

**TABLE 1.** Clinical features of patients with corticobasal syndrome during the entire disease course

Feature	CBD			PSP			AD			AT	No./total no. (%)
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	
Insidious onset	+	+	+	+	+	+	+	+	+	+	10/10 (100)
Gradual progression	+	+	+	+	+	+	+	+	+	+	10/10 (100)
Asymmetrical onset	+	+	+	+	+	+	-	+	+	+	9/10 (90)
Vertical gaze palsy	+	+	-	+	-	+	-	+	+	-	6/10 (60)
Limb apraxia	+	-	+	-	+	-	NE	+	+	+	6/9 (67)
Myoclonus	-	-	-	-	-	-	+	+	+	+	4/10 (40)
Alien limb syndrome	-	+	+	+	+	-	NE	-	-	-	4/9 (44)
Frontal signs	+	+	+	-	+	+	+	+	+	+	9/10 (90)
Aphasia	-	-	-	-	+	-	+	+	-	-	3/10 (30)
Dementia	+	+	+	+	+	+	+	+	+	-	9/10 (90)
Rigidity	+	+	+	+	+	+	+	+	+	+	10/10 (100)
Tremor	+	-	-	-	-	-	+	+	+	+	5/10 (50)
Dystonia	-	-	-	+	NE	+	-	-	-	-	2/9 (22)
Cerebellar ataxia	-	-	-	+	-	-	-	-	-	-	1/10 (10)
Upper and lower MN signs	-	-	-	-	-	-	-	-	-	+	1/10 (10)
Levodopa resistance	+	+	+	+	+	+	+	+	+	+	10/10 (100)
Asymmetry on MRI	+	+	-	+	-	+	-	-	+	-	5/10 (50)
Asymmetry on SPECT	+	+	-	+	NE	NE	NE	+	+	-	5/7 (71)
Mayo Clinic criteria	+	+	+	+	+	+	-	+	+	+	9/10 (90)
Modified Cambridge criteria	+	+	+	+	+	+	+	+	+	+	10/10 (100)

CBD, corticobasal degeneration; PSP, progressive supranuclear palsy; AD, Alzheimer's disease; AT, atypical tauopathy; +, sign present; -, sign absent; NE, not examined; MN, motor neuron; MRI, magnetic resonance imaging; SPECT, single photon emission computed tomography.

found a specific pattern of neurodegeneration in which neuronal loss was evident with microvacuolation in layers II and III of the primary motor and SMA cortices with no reference to underlying pathology (Fig. 1a-h). Neuronal loss with gliosis in the globus pallidus was moderate in patients with CBS-CBD (Fig. 1i), mild to severe in patients with CBS-PSP (Fig. 1j) and CBS-atypical tauopathy (Fig. 1l), but absent in patients with CBS-AD (Fig. 1k). Although free-melanin pigments with neuronal loss and gliosis in the substantia nigra were observed in patients with CBS-CBD (Fig. 1m), CBS-PSP (Fig. 1n), and CBS-atypical tauopathy (Fig. 1p), the overall pigmented neurons were well preserved in patients with CBS-AD (Fig. 1o). Moderate to severe neuronal loss with gliosis and tau pathology in the dentate nucleus were observed only in patients with CBS-PSP (Supporting Table 1).

### Frequency of Patients Satisfying CBS Clinical Criteria

The demographic features of all patients during the entire disease course are summarized in Table 1. All patients had an insidious onset and gradual progression, and none had a significant response to L-dopa. The most common features were rigidity (100% of patients) followed by frontal signs and dementia (90%). Dystonia and aphasia were not common even late in the disease course (22% and 30%, respectively). Although asymmetric atrophy of the cerebral cortex on MRI was observed in only five of the 10

patients (50%), asymmetric cerebral hypoperfusion on SPECT was observed in five of seven patients (71%).

Next, we investigated whether the 10 patients with CBS had satisfied Mayo Clinic or modified Cambridge criteria<sup>10,11</sup> within 2 years of disease onset (Table 2). Rigidity (50%) and limb apraxia (44%) were frequently observed. One patient developed cortical symptoms or signs, two patients developed extrapyramidal signs, five patients developed both, and two patients developed other symptoms. Only two patients fulfilled either set of criteria within 2 years of disease onset. Early clinical diagnosis included four patients with CBS, two with parkinsonism, one with Parkinson's or diffuse Lewy body disease, one with spinocerebellar degeneration, and one with progressive nonfluent aphasia. The final diagnoses included eight patients with CBS and two patients with CBS or PSP.

### Frequency of Patients Satisfying the Diagnostic Criteria for CBD

We also investigated whether the 10 patients with CBS had satisfied the diagnostic criteria for CBD<sup>12</sup> within 2 years of disease onset and during the entire disease course (Table 3). No patients with CBS-CBD fulfilled either set of criteria within 2 years of disease onset, whereas all three patients fulfilled them during the entire disease course. In contrast, one patient with CBS-PSP fulfilled the clinical criteria for possible CBD (p-CBD) during the entire disease course, and two patients with CBS-AD fulfilled the clinical research

**TABLE 2.** Clinical features of patients with corticobasal syndrome within 2 years of disease onset

Feature	CBD			PSP			AD		AT		No./total no. (%)
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	
Initial symptom	Clumsiness of R hand	Weakness of both hands	Clumsiness of L leg	Ataxic gait	Dysarthria	Slow movement	Small-stepped gait	Difficulty in writing, dressing	Dysarthria, tremor, limb apraxia	Tremulous movement of L hand	
Fall	-	-	+	+	-	-	-	-	-	-	2/10 (20)
Vertical gaze palsy	-	-	-	+	-	+	-	+	+	-	4/10 (40)
Limb apraxia	+	-	-	-	+	-	NE	+	+	-	4/9 (44)
Myoclonus	-	-	-	-	-	-	-	-	+	-	1/10 (10)
Alien limb syndrome	-	-	-	+	+	-	NE	-	-	-	2/9 (22)
Frontal signs	-	-	-	-	-	+	-	+	+	-	3/10 (30)
Aphasia	-	-	-	-	+	-	-	+	-	-	2/10 (20)
Dementia	-	-	-	+	-	+	-	+	+	-	4/10 (40)
Rigidity	-	-	+	+	-	+	-	+	-	+	5/10 (50)
Tremor	+	-	-	-	-	-	-	+	+	+	4/10 (40)
Cerebellar ataxia	-	-	-	+	-	-	-	-	-	-	1/10 (10)
Other symptoms			Spasticity, early fall	Apraxia of lid opening		Gait freezing		Memory impairment		Upper and lower MN signs	
Early clinical diagnosis	NA	Parkinsonism	Parkinsonism	SCD	PNFA	CBS	PD or DLB	CBS	CBS	CBS	
Final clinical diagnosis	CBS	CBS or PSP	CBS or PSP	CBS	CBS	CBS	CBS	CBS	CBS	CBS	
Mayo Clinic criteria	-	-	-	-	-	-	-	+	-	-	1/10 (10)
Modified Cambridge criteria	-	-	-	-	-	-	-	-	+	-	1/10 (10)

CBD, corticobasal degeneration; PSP, progressive supranuclear palsy; AD, Alzheimer's disease; AT, atypical tauopathy; R, right; L, left; -, sign absent; +, sign present; NE, not examined; MN, motor neuron; NA, not available; SCD, spinocerebellar degeneration; PNFA, progressive non-fluent aphasia; CBS, corticobasal syndrome; PD, Parkinson disease; DLB, dementia with Lewy body; PSP, progressive supranuclear palsy.

criteria for either probable sporadic CBD (cr-CBD) or p-CBD within 2 years of disease onset.

