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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.

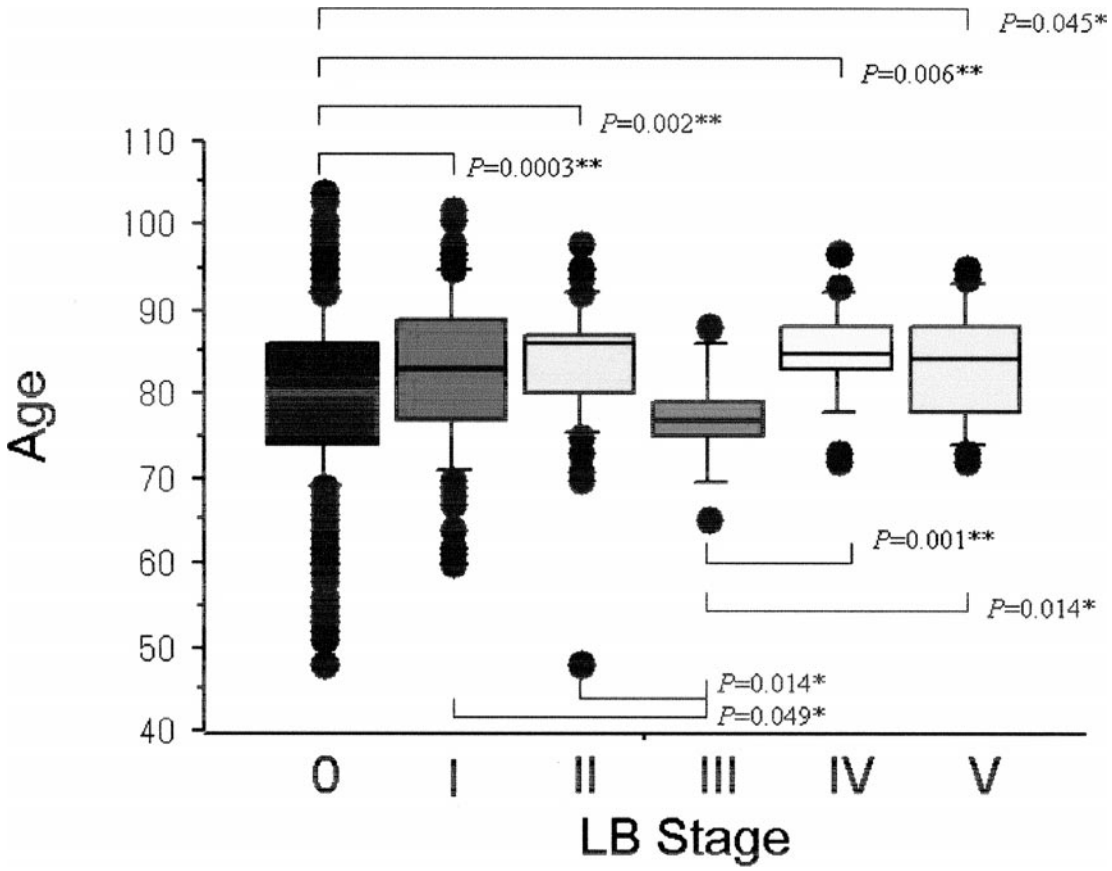

Fig. 1. Age distribution in each stage. The average age at death in Lewy body (LB) stages I, II, IV, and V was significantly greater than in LB stage 0 or LB stage III.

