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Most cases with Lewy pathology in a population-based cohort adhere to the Braak progression pattern but 'failure to fit' is highly dependent on staging system applied

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ABSTRACT

Braak et al.'s 2003 paper detailing the caudo-rostral progression of Lewy body pathology (LP) formed the foundation of current understanding of disease spread in Parkinson's disease (PD); however, its methods are difficult to recreate and consequently multiple new staging systems emerged to recapitulate Braak's staging system using standard neuropathological methods and to account for other patterns of LP. Studies using these systems have documented widely variable rates of cases that 'fail to fit' expected patterns of LP spread. This could be due to population differences, features of individual systems, or may constitute under-recognized patterns of disease. We examined 324 neuropathological cases from the Honolulu Asia Aging Study and applied four different LP staging systems to determine the proportion of cases adhering to different staging methodologies and those that 'fail to fit' expected patterns of LP. Of 141 cases with LP (24: PD, 8: Dementia with Lewy bodies (DLB), 109: Incidental Lewy body disease (ILBD)), our application of Braak et al., 2003 classified 83.7%, Müller et al., 2005 classified 87.9%, Beach et al., 2009 classified 100%, and Leverenz et al., 2008 classified 98.6%. There were significant differences in the cases classifiable by the Leverenz and Beach systems versus the Braak and Müller systems ($p < 0.001$ for each). In this population-based autopsy cohort with a high prevalence of ILBD, the majority of cases were consistent with the progression characterized by the Braak et al. however, the determination of cases as atypical is highly dependent on the staging system applied.

1. Introduction

In 2003, Braak and colleagues proposed a model for the progression of α-synuclein immunoreactive inclusions in the human brain including Lewy bodies (LBs) and Lewy neurites (LNs) in patients with Parkinson's disease (PD) [1]. This initial staging of disease led to a predilection theory that Lewy pathology (LP), which includes both LBs and LNs, advances from predisposed induction sites in the olfactory bulb and lower brainstem nuclei in a rostral pattern through the brainstem, limbic system, and then neocortical regions. The original Braak staging method was based on pathological assessment using 100 μm thick sections of whole brain hemispheres that, while extremely thorough and elegant, is not used in common practice in autopsy samples. Therefore, attempts have been made to develop staging systems that capture the essence of the Braak staging with more accessible methodologies. These systems have examined fewer brain regions, used standard neuropathological sampling methods, integrated severity of pathology into their classification schemata, and added designations for amygdala predominant LP, which is often found in the setting of Alzheimer's disease [2–5] all while improving classification ability and inter-rater reliability [3,4,6,7].

However, studies have reported that Braak staging may fail to classify between 14 and 43% of cases [1,8–10]. This wide range of the number of 'fail to fit' cases may depend on the population being studied

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Table 1
Classification Systems with definition of Stages of Lewy Pathology according to α-synuclein immunoreactivity in different brain regions.

Sampled Brain area	Olfactory Bulb	Medulla		Pons		Midbrain	Basal Forebrain		Hippocampus	Meso cortex		Neocortex		
Anatomical region	OLF	DMN	Irx	LC	CRN/RF	SN	NBM	AMYG	CA2/3	ENT	Cingulate Gyrus	Temporal Ctx	Frontal Ctx	Parietal Ctx
Braak et al.[a]														
1		1–2												
2		1–2		0–2	1–2									
3		1–3		1–3	1–2	1–3	1–3							
4		2–3		2–3	2–3	2–3	1–3	+	1–2	1–2				
5		2–3		2–3	2–3	2–3	2–3	+	2	2	1	1		
6		3		2–3	2–3	3	3	+	2–3	2–3	2	2	1	1
Müller et al.														
1		≥ +												
2		≥ +		≥ +										
3		≥ +		≥ +	≥ +	≥ +								
4		≥ +		≥ +	≥ +	≥ +			≥ +	≥ +				
5		≥ +		≥ +	≥ +	≥ +			≥ +	≥ +		≥ +		
6		≥ +		≥ +	≥ +	≥ +			≥ +	≥ +		≥ + +		
Beach et al.[b]														
I. Olfactory Only	1–4	0		0	0	0		0		0	0			
IIa. Brainstem Pred.	0–4	a. 1–2		a. 1–2	a. 1–2	a. 1–2		a. 0		a. 0	a. 0			
		b. 3-4		b. 3-4	b. 3-4	b. 3-4		b. 1-2		b. 1-2	b. 1-2			
IIb. Limbic Pred.	0–4	a. 0		a. 0	a. 0	a. 0		a. 1-2		a. 1-2	a. 1-2	0	0	0
		b. 1-2		b. 1-2	b. 1-2	b. 1-2		b. 3-4		b. 3-4	b. 3-4	0–1	0–1	0–1
III. Brainstem/Limbic	0–4	a. 1-2		a. 1-2	a. 1-2	a. 1-2		a. 1-2		a. 1-2	a. 1-2	0–1	0–1	0–1
		b. 3-4		b. 3-4	b. 3-4	b. 3-4		b. 3-4		b. 3-4	b. 3-4	0–1	0–1	0–1
IV. Neocortical	0–4	0–4		0–4	0–4	0–4		0–4		0–4	0–4	2–4	2–4	2–4
Leverenz et al.[c]														
Brainstem		1+				1+		0–2			0–1		0	
Amyg. Pred.		0–1				0–1		1+			0–1		0	
Limbic		1+				1+		2+			1–3		0–1	
Neocortical		1+				1+		2+			2+		2+	