### Characteristic Clinical Features Predicting Background Pathologies

We compared the clinical features among patients who had CBS with different pathologies (see Tables 2 and 3). Although we could not find any characteristic clinical features that were specific to CBD pathology, only patients who had PSP developed apraxia of eyelid opening, cerebellar ataxia, and dystonia. All three patients with AD pathology developed myoclonus of the extremities, whereas patients with CBD and PSP did not. Only one patient with AD pathology had a symmetric onset, and another patient with AD pathology developed memory impairment at an early stage of the disease. Only one patient classified with atypical four-repeat tauopathy had upper and lower motor neuron signs without dementia.

### Discussion

The present study has demonstrated several novel findings with regard to CBS. First, this study has con-

firmed, for the first time, a wide spectrum of pathological backgrounds in Japanese patients with CBS. We have demonstrated that the most frequent causes of CBS were CBD, PSP, and AD, consistent with previous reports from Western countries. Boeve et al. reported that the most common pathologies of CBS were CBD (18 of 34 patients; 52.9%), PSP (six of 34 patients; 17.6%), and AD (three of 34 patients; 8.8%)<sup>10</sup>; whereas Ling et al. reported that the most common pathologies were PSP (six of 21 patients; 28.6%), CBD (five of 21 patients; 23.8%), AD (five of 21 patients; 23.8%), and FTLT-TDP (two of 21 patients; 9.5%).<sup>19</sup> Lee et al. reported that the most common pathologies were CBD (14 of 40 patients; 35.0%), AD (nine of 40 patients; 22.5%), PSP (five of 40 patients; 12.5%), and FTLT-TDP (five of 40 patients; 12.5%).<sup>20</sup> Because our cohort was small and did not include patients who had CBS with FTLT-TDP or Pick's disease, further analysis of more patients with CBS may be required to determine the pathological backgrounds of Japanese patients with CBS. In addition, our study confirms that all included patients with CBS shared a common topographic distribution of neurodegeneration, which was maximal in the frontal and parietal cortical regions, especially in

**TABLE 3.** Criteria for the diagnosis of corticobasal degeneration

Variable	CBD			PSP			AD		AT	No./total no. (%)	
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9		Patient 10
Diagnosis (within 2 y) Syndrome	None	None	None	None	None	None	None	cr-CBD	p-CBD	None	
Probable CBS	-	-	-	-	-	-	-	-	-	-	0/10 (0)
Possible CBS	-	-	-	+	-	-	-	+	+	-	3/10 (30)
FBS	-	-	-	-	-	-	-	+	-	-	1/10 (10)
NAV	-	-	-	-	-	-	-	-	-	-	0/10 (0)
PSPS	-	-	-	-	-	-	-	-	-	-	0/10 (0)
Exclusion criteria	-	-	-	Prominent cerebellar signs	-	-	-	-	-	-	
Diagnosis (entire disease course) Syndrome	p-CBD	p-CBD	p-CBD	None	p-CBD	None	None	cr-CBD	p-CBD	None	
Probable CBS	-	-	-	-	-	-	-	-	-	-	0/10 (0)
Possible CBS	+	+	+	+	+	-	-	+	+	+	8/10 (80)
FBS	-	-	-	-	-	-	-	+	-	-	1/10 (10)
NAV	-	-	-	-	-	-	-	-	-	-	0/10 (0)
PSPS	-	-	-	-	-	-	-	-	+	-	1/10 (10)
Exclusion criteria	-	-	-	Prominent cerebellar signs	-	-	-	-	-	MN signs	

CBD, corticobasal degeneration; PSP, progressive supranuclear palsy; AD, Alzheimer's disease; AT, atypical tauopathy; cr-CBD, clinical research criteria for probable sporadic corticobasal degeneration; p-CBD, clinical criteria for possible corticobasal degeneration; CBS, corticobasal syndrome; -, did not fulfill the diagnostic criteria; +, fulfilled the diagnostic criteria; FBS, frontal behavioral-spatial syndrome; NAV, nonfluent/aromatic variant of primary progressive aphasia; PSPS, progressive supranuclear palsy syndrome; MN, motor neuron.

the primary motor cortex and SMA, and our findings reveal that CBS occurred in the absence of basal ganglia and nigral degeneration, as previously reported in Western populations.<sup>9</sup> This raises the possibility that dysfunction of the primary motor cortex and SMA could cause extrapyramidal symptoms in CBS, because both have been shown to play roles in voluntary muscle relaxation as well as muscle contraction.<sup>21-23</sup>

Second, we have also demonstrated that the sensitivity of the available clinical criteria for CBS is poor for classifying CBS within 2 years of disease onset. Although five patients developed symptoms or signs of both the cerebral cortex (cortical sensory motor symptoms/cognitive symptoms) and the extrapyramidal system (motor features) within 2 years, only two patients fulfilled the clinical criteria from the Mayo Clinic or Cambridge.<sup>10,11</sup> Mathew et al. also demonstrated that available criteria could be applied equally well in later disease stages, but not in the earlier stages.<sup>11</sup> Because available clinical criteria were established on the basis of clinical experience by experts in the field, future prospective studies need to be performed to determine natural history and clinicopathological correlations for the establishment of sensitive clinical criteria. With regard to the diagnostic criteria for CBD, we observed that their sensitivity within the first 2 years after disease onset may be low and that patients without CBD

pathology can fulfill the cr-CBD criteria. Further studies are required to determine the sensitivity and specificity of these criteria.

Third, despite the small number of patients studied, our results suggest that several clinical features may be helpful in predicting the pathological backgrounds of patients with CBS. The present study has demonstrated that only patients with CBS-PSP developed apraxia of eyelid opening, cerebellar ataxia, and dystonia; that all patients with CBS-AD had myoclonus; and that only one patient with CBS-AD had a symmetric onset. It has been demonstrated that apraxia of eyelid opening is a frequently observed ophthalmologic feature in PSP but not in CBD or AD.<sup>24</sup> Cerebellar ataxia also may indicate CBS-PSP; recent studies have indicated that patients with PSP, but not patients with CBD or AD, may develop cerebellar ataxia as the initial and principal symptom.<sup>25,26</sup> In contrast, it is not believed that dystonia can predict CBS-PSP, because dystonia is also observed in CBS-CBD<sup>27</sup> and CBS-AD.<sup>28</sup> Myoclonus and memory impairment, especially when they appear at an early stage of the disease, may predict CBS-AD according to a previous report.<sup>4,5</sup> However, symmetric CBS might not predict CBS-AD, because symmetric CBS is also observed in CBS-CBD<sup>29</sup> and in CBS caused by progranulin mutation.<sup>30</sup> Early diagnosis of CBS-CBD is still difficult,

because, to date, no characteristic features have been identified that can predict CBD pathology. However, our sample size was small, and we could not perform statistical analyses. In addition, studies with large sample sizes have proposed contrasting predictive clinical characteristics.<sup>19,31,32</sup> Future analyses of more patients may be required to draw more definitive conclusions.

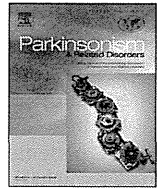
Finally, in our CBS cohort, there was a patient with atypical four-repeat tauopathy that did not satisfy the pathological diagnostic criteria for CBD or PSP despite the presence of neurodegeneration with tau-positive neuronal and glial cytoplasmic inclusions, as previously reported.<sup>17,18</sup> Clinical features in this patient were characterized by sporadic parkinsonism and motor neuron disease without dementia, and pathological features were four-repeat tauopathy with unique tau pathology: the astrocytic tau lesions were different in morphology from astrocytic plaques and tufted astrocytes, which are characteristic of CBD and PSP, respectively. Although the disease entity has not been established, this type of atypical four-repeat tauopathy may be considered a differential diagnosis for CBS.

