreased in heart tissues from patients with pathologically proven PD [7]. Recently, Orimo et al. examined heart tissues together with sympathetic ganglia from patients with pathologically proven PD, and concluded that although sympathetic ganglia were relatively preserved, TH-immunoreactive nerve fibers were markedly decreased in heart tissues [8].

Orimo's report is the only report describing an autopsy of a PD patient who had undergone MIBG scintigraphy in situ, because the examination is usually done in the very early clinical stage of the disorder. The purpose of this study was to examine in detail the neuropathological findings of the cardiac sympathetic nervous system in a patient with PD who was examined by MIBG scintigraphy 1 year prior to death.

2. Case report and methods

2.1. Case report

A 73-year-old right-handed man visited our outpatient clinic with chief complaints of progressive gait disturbance and bradykinesia. He had been well until 9 months before this visit, at which point he noticed slowness in walking and a tendency to fall backward. His gait disturbance and bradykinesia gradually deteriorated until he required help to rise from his bed. He had a past history of exposure to the atomic bomb in Hiroshima at age 19, at which time temporarily lost his hair. He also had an 11-year history of diabetes mellitus (DM) with excellent control using glibenclamide. On neurological examination, he showed mild rigidity in his neck and four extremities, severe bradykinesia and gait of short stride with loss of arm swing. His postural reflex was also impaired but resting tremor was absent. His deep tendon reflexes were preserved and no sensory disturbances were present and he did not have any symptoms of constipation, urinary disturbances or orthostatic hypotension.

The patient's fasting blood sugar was 106 mg/dl and his hemoglobin $A1_c$ was 6.0% (normal range: 4.3–5.8%). Magnetic resonance images of the brain were unremarkable except for mild cortical atrophy, and the electrocardiogram showed unremarkable results. The coefficient of variation of the R–R interval for the electrocardiogram was 1.03% (normal range: 1.27–3.69) but the head-up tilt test showed no evidence of orthostatic hypotension. Positron emission tomography (PET) studies showed reduced ^{18}F-fluorodopa uptake with mild laterality (right>left) and increased ^{11}C-*N*-methylspiperone uptake in the striatum with mild laterality (right<left), findings which were consistent with PD.

The patient received levodopa and experienced transient amelioration, but subsequently deteriorated into a wheelchair-bound state. At age 74, he had repeated hemorrhagic episodes from diverticulitis of the colon, subsequently followed by subacutely progressive dementia with a score by Mini-Mental Stage Examination of 3, one year and six months from the onset of Parkinsonism. He unexpectedly died of massive hemorrhage 5 months later. His clinical diagnosis was PD with dementia, following the "one year rule" of the Consensus Guidelines [9]. The total clinical course was 2 years.

2.2. MIBG myocardial scintigraphy

After the patient was in the supine position for 20 min, 111 MBq of ^{123}I-MIBG (Daiichi Radioisotope Laboratories Co, Tokyo, Japan) was intravenously injected. Planar imaging and single photon emission computed tomography were performed using a triple headed gamma camera (GCA9300A, Toshiba Co, Tokyo, Japan) after 15 min (early phase) and 3 h (late phase). Photopeak energy was centered at 159 keV with a 20% window and relative organ uptake of ^{123}I-MIBG was determined by setting the region of interest on the anterior planar image. Using average counts per pixel for the heart and mediastinum, the ratio of the uptake by the heart to that by the mediastinum was calculated.

2.3. Neuropathology

A postmortem examination was performed 18 h after death. The brain and spinal cord were fixed in 20% buffered formalin for two weeks and the appropriate areas were embedded in paraffin for routine morphological examinations. To study the cardiac sympathetic innervation in detail, the intermediolateral column at several levels of the thoracic spinal cord, the sympathetic trunk and ganglia, and the nerve plexus of the anterior wall of the left ventricle were carefully examined and compared with those of five age-matched controls.