Abbreviations: DMN: dorsal motor nucleus of the vagus, Irx: Intermediate Reticular zone, LC: Locus coeruleus, CRN/RF: caudal raphe nuclei/Reticular formation, SN: Substantia nigra pars compacta, NBM: Nucleus Basalis of Meynert, AMYG: Amygdala, CA 2/3: Ammon's horn, 2nd sector, ENT: entorhinal cortex, Ctx: cortex, +: present.

[a] Original publication details slight, moderate, and severe pathology, corresponding here with 1, 2, 3. + indicates immunoreactivity.

[b] For the IIa, Brainstem predominant, IIb. Limbid predominant, III Brainstem/Limbic, match a. or b. for pathology density across each region to determine whether brainstem or limbic regions are more heavily affected (IIa v IIb) or if they are equivalent (III).

[c] Cases that do not follow the above patterns are classified as 'Mixed'. LP may be present in *either* DMN/IRX or SN for brainstem, Limbic, or Neocortical designation but should be present in *both* for Amygdala predominant designation. Cases satisfying two criteria are assigned to the more rostral designation.

and which classification schemes are applied. 'Fail to fit' cases may warrant further study as they could occur due to under recognized pathways of LP spread or different points of LP inception due to unrecognized mechanisms, but detailed assessments comparing the behavior of classification systems are essential to understand the prevalence of cases that truly diverge from expected LP progression patterns.

The Honolulu-Asia Aging Study (HAAS) is a population-based study of the rates of dementia and neurodegenerative illnesses in Japanese American men which began in 1991 [11]. Here, a modified Braak staging protocol, developed to accommodate a standard neuropathological assessment, is applied along with three other published classification schemes [3,6,7] to a large population-based cohort of subjects from the HAAS spanning the spectrum of Lewy body disorders and the rates of 'fail to fit' cases are compared across staging systems.

2. Materials and methods

2.1. Subjects

324 brains obtained from a non-random sample from the HAAS were examined. The sample included all cases with known LP at the time this cohort was assembled, including 24 cases of clinicopathological Parkinson's disease (PD), and 8 cases of clinicopathological dementia with Lewy bodies (DLB). In addition, 292 cases with no clinical history of any neurodegenerative disorder were included. The asymptomatic cohort was slightly enriched by oversampling cases with low neuronal counts in the substantia nigra pars compacta [12].

All study participants were screened for parkinsonism during structured face-to-face interviews beginning in 1991 and occurring approximately every three years through 2012, and those with a history or signs of parkinsonism were referred to a study neurologist who administered standardized questions about symptoms and the onset of parkinsonism, previous diagnoses, and medication use, followed by a comprehensive and standardized neurologic examination. Final diagnosis of clinical PD was determined by consensus of two neurologists according to contemporary published criteria [13]. The study was approved by the Kuakini Medical Center Institutional Review Board and participants signed informed consents at all examinations in accordance with the Declaration of Helsinki.