In conclusion, we have established the wide spectrum of CBS clinicopathological manifestations in Japanese patients. We also have demonstrated that the sensitivity of the available clinical criteria for CBS and CBD was poor for detecting the disease within the first 2 years of onset. ■

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## References

- Mizuno T, Shiga K, Nakata Y, et al. Discrepancy between clinical and pathological diagnoses of CBD and PSP. *J Neurol* 2005;252:687-697.
- Tsuboi Y, Josephs KA, Boeve BF, et al. Increased tau burden in the cortices of progressive supranuclear palsy presenting with corticobasal syndrome. *Mov Disord* 2005;20:982-988.
- Chand P, Grafman J, Dickson D, Ishizawa K, Litvan I. Alzheimer's disease presenting as corticobasal syndrome. *Mov Disord* 2006;21:2018-2022.
- Hu WT, Rippon GW, Boeve BF, et al. Alzheimer's disease and corticobasal degeneration presenting as corticobasal syndrome. *Mov Disord* 2009;24:1375-1379.
- Shelley BP, Hodges JR, Kipps CM, Xuereb JH, Bak TH. Is the pathology of corticobasal syndrome predictable in life? *Mov Disord* 2009;24:1593-1599.
- Doran M, du Plessis DG, Enevoldson TP, Fletcher NA, Ghadiali E, Larner AJ. Pathological heterogeneity of clinically diagnosed corticobasal degeneration. *J Neurol Sci* 2003;216:127-134.
- Lang AE, Bergeron C, Pollanen MS, Ashby P. Parietal Pick's disease mimicking cortical-basal ganglionic degeneration. *Neurology* 1994;44:1436-1440.
- Tartaglia MC, Sidhu M, Laluz V, et al. Sporadic corticobasal syndrome due to FTL-D2P. *Acta Neuropathol* 2010;119:365-374.
- Boeve BF, Maraganore DM, Parisi JE, et al. Pathologic heterogeneity in clinically diagnosed corticobasal degeneration. *Neurology* 1999;53:795-800.
- Boeve BF, Lang AE, Litvan I. Corticobasal degeneration and its relationship to progressive supranuclear palsy and frontotemporal dementia. *Ann Neurol* 2003;54(suppl 5):S15-S19.
- Mathew R, Bak TH, Hodges JR. Diagnostic criteria for corticobasal syndrome: a comparative study. *J Neurol Neurosurg Psychiatry* 2012;83:405-410.
- Armstrong MJ, Litvan I, Lang AE, et al. Criteria for the diagnosis of corticobasal degeneration. *Neurology* 2013;80:496-503.
- Whitwell JL, Jack CR Jr, Parisi JE, et al. Imaging signatures of molecular pathology in behavioral variant frontotemporal dementia. *J Mol Neurosci* 2011;45:372-378.
- Dickson DW, Bergeron C, Chin SS, et al. Office of Rare Diseases neuropathologic criteria for corticobasal degeneration. *J Neuropathol Exp Neurol* 2002;61:935-946.
- Litvan I, Hauw JJ, Bartko JJ, et al. Validity and reliability of the preliminary NINDS neuropathologic criteria for progressive supranuclear palsy and related disorders. *J Neuropathol Exp Neurol* 1996;55:97-105.
- Hyman BT, Trojanowski JQ. Consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. *J Neuropathol Exp Neurol* 1997;56:1095-1097.
- Fu YJ, Nishihira Y, Kuroda S, et al. Sporadic four-repeat tauopathy with frontotemporal lobar degeneration, parkinsonism, and motor neuron disease: a distinct clinicopathological and biochemical disease entity. *Acta Neuropathol* 2010;120:21-32.
- Piao YS, Tan CF, Iwanaga K, et al. Sporadic four-repeat tauopathy with frontotemporal degeneration, parkinsonism and motor neuron disease. *Acta Neuropathol* 2005;110:600-609.
- Ling H, O'Sullivan SS, Holton JL, et al. Does corticobasal degeneration exist? A clinicopathological re-evaluation. *Brain* 2010;133:2045-2057.
- Lee SE, Rabinovici GD, Mayo MC, et al. Clinicopathological correlations in corticobasal degeneration. *Ann Neurol* 2011;70:327-340.
- Buccolieri A, Abbruzzese G, Rothwell JC. Relaxation from a voluntary contraction is preceded by increased excitability of motor cortical inhibitory circuits. *J Physiol* 2004;558:685-695.
- Terada K, Ikeda A, Yazawa S, Nagamine T, Shibasaki H. Movement-related cortical potentials associated with voluntary relaxation of foot muscles. *Clin Neurophysiol* 1999;110:397-403.
- Toma K, Honda M, Hanakawa T, et al. Activities of the primary and supplementary motor areas increase in preparation and execution of voluntary muscle relaxation: an event-related fMRI study. *J Neurosci* 1999;19:3527-3534.
- Lamberti P, De Mari M, Zenzola A, Aniello MS, Defazio G. Frequency of apraxia of eyelid opening in the general population and in patients with extrapyramidal disorders. *Neurol Sci* 2002;23(suppl 2):S81-s82.
- Iwasaki Y, Mori K, Ito M, Tatsumi S, Mimuro M, Yoshida M. An autopsy case of progressive supranuclear palsy presenting with cerebellar ataxia and severe cerebellar involvement. *Neuropathology* 2013;33:561-567.
- Kanazawa M, Shimohata T, Toyoshima Y, et al. Cerebellar involvement in progressive supranuclear palsy: a clinicopathological study. *Mov Disord* 2009;24:1312-1318.
- Stamelou M, Alonso-Canovas A, Bhatia KP. Dystonia in corticobasal degeneration: a review of the literature on 404 pathologically proven cases. *Mov Disord* 2012;27:696-702.
- Imamura A, Wszolek ZK, Lucas JA, Dickson DW. Corticobasal syndrome with Alzheimer's disease pathology. *Mov Disord* 2009;24:152-153; author reply 153.
- Hassan A, Whitwell JL, Boeve BF, Jack CR Jr, Parisi JE, Dickson DW, Josephs KA. Symmetric corticobasal degeneration (S-CBD). *Parkinsonism Relat Disord* 2010;16:208-214.
- Dopper EG, Seelaar H, Chiu WZ, et al. Symmetrical corticobasal syndrome caused by a novel C.314dup progranulin mutation. *J Mol Neurosci* 2011;45:354-358.
- Kouri N, Murray ME, Hassan A, et al. Neuropathological features of corticobasal degeneration presenting as corticobasal syndrome or Richardson syndrome. *Brain* 2011;134:3264-3275.
- Litvan I, Grimes DA, Lang AE, et al. Clinical features differentiating patients with postmortem confirmed progressive supranuclear palsy and corticobasal degeneration. *J Neurol* 1999;246(suppl 2):II1-II5.



## Short communication

## Early clinical features of patients with progressive supranuclear palsy with predominant cerebellar ataxia



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## ABSTRACT

**Background:** Patients who develop progressive supranuclear palsy with predominant cerebellar ataxia (PSP-C) develop cerebellar ataxia as the initial and principal symptom, may be misdiagnosed as having multiple system atrophy with predominant cerebellar features (MSA-C). Therefore, we investigated the clinical signs and symptoms between PSP-C and MSA-C early in their disease course.

**Methods:** We reviewed the medical records of 15 consecutive patients with pathologically proven PSP-C (4) and MSA-C (11). We recorded the presence or absence of clinical features that developed within 2 years of disease onset.

**Results:** The age at onset of PSP-C patients was older than that of MSA-C patients ( $p = 0.009$ ). The frequencies of falls were higher in PSP-C patients than in MSA-C patients ( $p = 0.026$ ). Additionally, the development of supranuclear vertical gaze palsy was higher in PSP-C patients than in MSA-C patients ( $p = 0.011$ ), whereas the frequency of dysautonomia was lower in PSP-C patients than in MSA-C patients ( $p = 0.035$ ).