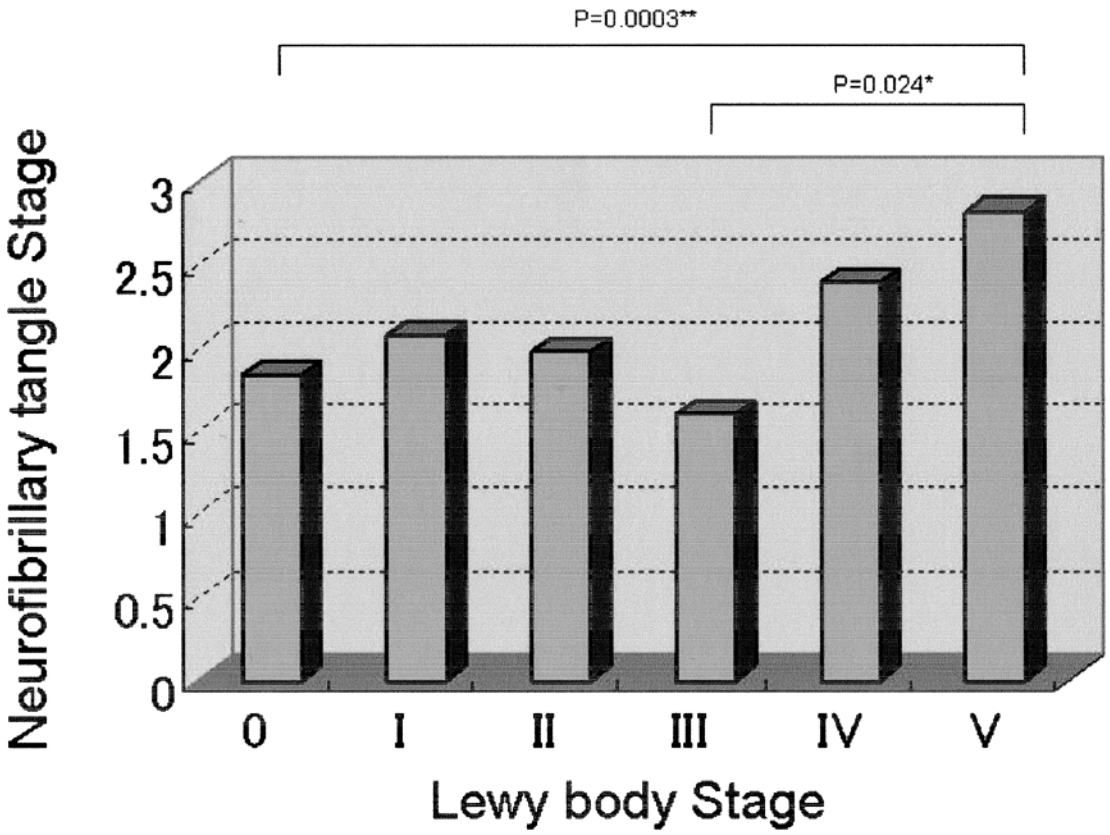

Fig. 2. Lewy body stage versus neurofibrillary tangle (NFT) stage. NFT stage is significantly higher in Lewy body (LB) stage V than in LB stage 0 or LB stage III.

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.

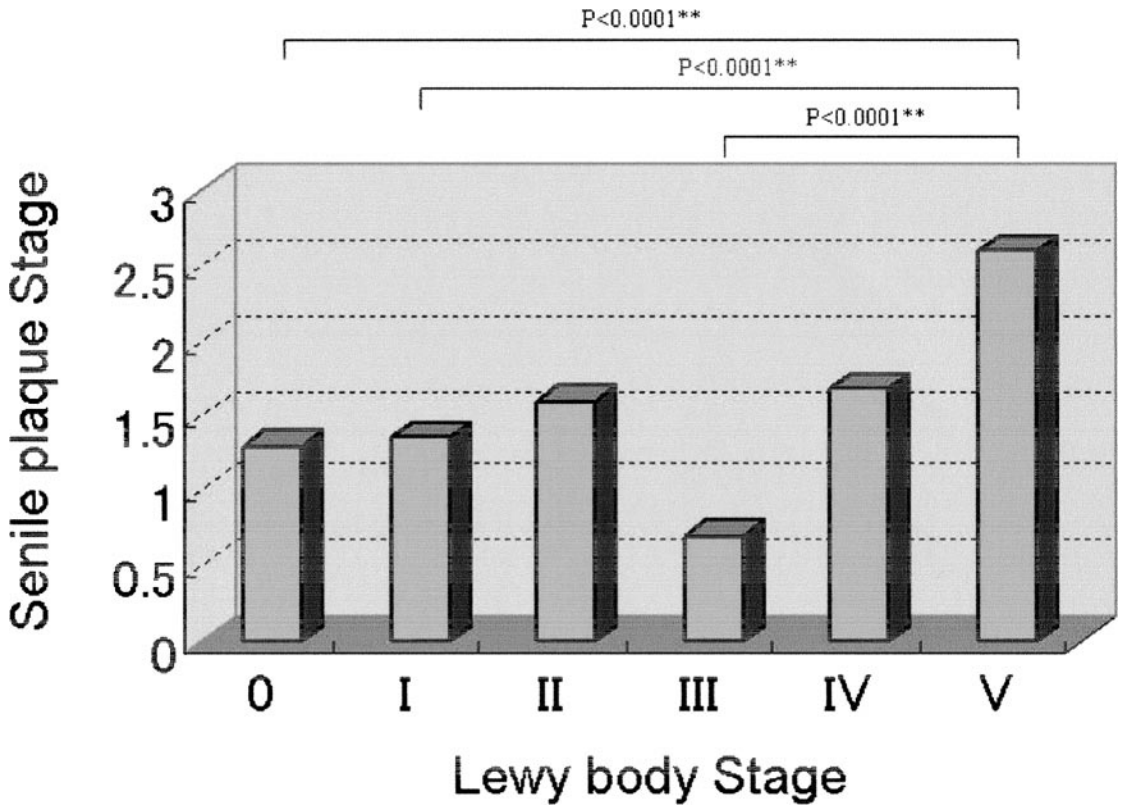

Fig. 3. Lewy body stage versus senile plaque (SP) stage. SP stage is significantly higher in Lewy body (LB) stage V than in LB stage 0, LB stage I or LB stage III.