2.2. Immunohistochemistry

Formalin-fixed, paraffin-embedded sections were used for assessment. Immunohistochemical staining for α-synuclein and semi-quantitative density analyses of pathology were performed on tissue collected from seventeen brain regions: the olfactory bulb, medulla, pons, midbrain, hippocampus, amygdala, striatum at the level of the nucleus accumbens, basal forebrain, and nine cortical regions (anterior cingulate cortex, anterior temporal mesocortex, entorhinal cortex, insular cortex, mid-frontal cortex, anterior superior and mid-temporal cortex, inferior parietal cortex, calcarine cortex, and superior pre- and postcentral gyri). The methods of immunostaining have been previously validated [7] and are published elsewhere [14]. Briefly, slides were deparaffinized, rehydrated in graded ethanols, and endogenous peroxidase activity was quenched in a 30% H_2O_2/methanol bath for 30 min. Antigen exposure was enhanced by sequential processing with a commercial antigen retriever (Antigen Retriever 2100, catalog #62700; EMS, Hatfield, PA) with commercial buffer (R-Buffer U, catalog #62706; EMS) overnight and then immersed in 88% formic acid for 30 min. Slides were blocked with 2% goat serum. The sections were immunostained for α-synuclein (Syn 303, 1:16,000; generously donated by Dr. Virginia Lee [15], overnight at 4 °C. The next day, a secondary antibody (Vector, Burlington, CA) was applied. After an hour incubation, sections were processed using the avidin-biotin-peroxidase (ABC) method with a Vectastain ABC Kit (Peroxidase Standard; Vector), and diaminobenzidine as chromogen. A modified version of the original Braak staging was developed to assess the distribution of pathology with the anatomic loci available (Supplementary Table 1). At least 2 foci from each stage were quantified: olfactory bulb and dorsal motor nucleus of the vagus (Stage 1); pontine raphe nucleus and locus coeruleus (Stage 2); substantia nigra pars compacta, and nucleus basalis of Meynert (Stage 3); basolateral nuclear complex of the amygdala, CA2/3 region of Ammon's horn, anterotemporal mesocortex, and transentorhinal cortex (Stage 4); insular, anterior cingulate (Stage 5); and motor, primary sensory, and middle temporal cortices (Stage 6). LP in each foci was assessed and assigned a semiquantitative density score of 0–3 regardless of LP morphology, as suggested previously [1,16].

A staging system proposed by Müller and colleagues [6] (Table 1) was also assessed. This system was developed to allow rapid and reproducible LP staging for cases that have undergone standard histological sampling. The foci for each stage include the dorsal motor nucleus of the vagus nerve or intermediate reticular zone (Stage 1), locus coeruleus (Stage 2), caudal raphe nucleus, reticular formation, and substantia nigra pars compacta (Stage 3), Ammon's horn (CA 2/3 of hippocampus) and anterior temporal mesocortex (Stage 4), neocortex of the first temporal gyrus (Stage 5; if pathology $\geq$1; Stage 6, if pathology $\geq$ 2).

Cases were determined to be consistent with the above staging systems if a case had LP present in at least one region representative of each stage lower than the stage assigned (for the Müller et al. system) and in an expected density progression (for Braak et al. system). This more permissive assessment was justified given the use of standard 10 μm thick sections as opposed to 100 μm thick sections used in Braak's original work and has been adopted previously [6].

Two alternative staging and classification systems were explored [3,4]. In contrast to the above systems, these two models classify cases based on the brain regions where LP predominates. The model proposed by Beach et al. [4] assesses LP distribution accordingly: (I) olfactory bulb only, (IIa) brainstem predominant, (IIb) limbic predominant, (III) brainstem/limbic, or (IV) neocortical. For this rating system, a reappraisal of pathology using a 0–4 rating system was required. Another model, proposed by Leverenz et al. [3], classifies LP as either (1) brainstem, (2) amygdala, (3) limbic, (4) or neocortical predominant (Table 1).

Braak tau stage [17] and CERAD stages [18] using neuritic plaque counts as previously described [19] were applied by expert neuropathologists (HP, LRW).

2.3. Statistical analysis

Demographics were compared between groups using one way analysis of variance with post hoc *t*-test with Bonferroni correction. Ability to classify cases across systems was compared using McNemar's χ^2 test. To ensure that the differences observed were not due to the ability of Beach et al. and Leverenz et al.'s ability to classify 'amygdala predominant' patterns LP [3,7] which are of greater likelihood to be unclassifiable by Braak et al. and Müller et al.'s systems [1,6], a subanalysis was performed with these cases removed. Comparisons of tau stage and CERAD stages between groups was accomplished using χ^2 test. All statistical testing was performed using Stata version 15.1 (College Station, TX).