**Conclusions:** Older onset, early falls, and supranuclear vertical gaze palsy without dysautonomia may predict the diagnosis of PSP-C in patients with late-onset sporadic cerebellar ataxia.

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Several clinical variants of progressive supranuclear palsy (PSP) have been identified [1,2]. Although cerebellar ataxia is one of the exclusion criteria of the National Institute of Neurological Disorders and Stroke and the Society for Progressive Supranuclear Palsy (NINDS-SPSP) [3], we have previously reported patients with pathologically confirmed PSP who developed truncal and limb ataxia as their initial and principal symptom, which were distinct from unsteadiness due to postural instability observed in Richardson syndrome [4]. Similar PSP patients have been reported from different Japanese groups and Brazil [5,1]. Although the cerebellar variant of PSP is very rare in Europe [6], the original report on PSP described four of nine PSP patients who developed truncal and limb ataxia and one of the nine patients who developed truncal ataxia as an initial and principal symptom [7]. The

pathology of PSP patients with cerebellar ataxia revealed more severe neuronal loss with gliosis and higher densities of coiled bodies in the cerebellar dentate nucleus compared to Richardson syndrome [4–7]. Additionally, these patients revealed tau-positive inclusion bodies in Purkinje cells and mild neuronal loss of Purkinje cells. On the basis of pathological examinations of PSP patients with cerebellar ataxia by us and others, we speculated that patients with tau-positive inclusion bodies in Purkinje cells and severely degenerated dentate nucleus can develop cerebellar ataxia [4–7]. Therefore, we proposed a new variant of PSP with truncal and limb ataxia as the initial and principal symptom, called PSP with predominant cerebellar ataxia (PSP-C) [4,8].

Although all patients with PSP-C developed the cardinal features of PSP during the course of their disease [4], it is possible that they might be clinically misdiagnosed as having multiple system atrophy with predominant cerebellar features (MSA-C) or other spinocerebellar degenerations as the result of presenting with cerebellar ataxia as the initial and principal symptom early in their disease course. In most Western clinicopathological PSP series, no patient with PSP-C has been reported [6]. In contrast, cerebellar features may be more common in Japanese PSP than in Western

**Abbreviations:** MSA-C, multiple system atrophy with predominant cerebellar features; NINDS-SPSP, National Institute for Neurological Diseases and Stroke – the Society for Progressive Supranuclear Palsy; PSP, progressive supranuclear palsy; PSP-C, progressive supranuclear palsy with predominant cerebellar ataxia.

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PSP like MSA [9]. The antemortem differential diagnosis between patients with PSP-C and those with MSA-C is required to predict their clinical course and prognosis and to realize future therapeutic intervention in Japanese patients.

We hypothesized that PSP-C patients can be differentiated from MSA-C patients on the basis of these signs and symptoms clinically even early in their disease course. Because a specific biomarker has not been yet found for use in PSP, signs and symptoms are very important for its diagnosis. Early falls have been regarded as a clinical hallmark of PSP since the first clinicopathological report of PSP [7]. Indeed, NINDS-SPSP diagnostic criteria require falls occurring within the first year of disease onset for a diagnosis of probable PSP [3]. In contrast, early falling is atypical in MSA patients [10,11]. Dysautonomia has been one of the cardinal features of MSA, but not of PSP [3,10]. In the present study, we compared the signs and symptoms between pathologically proven PSP-C and MSA-C patients.

### 1. Patients and methods

We retrospectively reviewed the clinical records of consecutive, hospitalized Japanese patients with pathologically proven PSP-C and MSA-C, from 1978 to 2011. We reviewed the clinical records to determine the age at onset, disease duration, gender, initial symptoms, and clinical features such as supranuclear vertical gaze palsy, gait disturbance, falls, postural instability, cognitive decline, asymmetric onset, and tremor of any type that developed within 2 years after disease onset. We defined cerebellar ataxia as the presence of both limb and truncal ataxia. We also determined the presence of dysautonomia [10] and cerebral cortical signs [4]. Other clinical symptoms and signs were defined as previously described [4]. In addition, we reviewed magnetic resonance imaging (MRI) findings of patients on whom MRI studies were performed within 2 years of disease onset. Parametric data were expressed as mean  $\pm$  standard deviation and analyzed using Student's *t*-test. Comparisons of the differences in clinical features were performed using Fisher's test. Statistical analyses were performed using IBM SPSS (ver. 21.0). A *p* value < 0.05 was considered statistically significant.

### 2. Results

We reviewed the medical records of four consecutive Japanese patients with pathologically proven PSP-C as well as 11 consecutive Japanese patients with pathologically proven MSA-C; the cases of 3 PSP-C and 11 MSA-C patients have been reported previously [4,9]. The age at onset of PSP-C patients ( $68.8 \pm 4.4$  years) was older than that of MSA-C patients (Table 1) ( $58.3 \pm 7.4$  years; *p* = 0.009). There were no significant differences in the initial symptoms between PSP-C patients and MSA-C patients (Table 2). All the PSP-C patients developed cerebellar ataxia and experienced falls (4/4, 100%), and three of them developed supranuclear vertical gaze palsy and postural instability (3/4, 75%) within 2 years after the disease onset

**Table 1**

Clinical features of PSP-C and MSA-C patients, which developed within 2 years of disease onset. MSA-C, multiple system atrophy with predominant cerebellar features; PSP-C, progressive supranuclear palsy with cerebellar ataxia. Bold: *p* < 0.05.

	PSP-C	MSA-C	<i>p</i> value
% of male	50.0 (2/4)	72.3 (8/11)	0.407
<b>Age at onset (years)</b>	<b>68.8 <math>\pm</math> 4.4</b>	<b>58.3 <math>\pm</math> 7.4</b>	<b>0.009</b>
Disease duration (years)	7.0 $\pm$ 5.4	7.0 $\pm$ 2.1	0.761
Asymmetric onset	75.0% (3/4)	20.0% (2/10)	0.670
Cerebellar ataxia	100% (4/4)	100% (10/10)	(–)
<b>Supranuclear vertical gaze palsy</b>	<b>75.0% (3/4)</b>	<b>0% (0/10)</b>	<b>0.011</b>
Gait disturbance	75.0% (3/4)	90.0% (9/10)	0.505
<b>Falls</b>	<b>100% (4/4)</b>	<b>27.3% (3/11)</b>	<b>0.026</b>
Postural instability	50.0% (2/4)	0% (0/4)	0.214
Rigidity	33.3% (1/3)	16.7% (1/6)	0.583
Tremor	0% (0/4)	28.6% (2/7)	0.382
Cognitive decline	25.0% (1/4)	0% (0/10)	0.286
Cerebral cortical signs	25.0% (1/4)	0% (0/10)	0.286
<b>Dysautonomia</b>	<b>0% (0/3)</b>	<b>90.0% (9/10)</b>	<b>0.035</b>

**Table 2**

Initial symptoms in patients with PSP-C and MSA-C.

	PSP-C	MSA-C	<i>p</i> value
Wobbling	75.0% (3/4)	54.5% (6/11)	0.604
Fall	25.0% (1/4)	27.3% (3/11)	0.725
Urinary disturbance	0% (0/4)	18.2% (2/11)	0.524
Lightheadedness upon standing	0% (0/4)	9.1% (1/11)	0.733

(Table 1). In contrast, in MSA-C patients, ten of the ten patients (100%) developed cerebellar ataxia, three of the 11 patients (27.3%) experienced falls, and none of the patients developed supranuclear vertical gaze palsy and postural instability within 2 years of disease onset. The frequencies of falls were higher in PSP-C patients than in MSA-C patients within 2 years of disease onset (*p* = 0.026). Additionally, The development of supranuclear vertical gaze palsy was higher in PSP-C patients than in MSA-C patients within 2 years of disease onset (*p* = 0.011). The frequency of dysautonomia in MSA-C was higher in MSA-C patients than in PSP-C patients (*p* = 0.035). There was no significant difference in the presence of gait disturbance, rigidity, tremor, cognitive decline, or asymmetric onset between PSP-C and MSA-C patients.