Six micron-thick sections were stained with hematoxylin and eosin by the Klüver–Barrera method. Antibodies raised against Aβ (12B2, monoclonal, aa. 11–28, IBL, Maebashi, Japan); phosphorylated τ (ptau) (AT8, Innogenetics, Temse, Belgium); phosphorylated α-synuclein (psyn) (psyn#64, monoclonal, and Pser129, polyclonal, kind gifts from Dr T. Iwatsubo), phosphorylated neurofilament (SMI31, Sternberger Immunochemicals, Bethesda, MD); HLA-DR (CD68, Dako, Glostrup, Denmark); tyrosine hydroxylase (TH, polyclonal, Calbiochem, Parmstadt, Germany); and glial fibrillary acidic protein (GFAP, polyclonal, Dako, Glostrup, Denmark) were employed. The sections were visualized with a Ventana NX20 system as previously reported [10].

The control cases died of systemic disorders that did not affect the heart.

3. Results

3.1. MIBG myocardial scintigraphy

MIBG myocardial scintigraphy revealed that the uptake ratio of the heart to that of the mediastinum was 1.58 (normal mean of 2.76) during the early phase and 1.35 (normal mean of 3.45) during the late phase.

3.2. Neuropathology

The brain weighed 1250 g and the temporal lobe was slightly atrophic. Serial coronal slices of the brain showed mild dilatation of the lateral and third ventricles and serial axial sections revealed the loss of pigmentation in the substantia nigra and locus ceruleus. Histologically, neuronal loss and gliosis were present in the substantia nigra, locus ceruleus, and basal nucleus of Meynert. Lewy bodies (LBs) were present in the substantia nigra, locus ceruleus, dorsal vagal motor nucleus, raphe nucleus, hypothalamus, basal nucleus of Meynert, amygdala, anterior cingulate gyrus, transentorhinal region and second temporal gyrus, but not present in the frontal or parietal cortex. The LB score of this case was 4, following the Consensus Guidelines for DLB [9]. Senile plaques were absent and neurofibrillary tangles were only scattered in the transentorhinal cortex (Braak Stage I).

In the sympathetic nerves innervating the heart, LBs were present in the intermediolateral column of the thoracic spinal cord and sympathetic ganglia. In contrast, LBs were completely absent in the control subjects. Multiple levels of the intermediolateral column of the thoracic spinal cord were examined with anti-phosphorylated α-synuclein antibody (psyn). Scattered psyn-immunoreactive neuronal intracytoplasmic inclusions, threads and dots were present there. Immunohistochemistry with anti-psyn antibodies showed positive axons in the thoracic ventral roots, sympathetic trunk and cardiac plexus (Fig. 1D–F) and Nageotte's residual nodules were scattered among relatively preserved sympathetic ganglia (Fig. 1A). In the cardiac plexus, total loss of TH-immunoreactivity (Fig. 1H) compared with the normal control (Fig. 1G) and a marked decrease of axons (Fig. 1C) compared with the normal control (Fig. 1B) were evident. In contrast, the dorsal root ganglia and the sural nerve, including unmyelinated fibers, were well preserved, as shown by ultrastructural studies (data not shown). The heart itself did not show any valvular, coronary or myocardial change.

4. Discussion

This study found cardiac sympathetic denervation in a patient with PD, which was well correlated with severely decreased uptake in MIBG scintigraphy.

Previous studies demonstrated that neuronal degeneration with LBs occurs in broad areas of the sympathetic nervous system, including the sympathetic ganglia and the cardiac plexus, in patients with PD [11]. In the cardiac plexus, LBs and α-synuclein positive axons [12] or