3. Results

The mean time between last assessment and autopsy was 1.52 years (minimum: 0.003 years, maximum 12.2 years). Among 324 autopsied cases, 141 (43.5%) had LP, including all 24 cases with clinically diagnosed PD, 8 cases of clinically DLB, and 109 additional cases of ILBD. The frequency of ILBD was 33.6% in this cohort. Tissue was missing for 37 olfactory bulbs, 1 Locus Coeruleus, 1 Substantia Nigra, 2 Nucleus

Table 2
Characteristics of Cases assessed for α-Synuclein staging.

Clinical Characteristic	PD(N = 24)			DLB (N = 8)			ILBD (N = 109)			No LP (N = 183)			P
	Mean (SD)	Range	N	Mean (SD)	Range	N	Mean (SD)	Range	N	Mean (SD)	Range	N	
Age at Death	85.6 (3.5)	79–92	24	87.5 (3.7)	81–92	8	87.0 (5.4)	74–102	109	86.3 (5.4)	73–99	183	.52
Education	10.8 (3.2)	7–17	22	10.5 (3.9)	6–17	8	10.4 (3.4)	3–20	102	10.9 (3.3)	4–20	169	.69
Most recent CASI score	49.5[a] (30.1)	0–88	21	37.0[a] (30.0)	0–79	8	58.8 (30.2)	0–98	101	65.7[a] (27.3)	0–98	168	.003[a]
Neuropathologic Staging	Stage	N (%)		Stage	N (%)		Stage	N (%)		Stage	N (%)		
Tau Stage													
	0	0		0	0		0	1 (0.9)		0	0		
	I,II	5 (20.8)		I,II	2 (25.0)		I,II	24 (22.0)		I,II	45 (24.6)		
	III,IV	16 (66.7)		III,IV	2 (25.0)		III,IV	54 (49.5)		III,IV	94 (51.4)		
	V,VI	3 (12.5)		V,VI	4 (50.0)		V,VI	30 (27.5)		V,VI	44 (24.0)		
CERAD Stage													
	0	10 (41.7)		0	2 (25.0)		0	47 (43.1)		0	76 (41.5)		
	1	12 (50.0)		1	4 (50.0)		1	43 (39.5)		1	86 (47.0)		
	2	1 (4.2)		2	2 (25.0)		2	15 (13.8)		2	19 (10.4)		
	3	1 (4.2)		3	2 (25.0)		3	3 (3.67)		3	2 (1.1)		
α-Synuclein Staging													
Modified Braak	1	0		1	0		1	16 (15)					
	2	0		2	0		2	5 (4.6)					
	3	0		3	0		3	2 (1.8)					
	4	1 (4.2)		4	0		4	15 (14)					
	5	8 (33)		5	3 (38)		5	35 (32)					
	6	14 (58)		6	5 (62)		6	14 (13)					
	Absent[b]	0		Absent	0		Absent	0					
	Unclassifiable	1 (4.2)		Unclassifiable	0		Unclassifiable	22 (20)					
Muller et al.	1	0		1	0		1	7 (6.5)					
	2	0		2	0		2	2 (1.9)					
	3	1 (4.2)		3	0		3	20 (19)					
	4	4 (17)		4	0		4	26 (24)					
	5	5 (21)		5	3 (38)		5	10 (9.3)					
	6	13 (54)		6	5 (62)		6	13 (12)					
	Absent	0		Absent	0		Absent	14 (13)					
	Unclassifiable	1 (4.2)		Unclassifiable	0		Unclassifiable	16 (15)					
Leverenz et al.	Brainstem	4 (17)		Brainstem	0		Brainstem	32 (29)					
	Limbic	16 (67)		Limbic	4 (50)		Limbic	28 (26)					
	Amydgala Predominant	1 (4.2)		Amydgala Predominant	0		Amydgala Predominant	12 (11)					
	Neocortical	3 (13)		Neocortical	4 (50)		Neocortical	3 (2.8)					
	Absent	0		Absent	0		Absent	32 (29)					
	Unclassifiable	0		Unclassifiable	0		Unclassifiable	2 (1.8)					
Beach et al.	I. Olfactory bulb only	0		I. Olfactory bulb only	0		I. Olfactory bulb only	13 (12)					
	IIa. Brainstem Predominant	6 (25)		IIa. Brainstem Predominant	0		IIa. Brainstem Predominant	38 (35)					
	IIb. Limbic Predominant	1 (4.2)		IIb. Limbic Predominant	0		IIb. Limbic Predominant	12 (11)					
	III. Brainstem/ Limbic	16 (67)		III. Brainstem/ Limbic	4 (50)		III. Brainstem/ Limbic	41 (38)					
	IV. Neocortical	1 (4.2)		IV. Neocortical	4 (50)		IV. Neocortical	3 (2.8)					
	Absent	0		Absent	0		Absent	2 (1.8)					
	Unclassifiable	0		Unclassifiable	0		Unclassifiable	0					

[a] Post hoc comparisons Bonferroni corrected for multiple comparisons (alpha for significance set at .0083) show significant differences in CASI score between PD and No LP (p = 0.0075) and DLB and No LP (p = 0.0042).