The two PSP-C patients, who underwent brain MRI, showed no hot cross bun sign or putaminal slit sign [12], although one PSP-C patient underwent brain MRI twice, as previously reported [8]. They also showed no obvious atrophy of the cerebellum within 2 years of disease onset. None of the MSA-C patients underwent MRI studies within 2 years of disease onset, because most of them were hospitalized before the 1990s.

### 3. Discussion

Our study demonstrated that early falls and supranuclear vertical gaze palsy were significantly more frequent in PSP-C patients than in MSA-C patients within 2 years of disease onset. In addition, the age at onset of PSP-C patients was higher than that of MSA-C patients, whereas dysautonomia was less frequent in PSP-C patients than in MSA-C patients. Taken together, the early falls and supranuclear vertical gaze palsy without dysautonomia are considered important features that can differentiate PSP-C from MSA-C.

We demonstrated that clinical dissimilarities between PSP and MSA might be helpful in differentiating PSP-C from MSA-C. It has been demonstrated that the age at onset of PSP is significantly older than that of MSA [13]. Early falling is one of the cardinal features of PSP [3], although it is uncommon in MSA [10]. The median time to first fall from disease onset is significantly shorter in classical PSP, Richardson syndrome (mean 12 months; range 0–95 months), than in MSA (mean 42 months; 0–165 months) [11]. Additionally, supranuclear vertical gaze palsy is frequent in PSP patients, but not in MSA patients, although gaze-evoked nystagmus and abnormal eye pursuit movements are discriminative in MSA patients [13]. Moreover, dysautonomia is one of the cardinal features of MSA [9,10], although it is one of the mandatory exclusion criteria in NINDS-SPSP [3]. These dissimilarities between PSP and MSA might be applicable to PSP-C and MSA-C as well. That is, PSP-C patients might show cardinal features of PSP even early in their disease course.

On the other hand, MRI findings of PSP-C patients showed no obvious atrophy of the cerebellum, hot cross bun sign, or putaminal slit signs, which are characteristics of MSA-C that develop within 2 years of disease onset. A recent study also reported an autopsied case of PSP-C who had not developed hot cross bun sign or putaminal slit sign during the course of his disease [5]. Although we were review MRI findings in our MSA-C patients obtained within 2 years of disease onset, Horimoto et al. reported that MSA-C patients exhibited the 'partial' hot cross bun sign, which is a vertical line

without a horizontal line, within 2 or 3 years of disease onset, and the 'complete' hot cross bun sign, which is the presence of both the horizontal line and the vertical line, within 5 years [12], and that they exhibited no bilateral or a unilateral putaminal slit sign within 4 years of disease onset. Taken together, these findings raise the possibility that conventional MRIs can not reliably distinguish between PSP-C and MSA-C early in the disease course.

In conclusion, older onset, early falls, and supranuclear vertical gaze palsy without dysautonomia predict the diagnosis of PSP-C in patients with late-onset sporadic cerebellar ataxia.

## Disclosure

The authors report no conflicts of interest.

## References

- [1] Barsottini OG, Felício AC, Aquino CC, Pedroso JL. Progressive supranuclear palsy: new concepts. *Arq Neuropsiquiatr* 2010;68:938–46.
- [2] Srulijes K, Mallien G, Bauer S, Dietzel E, Gröger A, Ebersbach G, et al. In vivo comparison of Richardson's syndrome and progressive supranuclear palsy-parkinsonism. *J Neural Transm* 2011;118:1191–7.
- [3] Litvan I, Agid Y, Calne D, Campbell G, Dubois B, Duvoisin RC, et al. Clinical research criteria for the diagnosis of progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome): report of the NINDS-SPSP international workshop. *Neurology* 1996;47:1–9.
- [4] Kanazawa M, Shimohata T, Toyoshima Y, Tada M, Kakita A, Morita T, et al. Cerebellar involvement in progressive supranuclear palsy: a clinicopathological study. *Mov Disord* 2009;24:1312–8.
- [5] Iwasaki Y, Mori K, Ito M, Tatsumi S, Mimuro M, Yoshida M. An autopsied case of progressive supranuclear palsy presenting with cerebellar ataxia and severe cerebellar involvement. *Neuropathology* 2013 published online on Jan 16.
- [6] Jellinger K. Cerebellar involvement in progressive supranuclear palsy. *Mov Disord* 2010;25:1104–5.
- [7] Steele JC, Richardson JC, Olszewski J. Progressive supranuclear palsy – a heterogeneous degeneration involving the brain stem, basal ganglia and cerebellum, with vertical gaze and pseudobulbar palsy, nuchal dystonia and dementia. *Arch Neurol* 1964;10:333–59.
- [8] Kanazawa M, Shimohata T, Endo K, Koike R, Takahashi H, Nishizawa M. A serial MRI study in a patient with progressive supranuclear palsy with cerebellar ataxia. *Parkinsonism Relat Disord* 2012;18:677–9.
- [9] Tada M, Onodera O, Tada M, Ozawa T, Piao YS, Kakita A, et al. Early development of autonomic dysfunction may predict poor prognosis in patients with multiple system atrophy. *Arch Neurol* 2007;64:256–60.
- [10] Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, et al. Second consensus statement on the diagnosis of multiple system atrophy. *Neurology* 2008;71:670–6.
- [11] Williams DR, Watt HC, Lees AJ. Predictors of falls and fractures in bradykinetic rigid syndromes: a retrospective study. *J Neurol Neurosurg Psychiatry* 2006;77:468–73.
- [12] Horimoto Y, Aiba I, Yasuda T, Ohkawa Y, Katayama T, Yokokawa Y, et al. Longitudinal MRI study of multiple system atrophy – when do the findings appear, and what is the course? *J Neurol* 2002;249:847–54.
- [13] Litvan I, Campbell G, Mangone CA, Verny M, McKee A, Chaudhuri KR, et al. Which clinical features differentiate progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome) from related disorders? A clinicopathological study. *Brain* 1997;120:65–74.

## Original Article

Neuropathologic analysis of Lewy-related  $\alpha$ -synucleinopathy in olfactory mucosa

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We analyzed the incidence and extent of Lewy-related  $\alpha$ -synucleinopathy (LBAS) in the olfactory mucosa, as well as the central and peripheral nervous systems of consecutive autopsy cases from a general geriatric hospital. The brain and olfactory mucosa were immunohistochemically examined using antibodies raised against phosphorylated  $\alpha$ -synuclein. Thirty-nine out of 105 patients (37.1%) showed LBAS in the central or peripheral nervous systems. Seven patients presented LBAS (Lewy neurites) in the olfactory lamina propria mucosa. One out of the seven cases also showed a Lewy neurite in a bundle of axons in the cribriform plate, but  $\alpha$ -synuclein deposits were not detected in the olfactory receptor neurons. In particular, high incidence of  $\alpha$ -synuclein immunopositive LBAS in the olfactory mucosa was present in the individuals with clinically as well as neuropathologically confirmed Parkinson's disease and dementia with Lewy bodies (6/8 cases, 75%). However, this pathologic alteration was rare in the cases with incidental or subclinical Lewy body diseases (LBD) (one out of 31 cases, 3.2%). In the olfactory bulb, the LBAS was usually present in the glomeruli and granular cells of most symptomatic and asymptomatic cases with LBD. Our studies further confirmed importance of the olfactory entry zone in propagation of LBAS in the human aging nervous system.