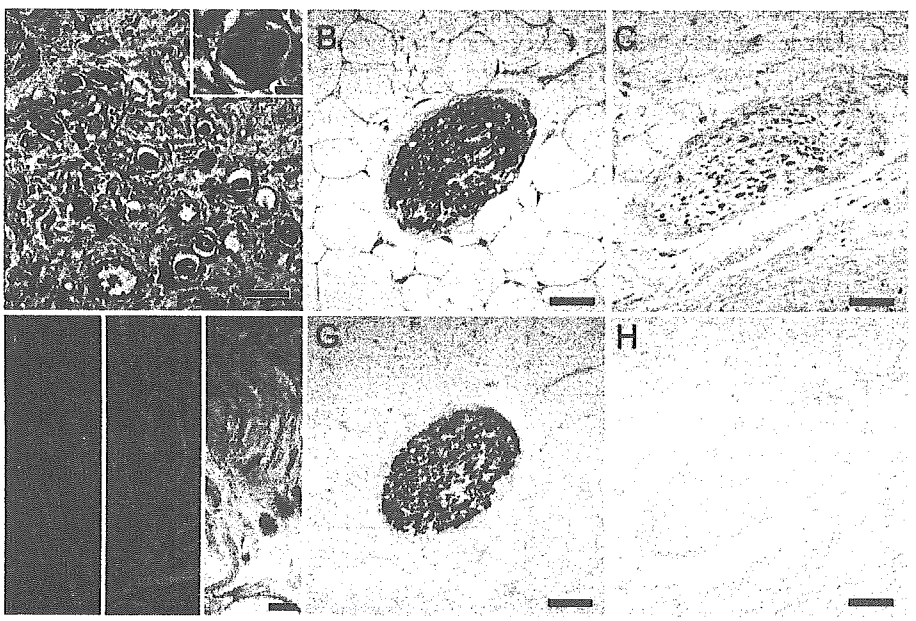

Fig. 1. Pathology of the sympathetic nervous system of a case of Parkinson disease A: a sympathetic ganglion showing a Nageotte's residual nodule (arrows) with Lewy bodies (LBs) (arrowhead) (hematoxylin and eosin staining, bar=50 μm). Inset: a typical LB in the sympathetic ganglion (bar=10 μm). B and C: unmyelinated fibers in the epicardial fatty tissue immunostained with anti-phosphorylated neurofilament antibody (SMI 31). Abundant axons from a control (B) and marked loss of axons from the case (C) (bar=50 μm). D–F: Lewy axons visualized by immunohistochemistry with anti-phosphorylated α-synuclein antibody (psyn#64) in the same fascicle as in section C (bar=10 μm). G and H: serial sections from section B (G) and C (H) immunoassayed with anti-tyrosine hydroxyls (TH) antibody. Abundant TH-immunoreactive fibers from the control (G) and total loss of immunoreactivity from the patient (H) (bar=50 μm).

markedly decreased TH-positive nerve fibers [7,8] were reported, which is consistent with our findings.

The present study found that the pathology of the sympathetic ganglia consisted of prominent α-synucleinopathy with a relatively preserved neuronal population. This was in sharp contrast with the severe axonal loss of sympathetic nerves in the cardiac muscle. Thus, LB-related α-synucleinopathy may cause distal axonopathy of the postganglionic sympathetic nerves.

It is difficult to exclude the possibility that the clinical history of DM may have made some contribution to the findings of MIBG scintigraphy and the pathology of the peripheral autonomic nervous system in this case, although the extremely low MIBG uptake and intact unmyelinated fibers in the sural nerve and dorsal root ganglia as well as pathologically unremarkable heart itself suggest that this possibility is not likely.

This study suggested that MIBG scintigraphy could be used to detect the presence of LB-related α-synucleinopathy in the cardiac sympathetic nervous system. Further prospective pathological studies on cardiac sympathetic innervation in PD or DLB patient who underwent MIBG scintigraphy should be carried out.