[b] Cases marked as 'Absent' had no LP in regions used for assessment in that particular staging system. LP was present elsewhere.

Basalis of Meynert, 2 Amygdala, 24 Anterotemporal mesocortex, 4 Cornu Ammonis 2/3, 8 Entorhinal cortices, 2 Cingulate gyri, and 2 insular cortices. Missing tissue only affected the ability to assess the fit of one case with LP by the Müller et al. staging system and this cases was excluded from the corresponding analysis. All DLB cases were either Braak stage 5 (n = 3) or 6 (n = 5); PD cases were either Braak stages 4 (n = 1), 5 (n = 8), or 6 (n = 14) with one case being unstageable; ILBD cases were highly variable, representing all Braak LB stages 1–6 (Table 2, Fig. 1A). Frequency of stages in each system are detailed in Fig. 1A–D. Many ILBD cases demonstrated trace or low levels of LP in regions throughout the brainstem, limbic, and neocortical areas as opposed to the PD and DLB cases where moderate to severe pathology at each stage was the norm (Fig. 1E).

Tau and CERAD stages are detailed in Table 2. CERAD stage was similar between all groups. There was a trend for higher tau stage in DLB compared to PD ($\chi^2 = 5.8$, p = 0.056). High tau stage ≥2 and high CERAD stage ≥2 were not more common in ILBD cases compared to those without LP (p = 0.14).

Of the 141 cases with Lewy body pathology present, 118 cases (83.7%) conformed to our application of the Braak staging system with