**Key words:**  $\alpha$ -synuclein, Lewy body, neuropathology, olfactory mucosa, Parkinson's disease.

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## INTRODUCTION

Sporadic Parkinson's disease is a neurodegenerative disorder characterized clinically by resting tremor, rigidity, bradykinesia and gait disturbance, as well as neuropathologically by the loss of neurons in several brainstem nuclei and the presence of Lewy bodies formed by abnormal accumulation of  $\alpha$ -synuclein.<sup>1–5</sup> Of the many types of neurons in the central and peripheral nervous systems, a specific subset of neurons is vulnerable to accumulation of  $\alpha$ -synuclein, which takes the form of aggregates such as Lewy bodies and Lewy neurites (LBs/LNs).<sup>6–8</sup>

Based on studies of a large number of autopsy cases, the initial sites involved in Lewy-related pathology are reported to be the dorsal motor nucleus of the vagus, the intermediate reticular zone in the lower brainstem and olfactory bulb.<sup>9,10</sup> We previously reported that in the earliest stage of Lewy-related  $\alpha$ -synucleinopathy (LBAS), abnormal  $\alpha$ -synuclein accumulation extends from the peripheral part of the olfactory bulb to the anterior olfactory nucleus as well as the amygdala.<sup>11</sup> From a clinical standpoint, impaired olfactory function constitutes one of the earliest symptoms of sporadic Parkinson's disease.<sup>12,13</sup> Therefore, the olfactory system may be one of the vital regions in the development of Lewy body disease (LBD).

In the olfactory bulb,  $\alpha$ -synuclein accumulation is observed in the anterior olfactory nucleus as well as the mitral, tufted, and granular cells of individuals with clinical Parkinson's disease or dementia with Lewy bodies (DLB). Even in the early stages of these diseases, LNs, LBs or both, can be seen in the olfactory bulbs.<sup>11,14,15</sup> Based on the results of a neuropathologic study, Beach *et al.* suggested that the olfactory bulb may be a candidate region of biopsy study to



confirm the diagnosis of LBD.<sup>16</sup> However, the biopsy of olfactory bulb is too invasive and difficult to carry out for patients without risk.<sup>17,18</sup>

The olfactory epithelium is composed of paraneurons and neurites from which the glomeruli of the olfactory bulb originate. However, a neuropathologic analysis of LBAS has not been carried out adequately for LBD. Duda *et al.* reported that normal  $\alpha$ -synuclein is expressed in the basal cells, olfactory receptor neurons, supporting cells, and Bowman's glands of the olfactory epithelium in normal controls, as well as patients with Parkinson's disease, Alzheimer disease and multiple system atrophy.<sup>19</sup> However, pathologic  $\alpha$ -synuclein accumulation is rare (3.7%) among both normal controls and individuals affected by DLB, Alzheimer disease or Parkinson's disease.<sup>20</sup> According to a biopsy study of the olfactory epithelium in individuals with Parkinson's disease and younger hyposmic controls, no specific pathologic alteration was found.<sup>21</sup>

Therefore, it is still controversial whether abnormal  $\alpha$ -synuclein accumulation in the olfactory epithelium precedes the formation of LBs/LNs in the olfactory bulb and contributes to olfactory dysfunction in sporadic Parkinson's disease. The aim of this study was to clarify the neuropathologic alterations of the olfactory mucosa in LBD by immunohistochemical analysis of a series of autopsied individuals.

## MATERIALS AND METHODS

### Tissue source

Tissue samples were obtained from autopsy materials that were collected at the Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology between October 2008 and August 2010. This hospital is located at the center of Tokyo city and is a geriatric general emergency hospital with 579 beds. This hospital provides community-based medical service to the aged population 24 h/day in cooperation with local general practitioners. The number of autopsy cases was 162 in the above duration. In addition to the general organs, we could obtain the brains and spinal cords from 105 cases in that period, that were registered to the Brain Bank for Aging Research (BBAR) with the deceased's relatives' informed consent. The BBAR is approved by the ethics committee of the Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology to carry out comprehensive research.

### Clinical information

All clinical information, including the presence or absence of Parkinsonism as well as dementia, was retrospectively

obtained from medical charts and reviewed by two board-certified neurologists.<sup>11,22–26</sup> First, we evaluated Parkinsonism such as bradykinesia, resting tremor, rigidity and postural instability. In this study, when individuals had two or more of these four clinical symptoms, we defined them as having Parkinson's disease-related symptoms.<sup>27</sup> Second, we analyzed scores for the Mini-Mental State Examination<sup>28</sup> or the Hasegawa Dementia Scale (or its revised version),<sup>29,30</sup> the Instrumental Activities of Daily Living,<sup>31</sup> and the Clinical Dementia Rating (CDR).<sup>32</sup> When individuals were not assigned to a category of CDR, we retrospectively determined CDR using medical records, including the battery of cognitive tests above, as well as interviews with attending physicians and caregivers when necessary. Based on these results, we assigned a clinical diagnosis to each patient. The clinical diagnosis of Alzheimer disease was carried out based on the criteria of the National Institute of Neurological and Communication Disorders and Stroke-Alzheimer Disease and Related Disorders Association.<sup>33</sup> The diagnosis of DLB and Parkinson's disease with dementia conformed to the third report of the DLB consortium.<sup>34</sup>

### Histology

We examined the brain and olfactory epithelium, olfactory bulb, esophagogastric mucosal junction, sympathetic ganglia, thoracic spinal cord, adrenal glands, anterior wall of the left ventricle of the heart, and abdominal skin.<sup>22,26</sup> The brains and spinal cords were examined as previously reported.<sup>22,24,25</sup> Briefly, the cerebral and cerebellar hemispheres as well as brainstem were dissected in the sagittal plane at the time of autopsy. In each case, half of the brain was preserved at  $-80^{\circ}\text{C}$  for further biochemical and molecular analyses. The other half of the brain and abdominal skin were fixed in 20% buffered formalin (WAKO, Osaka, Japan) for 7–13 days and sliced in the same manner as the contralateral hemisphere. The adrenal gland and anterior wall of the left ventricle of the heart were fixed in 20% formalin. The representative areas were embedded in paraffin. Six-micrometer-thick serial sections were cut and stained with HE and KB. Sections of the amygdala, hippocampus, parahippocampal gyrus and temporal cortex were stained with the modified Gallyas-Braak method for senile plaques, NFTs and argyrophilic grains.<sup>35</sup>

### Immunohistochemistry

Sections were immunostained using the following antibodies raised against phosphorylated tau protein (p-tau) (AT8, monoclonal; Innogenetics, Temse, Belgium): synthetic peptide corresponding to amino acids 11–28 of amyloid-beta protein (12B2, monoclonal; IBL, Maebashi, Japan); phosphorylated  $\alpha$ -synuclein (pSyn#64, monoclonal<sup>25</sup> and

**Table 1** Antibodies used for immunohistochemistry

Antibody	Epitope	Source	Clone	Dilution ratio	Antigen method	Retrieval (min)
pSyn#64	$\alpha$ -synuclein phosphorylated ser 129	T. Iwatsubo	Monoclonal	1:20000	99% formic acid	5
PSer129	$\alpha$ -synuclein phosphorylated ser 129	T. Iwatsubo	Polyclonal	1:100	None	
PGP9.5	PGP9.5	Biomol	Polyclonal	1:5000	microwave	30
SMI31	phosphorylated neurofilament	Sternberger	Monoclonal	1:20000	None	
Tyrosine hydroxylase	Anti-tyrosine hydroxylase, rat	CALBIOCHEM	Monoclonal	1:10	microwave	30
AT8	Phosphorylated tau protein	Innogenetics	Monoclonal	1:1000	None	
12B2	A $\beta$ 11–28	IBL	Monoclonal	1:50	99% formic acid	5

PSer129 polyclonal<sup>36</sup>), ubiquitin (polyclonal, Sigma-Aldrich, St. Louis, MO), Protein Gene Product 9.5 (PGP9.5, polyclonal; ENZO Life Sciences International, Farmingdale, NY USA); phosphorylated neurofilament (SMI31, monoclonal; Sternberger Immunochemicals, Bethesda, MA, USA); and tyrosine hydroxylase (Anti-Tyrosine Hydroxylase, Rat, monoclonal; Calbiochem-Novabiochem Corporation, Darmstadt, Germany) (Table 1). The signals from monoclonal and polyclonal antibodies were detected by using the automatic system on a VENTANA NX20 with the I-View DAB Universal Kit (Roche, Basel, Switzerland) according to the manufacturer's instructions. Sections were counter-stained with hematoxylin.