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付 2.　　当該年度別刷

HIGH LIGHT
CME

Clinical diagnosis of MM2-type sporadic Creutzfeldt–Jakob disease

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Abstract—*Background:* No method for the clinical diagnosis of MM2-type sporadic Creutzfeldt–Jakob disease (sCJD) has been established except for pathologic examination. *Objective:* To identify a reliable marker for the clinical diagnosis of MM2-type sCJD. *Methods:* CSF, EEG, and neuroimaging studies were performed in eight patients with MM2-type sCJD confirmed by neuropathologic, genetic, and western blot analyses. *Results:* The eight cases were pathologically classified into the cortical (n = 2), thalamic (n = 5), and combined (corticothalamic) (n = 1) forms. The cortical form was characterized by late-onset, slowly progressive dementia, cortical hyperintensity signals on diffusion-weighted imaging (DWI) of brain, and elevated levels of CSF 14-3-3 protein. The thalamic form showed various neurologic manifestations including dementia, ataxia, and pyramidal and extrapyramidal signs with onset at various ages and relatively long disease duration. Characteristic EEG and MRI abnormalities were almost absent. However, all four patients examined with cerebral blood flow (CBF) study using SPECT showed reduction of the CBF in the thalamus as well as the cerebral cortex. The combined form had features of both the cortical and the thalamic forms, showing cortical hyperintensity signals on DWI and hypometabolism of the thalamus on [^{18}F]2-fluoro-2-deoxy-D-glucose PET. *Conclusion:* For the clinical diagnosis of MM2-type sporadic Creutzfeldt–Jakob disease, cortical hyperintensity signals on diffusion-weighted MRI are useful for the cortical form and thalamic hypoperfusion or hypometabolism on cerebral blood flow SPECT or [^{18}F]2-fluoro-2-deoxy-D-glucose PET for the thalamic form.

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Sporadic Creutzfeldt–Jakob disease (sCJD) has been classified based on the genotype at polymorphic codon 129 of the prion protein gene (*PrP*) and the physicochemical properties of the pathologic PrP (PrP^{Sc}); various classification systems have been proposed.[1-5] A simple classification[1,2] has been widely accepted and recognizes at least six phenotypes in sCJD: MM1, MV1, VV1, MM2, MV2, and VV2.[3] About 70% of patients with sCJD show the classic CJD phenotype and are mostly classified as MM1 or MV1 types.[3] Their clinical diagnosis relies on the detection of periodic sharp-wave complexes (PSWCs) in EEG, elevated levels of CSF 14-3-3 protein, and typical hyperintensity signals of brain MRI in addition to the classic clinical manifestations.[3,6]

Other phenotypes of sCJD do not present with typical clinical symptoms of classic CJD or PSWCs on EEG.[3,6] Both VV2 and MV2 patients are characterized by ataxia and dementia,[3] and brain MRI and CSF 14-3-3 protein are useful for their clinical diagnosis.[6] As for several reported patients with MM2-type sCJD, some showed positive CSF 14-3-3 protein, but others did not.[6-9] Thus, for MM2 as well as VV1, diagnostic markers have not been established yet.

The clinical features of MM2-type sCJD in some patients were previously reported.[1,3,6,8,10,11] MM2-type sCJD presents with at least two pathologic phenotypes: MM2 cortical and MM2 thalamic forms.[3] In the MM2 cortical phenotype, dementia is a major symptom, and visual or cerebellar signs are usually

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Table 1 Clinical features of eight patients with MM2-type sCJD

Patient no.	Sex	Onset, y	Course, mo	Onset symptoms	Clinical manifestations during illness	Initial diagnosis
1	F	65	Alive (13)	Dementia	Dementia, pyramidal signs, insomnia	CJD
2	F	75	Alive (28)	Depression	Psychiatric symptoms, dementia, myoclonus	CJD
3	M	65	14	Falling to left side	Dementia, myoclonus, cerebellar ataxia, akinetic mutism	CJD
4	F	49	30	Insomnia	Insomnia, dementia, psychiatric symptoms, pyramidal signs, extrapyramidal signs, autonomic symptoms, myoclonus, akinetic mutism	PSP
5	M	64	53	Photophobia	Visual symptoms, extrapyramidal signs, dementia, autonomic symptoms, psychiatric symptoms, myoclonus, akinetic mutism	PSP
6	F	30	73	Blurred vision	Visual symptoms, psychiatric symptoms, cerebellar ataxia, dementia, pyramidal signs, extrapyramidal signs, myoclonus, akinetic mutism	SCD
7	M	71	25	Ataxic gait	Cerebellar ataxia, autonomic symptoms, dementia	SCD
8	M	58	13	Dementia	Dementia, cerebellar ataxia, myoclonus, pyramidal signs, psychiatric symptoms	AD

sCJD = sporadic Creutzfeldt–Jakob disease; PSP = progressive supranuclear palsy; SCD = spinocerebellar degeneration; AD = Alzheimer disease.