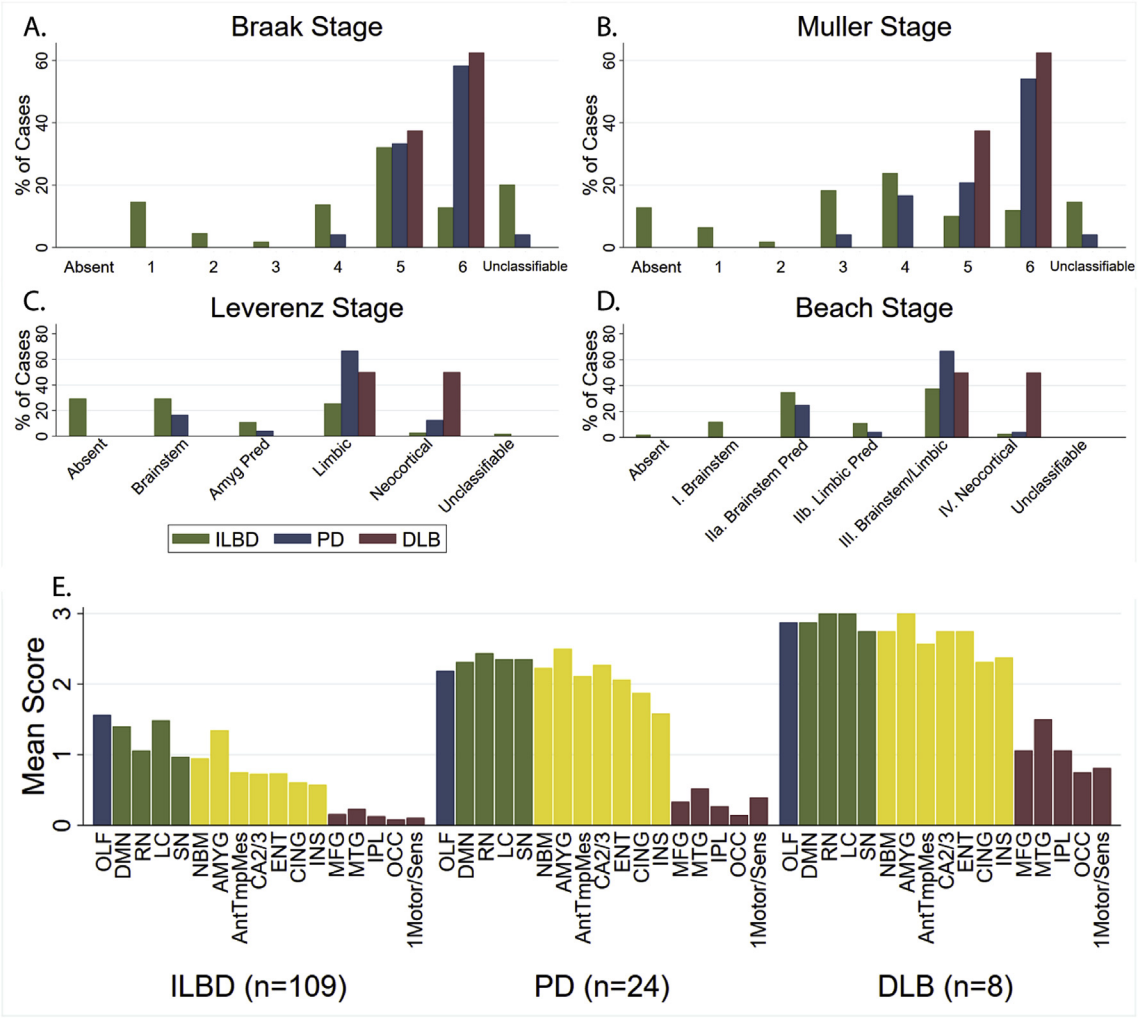

Fig. 1. Lewy Pathology Staging and Distribution. 1) A–D: Percent of cases in each pathology stage (Green: ILBD, Blue: PD, Red: DLB) as measured by each staging system A: Braak et al., 2003, B: Müller et al., 2005, C: Leverenz et al., 2008, D: Beach et al., 2008. PD and DLB had uniformly higher stages of pathology whereas ILBD had a wide range of stages. E: Average ordinal score for each region by disease (0–3 scale): PD and DLB cases had higher LP severity in brainstem, limbic, and neocortical regions whereas ILBD had low level LP scattered throughout. (Blue: Olfactory bulb, Green: brainstem regions, Yellow: Limbic regions, Red: neocortical regions).

Abbreviations: OLF: Olfactory bulb, DMN: dorsal motor nucleus of the vagus nerve, RN: raphe nuclei, LC: locus coeruleus, SN: substantia nigra, NBM: nucleus basalis of Meynert, AMYG: amygdala, AntTmpMeso: Anterior temporal mesocortex, CA2/3; cornu ammonis regions 2 and 3, ENT: entorhinal cortex, CING: anterior cingulate cortex, INS: insular cortex, MFG: Middle Frontal Gyrus, MTG: Middle Temporal Gyrus, IPL: inferior parietal lobe, OCC: Occipital Lobe, calcarine cortex, 1 Motor/Sens: Primary motor and Sensory cortex. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

23 cases (16.3%) not conforming to the caudo–rostral progression of disease through six distinct stages. Instead, pathology was present in higher-staged foci without LP in any foci representing a lower stage. When we modified our criteria to require the presence of LP in all nuclei examined within a stage (as opposed to having α-synuclein immunopositive inclusions in at least one area of a stage), the number of cases conforming cases fell to 96 (68.1%).

Similar to our modified application of Braak staging, a significant proportion of LP cases (124, 87.9%) conformed to the Müller staging proposal, leaving 17 cases that failed to fit the model. The majority of these cases did not fit because the locus coeruleus, the sole brain area examined for stage 2 pathology in that staging system, did not exhibit LP. Although the Müller staging proposal sought to mirror the results of the gold standard Braak staging, discrepancies between the stage assigned by our adapted Braak staging versus the stage assigned by the Müller model existed in 93 (66.9%) of cases. (See Supplementary Material).