## LBAS

### CNS

In order to analyze LBAS,<sup>22</sup> we carried out immunohistochemical analysis with phosphorylated  $\alpha$ -synuclein antibodies for the following sections: the medulla oblongata at the level of the dorsal motor nucleus of the vagus, the upper pons at the level of the locus coeruleus, and the midbrain including the substantia nigra, amygdala, anterior hippocampus and the peripheral nervous system from all cases (described in the next section). When immunopositive deposits were observed in these anatomic regions, we carried out additional immunohistochemical analysis for sections of the basal nucleus of Meynert, anterior cingulate gyrus, entorhinal cortex, the second frontal and temporal gyri and the supramarginal gyrus, using antibodies raised against phosphorylated  $\alpha$ -synuclein.

### Peripheral nervous system

To analyze LBAS of the peripheral nerve, tissue sections from epicardium and epicardial fat of the left ventricle of the heart, sympathetic ganglia, esophagogastric mucosal junction, adrenal gland<sup>22</sup> and abdominal skin<sup>26</sup> were examined by using antibodies raised against phosphorylated  $\alpha$ -synuclein.

### Olfactory mucosa

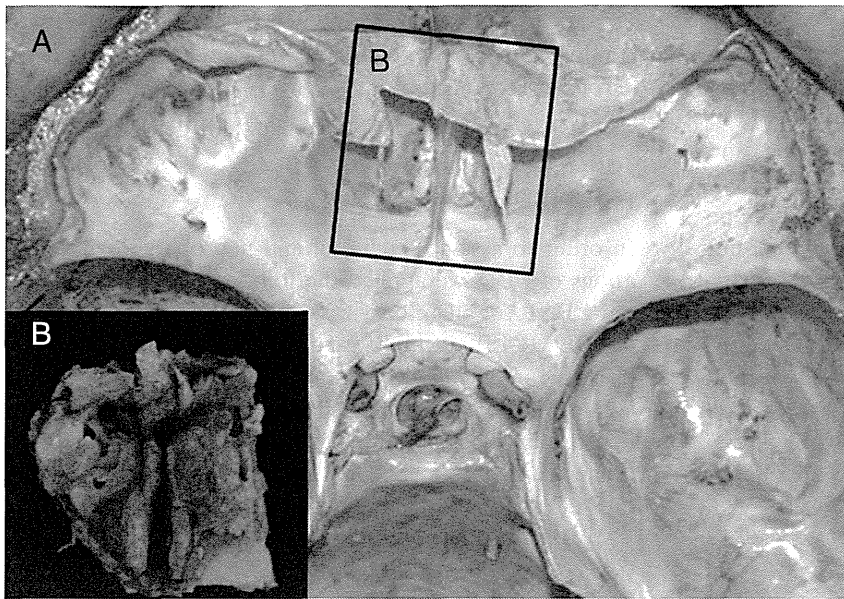
At the time of autopsy, the olfactory mucosa, bony septae and contiguous cribriform plate were removed en bloc (Fig. 1). The cribriform plate was dissected in the sagittal plane of the midline by using an electric jigsaw. The left side was fixed for 24 h in 4% paraformaldehyde. After fixation, the olfactory mucosa was removed, dehydrated in a graded alcohol series, cleared in xylene and embedded in paraffin. The right side was fixed for 24 h in 4% paraformaldehyde, decalcified with EDTA for 2 weeks, and dehydrated and embedded in paraffin. Serial 6- $\mu$ m-thick sections were stained with HE and immunolabeled with antibodies against phosphorylated  $\alpha$ -synuclein, PGP9.5, phosphorylated neurofilament, tyrosine hydroxylase, phosphorylated tau and amyloid  $\beta$  (Table 1). In particular, the olfactory receptor neurons of the olfactory epithelium were identified by using PGP9.5 immunohistochemistry.<sup>19</sup> The normal anatomical appearance of the olfactory system is shown in Figure 2.

### Olfactory bulb

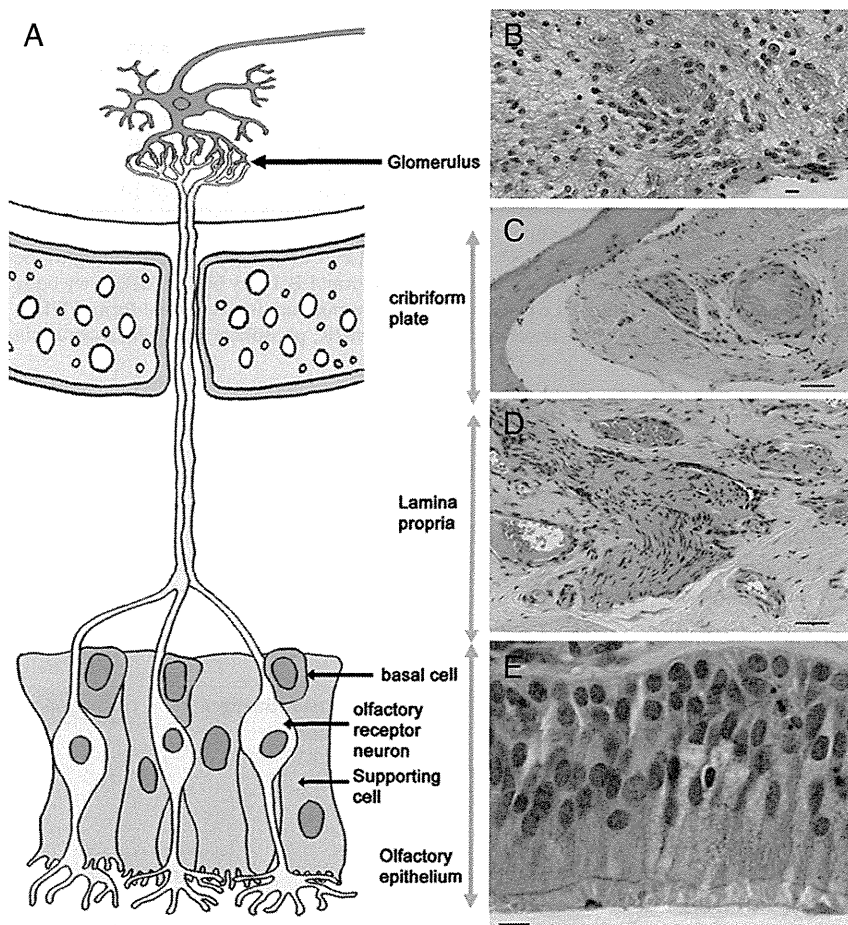
The olfactory bulbs were prepared for histologic sections to analyze the presence of LBAS. By using HE stain and  $\alpha$ -synuclein antibodies, LBAS were identified in the glomeruli, mitral cells, tufted cells and granular cells as previously reported.<sup>11</sup> Mitral and tufted cells were distinguished by their specific shapes. Each neuron was identified when it had an apparent nucleus containing a prominent nucleolus and Nissl substance.