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 $\varepsilon 3/\varepsilon 4$ and the allelic frequency of $\varepsilon 4$ 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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REFERENCES

1. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003;24:197–211
2. Folstein MF, Folstein SE, McHugh PR. ''Mini-mental state''. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98
3. Hasegawa K, Inoue K, Moriya K. An investigation of dementia rating scale for the elderly. Seishin Igaku 1974;16:965–69
4. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the staging of dementia. Br J Psychiatry 1982; 140:566–72
5. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991;41:479–86
6. McKeith IG, Galasko D, Kosaka K, et al. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies

(DLB): Report of the consortium on DLB international workshop. Neurology 1996;47:1113–24
7. Yamaguchi H, Haga C, Hirai S, Nakazato Y, Kosaka K. Distinctive, rapid, and easy labeling of diffuse plaques in the Alzheimer brains by a new methenamine silver stain. Acta Neuropathol 1990;79: 569–72
8. Gallyas F. Silver staining of Alzheimer's neurofibrillary changes by means of physical development. Acta Morphol Acad Sci Hung 1971;19:1–8
9. Saito Y, Nakahara K, Yamanouchi H, Murayama S. Severe involvement of ambient gyrus in dementia with grains. J Neuropath Exp Neurol 2002;61:789–96
10. Saito Y, Kawashima A, Ruberu NN, et al. Accumulation of phosphorylated α-synuclein in aging human brain. J Neuropathol Exp Neurol 2003;62:644–54
11. Fujiwara H, Hasegawa M, Dohmae N, et al. α-Synuclein is phosphorylated in synucleinopathy lesions. Nat Cell Biol 2002;4:160–64
12. Murayama S, Saito Y. Neuropathological diagnostic criteria for Alzheimer disease. Neuropathology (in press)
13. The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. Neurobiol Aging 1997;18:S1–S2
14. Jellinger KA. Dementia with grains (argyrophilic grain disease). Brain Pathol 1998;8:377–86
15. Jellinger KA, Bancher C. Senile dementia with tangles (tangle predominant form of senile dementia). Brain Pathol 1998;8:367–76
16. Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: Diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 1993;43:250–60
17. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 1991;82:239–59
18. Garin MC, James RW, Dussoix P, et al. Paraoxonase polymorphism Met-Leu54 is associated with modified serum concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. J Clin Invest 1997;99:62–66
19. Humbert R, Adler DA, Disteche CM, Hassett C, Omiecinski CJ, Furlong CE. The molecular basis of the human serum paraoxonase activity polymorphism. Nat Genet 1993;3:73–76
20. Leviev I, James RW. Promoter polymorphisms of human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. Arterioscler Thromb Vasc Biol 2000;20:516–21
21. Suehiro T, Nakamura T, Inoue M, et al. A polymorphism upstream from the human paraoxonase (PON1) gene and its association with PON1 expression. Atherosclerosis 2000;150:295–98
22. Kosaka K, Iseki E. Diffuse Lewy body disease within the spectrum of Lewy body disease. In: Perry RH, McKeith IG, Perry EK, eds. Dementia with Lewy bodies. Cambridge: Cambridge University Press, 1996;238–47
23. Jellinger KA. α-Synuclein pathology in Parkinson's and Alzheimer's disease brain: Incidence and topographic distribution–a pilot study. Acta Neuropathol (Berl) 2003;106:191–201
24. Parkkinen L, Soininen H, Alafuzoff I. Regional distribution of α-synuclein pathology in unimpaired aging and Alzheimer disease. J Neuropathol Exp Neurol 2003;62:363–67
25. Wakabayashi K, Kakita A, Hayashi S, et al. Apolipoprotein E ε4 and allele and progression of cortical Lewy body pathology in Parkinson's disease. Acta Neuropathol (Berl) 1998;95:450–54
26. Lippa CF, Smith TW, Saunders AM, et al. Apolipoprotein E genotype and Lewy body disease. Neurology 1995;45:97–103
27. Harrington CR, Louwagie J, Rossau R, et al. Influence of apolipoprotein E genotype on senile dementia of the Alzheimer and Lewy body types. Significance for etiological theories of Alzheimer's disease. Am J Pathol 1994;145:1472–84
28. Ruberu NN, Saito Y, Koyama S, et al. Alzheimer-type senile changes and ApoE genotyping. Neuropathology 2003;23:A42

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