In contrast, 100% cases with LP were classifiable according to the Beach et al. staging system, and 2 cases failed to conform to the Leverenz et al. model (1.4% of LP cases). Unclassifiable cases from all systems are detailed in Supplementary Material. The Leverenz et al. system was able to classify significantly more LP cases than our modified Braak staging and Müller et al.'s system (McNemar's $\chi^2 = 17.6$ and 11.8 respectively, $p < 0.001$ for both). The Beach et al. system also was able to classify more cases than both our modified Braak system and Müller et al.'s system (McNemar's $\chi^2 = 23.0$, 17.0 respectively, $p < 0.001$ for each). These differences remained after amygdala-predominant cases were removed from analysis ($n = 6$) (McNemar's $\chi^2 = 23.00$, 8.1 respectively, $p < 0.001$ and 0.005 respectively). Our application of a modified Braak system classified similar numbers of cases to Müller et al.'s system (McNemar's $\chi^2 = 2.6$, $p = 0.11$). Similarly, the Beach et al. system classified similar numbers of cases to the Leverenz et al. system (McNemar's $\chi^2 = 2.0$, $p = 0.16$). Of the 29 cases that failed to fit one of the one staging systems, 15 failed to fit both our modified Braak staging system and the Müller system, 10 cases failed to fit our modified Braak staging system alone, 2 cases failed to fit Müller system alone, and 2 cases would have been considered 'Mixed' by the Leverenz system while being classifiable by the others (Table 3).

Table 3
Summary of classification systems.

	Modified Braak[a]	Strict Braak[b]	Müller et al.	Beach et al.	Leverenz et al.
Classifiable[c]					
LP n = 141	118 (83.7)	96 (68.1)	124 (87.9)	141 (100)	139 (98.6)
ILBD n = 109	87 (79.8)	66 (60.6)	93 (85.3)	109 (100)	107 (98.2)
DLB n = 8	8 (100)	8 (100)	8 (100)	8 (100)	8 (100)
PD n = 24	23 (95.8)	22 (91.7)	23 (95.8)	24 (100)	24 (100)
'Failure to Fit'[c]	23 (16.3)	45 (31.9)	17 (12.1)	0 (0)	2 (1.4)[d]

[a] n (%) of cases classified using our modified application of the staging system proposed by Braak et al., 2003. which required presence of pathology in *one* of the regions examined to satisfy a given stage.

[b] n (%) of cases classified using a strict application the staging system proposed by Braak et al., 2003 requiring presence of pathology in *all* regions examined to fully satisfy a given stage.

[c] n (percent of cases with LP, percent of total cases).

[d] These cases would be determined to be 'Mixed' in the criteria proposed by Leverenz et al., 2008.

All cases assessed fit at least two of the classification schemata. There was no significance in the likelihood of failing to fit at least one LP staging system when higher levels of tau and higher CERAD stages were present (i.e. Braak tau stage ≥ 2 and CERAD stage ≥ 2) ($\chi^2 = 2.5$, $p = 0.12$).

4. Discussion

Braak and colleagues were the first to suggest a staging system of LP in Parkinson's disease based on the caudal to rostral spread of LP in specific susceptible brain areas [1]. Such observations suggested the hypothesis, subsequently supported by cell and animal models, of cell to cell trans-synaptic spread of LP [20–22]. The validity of this pattern of spread in humans has been verified primarily in hospital based cohorts [8–10,16], but fewer studies in population based cohorts have been performed [23]. Some of these prior studies found that large percentages of cases were unclassifiable by the Braak staging system [9,16]. Such aberrant patterns of LP could be suggestive of alternative mechanisms of pathologic spread or alternative sites of inception; however, there are several possible alternative explanations including different features of the staging systems that were utilized and the populations that were examined.

In this current study, we examined a large cohort of patients from the Honolulu Asian Aging Study and applied four systems of classifying LP to determine the proportion of cases that fit the typical caudo-rostral progression of LP and to compare the ability of each system to classify cases. We analyzed cases using a modified form of the original Braak staging and the system applied by Müller et al. which are both attempts to recapitulate Braak's original work in standard neuropathological assessments. We also applied Leverenz et al.'s criteria which was developed for patients with dementia, and classifies cases into brainstem, limbic, neocortical, with a special designation for amygdala predominant cases [3] in a similar manner to McKeith et al.'s typing of LP [24]. We also applied Beach et al.'s criteria which also integrates severity of LP to make designations [4].

We found in this series that a large majority of cases with LP could be classified by any individual staging system that was applied. Furthermore, we found that many cases that 'fail to fit' one classification system could be adequately staged using others. We found significant discordance between the number of cases that could be classified by our application of the Braak et al. and Müller et al. schemata versus the Leverenz et al. and Beach et al. systems even after 'amygdala only' cases were removed from analysis. These findings raise the possibility that previously reported high numbers of 'fail to fit' cases could be classified if a different staging system was applied.