absent.[3] There have been no reports of laboratory or neuroimaging studies in MM2 cortical sCJD. In the MM2 thalamic phenotype, the clinical features are insomnia and psychomotor hyperactivity in addition to ataxia and cognitive impairment.[3] This phenotype may be called sporadic fatal insomnia (SFI) because the clinical and pathologic features resemble those of fatal familial insomnia (FFI).[8,10] In the MM2 thalamic form or SFI, no PSWCs on EEG, positive or negative 14-3-3 protein in CSF, and normal brain MRI have been reported.[6,8,10]

Since a report of variant CJD (vCJD) in the United Kingdom in 1996,[12] identification of vCJD has been an important part of the surveillance of prion diseases. The clinical features of vCJD are similar to those of MM2-type sCJD, including young age at onset, long disease duration, absence of PSWCs on EEG, and methionine homozygosity on codon 129 of *PrP* gene.[3,13] Therefore, MM2-type sCJD is important in the differential diagnosis for vCJD.

We have investigated the clinical and neuroimaging features of eight patients with MM2-type sCJD in an attempt to identify a reliable marker for the clinical diagnosis of this type of sCJD.

Methods. *Subjects.* We investigated eight patients with MM2-type sCJD confirmed by neuropathologic, genetic, and western blot analyses. The clinical features, results of EEG, CSF 14-3-3 protein, MRI, cerebral blood flow (CBF) studies using SPECT, and brain glucose metabolism studies using [^{18}F]2-fluoro-2-deoxy-D-glucose (FDG) PET were reviewed. CBF-SPECT using ^{99m}Tc-ethyl cysteinate dimer (^{99m}Tc-ECD) was performed in three (Patients 1, 2, and 8), CBF-SPECT using *N*-isopropyl-*p*-[^{123}I]iodoamphetamine in three (Patients 4, 5, and 6), and FDG-PET in one (Patient 3). MRI, CBF-SPECT, and FDG-PET were evaluated by two neurologists and a neuroradiologist without knowledge of the clinical findings. None of these subjects had a family history of CJD or known exposure to prion contamination in the past.

Analysis of PrP *gene.* DNA was extracted from the blood, and the open reading frame of the *PrP* gene was analyzed as previously described.[14,15]

Neuropathology. The brain tissues were obtained by brain biopsy (Patients 1 and 2) or autopsy (Patients 3 to 8). Brain tissue sections were stained with routine neuropathologic techniques. Immunohistochemistry was performed with a monoclonal antibody to PrP (3F4), as previously described.[16]

Western blot analysis. Brain tissue from the frontal lobe was homogenized, and western blot analysis of protease K-resistant PrP was performed with 3F4 as previously described.[17]

Results. *Clinical features and laboratory and neuroimaging studies.* The clinical features are summarized in table 1 and the laboratory and neuroimaging studies in table 2. The details of the clinical and pathologic features of Patients 5[18] and 6[11] were previously reported, and Patient 8 was included in a previous MRI study.[19] The details of the clinical features of three representative patients (Patients 2, 3, and 8) are described below.

Patient 2. A 75-year-old woman developed forgetfulness and depression. Her mental symptoms gradually deteriorated, and she was admitted to our hospital 14 months after the onset. Neurologically, she showed only dementia (Mini-Mental State Examination 9/30) with depressed mood.

Sixteen months after the onset, the MRI showed gyriform hyperintensity in the bilateral frontal, temporal, parietal, and occipital cortices on diffusion-weighted imaging (DWI) (figure 1A). Less intense and smaller cortical lesions were shown on fluid-attenuated inversion recovery imaging (see figure 1B). On the EEG, diffuse slowing was found without PSWCs. CBF-SPECT using ^{99m}Tc-ECD showed re-