The original methods used in Braak et al., 2003 were highly sensitive and included brain regions not typically covered in modern standard autopsy sampling; consequently, several different staging systems utilizing standard neuropathologic sampling techniques have been subsequently developed. It is unclear which system is able to classify the most cases with the least inter-rater variability. In the BrainNet European (BNE) Consortium study [16] 31 cases of α-synuclein positive autopsies were assessed by 22 raters, and found the greatest absolute inter-rater agreement when the Leverenz et al. criteria were applied (82%), followed by Müller's system (78%), and Braak's original staging system (74%).

Since Braak et al.'s initial manuscript, other patterns of LP distribution have been appreciated including the so called 'amygdala only' LP pathologic distribution type which is strongly associated with co-occurring Alzheimer's disease pathology [5]. Patients with this distribution of LB pathology in the setting of high AD co-pathology are less likely to have a DLB or PD clinical phenotype and are more likely to have an Alzheimer's disease like presentation [24]. Indeed, LB pathology in Alzheimer's disease may follow a different pattern and may represent global proteostatic dysregulation in AD versus primary pathologic processes in PD and DLB [2,5,25]. The inclusion of these cases into studies before this distribution was recognized may contribute to higher numbers of 'fail to fit' cases in certain studies [23,24]. The frequency of incidental Lewy pathology in this cohort of aged Japanese-American men was 33.6%, with high variability in the distribution of pathology. ILBD cases with high Braak and Müller stages achieved these designations due to mild pathology that was topographically widespread as opposed to cases of DLB and PD which were uniformly high Braak and Müller stage and had higher severity of LP. Our findings show similar ILBD prevalence to other previously reported series [23,26]. Many studies have shown a correlation of LP with a variety of symptoms and stages of disease in PD and DLB [4,27,28] whereas others have shown widespread LP in non-demented PD patients and in neurologically healthy subjects [10,29,30]. One large study from Finland noted that 55% of 226 subjects with LP subjects with Braak synuclein stage 5 and 6 did not show signs of dementia or parkinsonism [10]. While neocortical LP is associated with dementia in PD [27,31], significant degrees of AD co-pathology is thought to occur in 10% of PD, 35% of PDD, and 70% of DLB [32–36] and this co-pathology also influences the occurrence of dementia and specific cognitive features therein [34,37–40]. We similarly observed a nearly significant association of higher tau stages with DLB cases which likely did not reach significance because of the small number of DLB cases. AD co-pathology was not associated with the presence of LP pathology or a greater likelihood of 'failing to fit' staging systems in this cohort.

There are limitations to the current study. While patients were screened in the HAAS cohort for the development of parkinsonism and other neurodegenerative illnesses, subtle findings could have been missed, or developed in between the last clinical exam and autopsy. There were cases where tissue was not available, but this occurred in a manner that would have potentially affected the fit designation only for one case in one staging system. The absence of 37 olfactory bulbs could have led to an underestimation of olfactory bulb only cases. We did not have access to Thal phases to fully assess Alzhiemer's disease neuropathologic change (ADNPC) [41]; however, we chose to analyze cases with Braak tau stage ≥ 2 and CERAD stage ≥ 2 to guarantee either intermediate or high level of ADNPC by that criteria [41]. In this population based cohort, there were relatively fewer numbers of PD and DLB patients, compared to ILBD which could affect our conclusions and validation in larger dedicated cohorts would be beneficial. Finally, the HAAS study is limited to Japanese-American men from Hawaii and genetic and environmental factors specific to this group may influence the occurrence and distribution of pathology and limit generalizability. While this type of neuropathological staging is probably unnecessary for routine diagnostic assessment, for research purposes, consideration

of utilizing either the Leverenz or Beach staging systems, that have fewer unclassifiable cases, is warranted. Given that there were few cases that failed to fit those staging systems, it is unlikely that further refinement of existing systems would lead to dramatic improvements in the number of cases classified, but when cases are identified that appear to be unclassifiable then applying an alternative staging system may be appropriate.

5. Conclusion

We found that in cases with LP from the HAAS cohort, there was high conformity to the caudo-rostral progression of LP described originally in Braak et al. in contrast to several prior studies [10,23]. PD and DLB cases had higher densities of LP pathology compared to ILBD where pathology was not severe, but was topographically widely distributed. There was significant discordance between the modified Braak and Müller staging schemes and the Leverenz and Beach staging schemes, indicating that the designation of a case as unclassifiable is often reliant on the staging system applied. Further studies may help to understand why some aged subjects with widespread LP may be asymptomatic.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.03.023.

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