### Semiquantitative scoring system of Lewy-related pathology

For each section, we semi-quantitatively graded the immunohistochemical staining with antibody raised against phosphorylated  $\alpha$ -synuclein. Our grading system was modified based on the scoring system of the third report of the DLB consortium<sup>34</sup> because we used both the HE stain and immunohistochemistry using monoclonal antibody for phosphorylated  $\alpha$ -synuclein to identify LBAS.



**Fig. 1** (a) The anterior cranial fossa after removal of the brain. In order to obtain the olfactory mucosa, the bony septae and contiguous cribriform plate (the rectangular area) were dissected using an electric jigsaw. (b) An inset shows the olfactory mucosa and cribriform plate from the opposite side of the rectangular area.



**Fig. 2** Scheme of the normal olfactory pathway (a) and photomicrographs of representative histologies of each region (b–e). The olfactory epithelium is composed of three cell types: the basal cells, olfactory receptor neurons and supporting cells (a). The basal cells are the progenitor of the olfactory receptor neurons (a, e). In general, the turnover rate of the olfactory receptor neurons is approximately 30–90 days. Nerve fibers are present in the lamina propria and cribriform plate (c and d, respectively). They consist of either the axons of the olfactory receptor neurons or postganglionic sympathetic nerve fibers. There are glomeruli in the olfactory bulb (b). Glomeruli are the synaptically connected structures of the axons of the olfactory receptor neurons and mitral/ tufted cells in the olfactory bulb. (b, e), scale bar = 10  $\mu$ m; (c), scale bar = 50  $\mu$ m; (d), scale bar = 100  $\mu$ m.

For example, ‘Stage 1’ of the original scoring system was defined as ‘sparse Lewy bodies or neurites.’ On the other hand, ‘Grade 1’ of our methodology is defined as ‘sparse Lewy neurites without Lewy bodies.’

Grade 0 = neither LNs nor LBs detected using anti-phosphorylated  $\alpha$ -synuclein antibody.

Grade 1 = sparse phosphorylated  $\alpha$ -synuclein immunopositive dots or neurites, or diffuse granular cytoplasmic stain in the neuron, neither LBs nor phosphorylated  $\alpha$ -synuclein-immunopositive neuronal intracytoplasmic dense aggregations.

Grade 2 = 1–3 LBs or phosphorylated  $\alpha$ -synuclein-immunopositive intracytoplasmic dense aggregations and scattered LNs in a low-power field ( $\times 10$ ).

Grade 3 = more than four LBs and scattered LNs in a low-power field ( $\times 10$ ).

Grade 4 = numerous LBs and neurites with severe immunoreactivity for phosphorylated  $\alpha$ -synuclein in the neuropil or background.

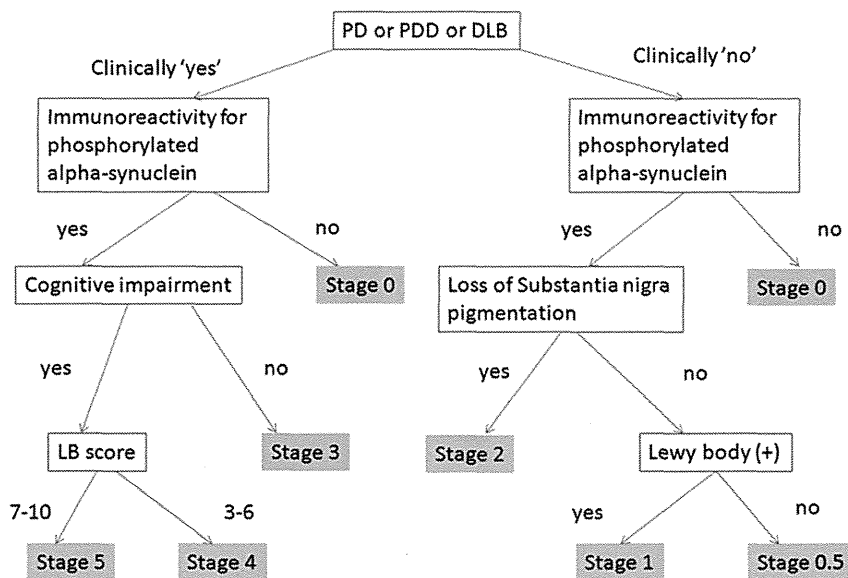
### LB staging system of our BBAR (BBAR LB stage)

In order to assess the clinical and neuropathologic alterations of LBD, we applied the following rating system to our BBAR for all autopsy cases (Table 2, Fig. 3). The original BBAR LB staging system was developed in order to track the individual data of our brain bank.<sup>24,25</sup> This rating system requires clinical symptoms, gross and microscopic neuropathologic alterations, and LB scores used in the consensus guidelines for the clinical and pathologic diagnosis of DLB.<sup>27</sup> In this staging system, Parkinson’s disease with

**Table 2** Lewey body stage of Brain Bank for Aging Research

Stage	Psyn-IR	LB	SN: loss of pigmentation	LB score	Dementia	Parkinsonism	Diagnosis
0	–	–	–				
0.5	+	–	–				
1	+	+	–				Incidental LBD
2	+	+	+	0–10	–†	–†	Subclinical LBD
3	+	+	+	0–10	–	+	PD
4	+	+	+	3–6	+	+	PDDL
	+	+	+	3–6	+	+ or –	DLBL‡
5	+	+	+	7–10	+	+	PDDN
	+	+	+	7–10	+	+ or –	DLBN‡

†Neither dementia nor Parkinsonism associated with Lewy body-related  $\alpha$ -synucleinopathy. ‡Differential diagnosis of PDD and DLB was based on the ‘1-year rule’ according to the consensus guidelines (34). DLBL, dementia with Lewy bodies and a Lewy body score corresponding to the limbic form; DLBN, dementia with Lewy bodies and a Lewy body score corresponding to the neocortical form; LB, Lewy body; LBD, Lewy body disease; PD, Parkinson’s disease; PDDL, Parkinson’s disease with dementia and a Lewy body score corresponding to the limbic form; PDDN, Parkinson’s disease with dementia and a Lewy body score corresponding to the neocortical form; Psyn-IR, phosphorylated alpha-synuclein immunoreactivity; SN, substantia nigra.



**Fig. 3** Flow-chart of the Lewy body staging system of the Brain Bank for Aging Research (BBAR). PD, Parkinson’s disease; PDD, Parkinson’s disease with dementia; DLB, dementia with Lewy bodies; SN, substantia nigra; LB score, Lewy body score. See Table 2 for detailed description of each stage.

dementia was differentiated from DLB by applying the '12-month (1-year)' rule noted in the Consensus Guidelines (i.e., 'dementia appears more than one year after the onset of Parkinsonism').<sup>27</sup>

### Evaluation of senile changes and neuropathologic diagnosis

NFTs were classified according to Braak and Braak's staging system using modified Gallyas-Braak staining<sup>37</sup> and AT8 immunohistochemistry.<sup>38</sup> The staging system for senile plaques (SPs) comprises four stages (0–C). Argrophilic grains were classified into our four stages (0–III), as reported previously.<sup>23</sup> The neuropathologic diagnosis of Alzheimer disease was based on our previous definition,<sup>39</sup> which proposed a modification of the National Institute on Aging and Reagan Institute criteria.<sup>40,41</sup> The diagnoses of dementia with grains and NFT-predominant forms of dementia were based on the previously described definitions.<sup>42,43</sup>

### Statistical analysis

Fisher's exact test was carried out to compare the number of cases having LBAS pathology in the olfactory mucosa.

## RESULTS

### Clinical information

Of the 105 consecutive autopsy patients, 58 were men and 47 were women. The patient ages at death ranged from 65 to 104 years ( $82 \pm 37$ , mean  $\pm$  SD). Twelve patients showed Parkinson's disease-related symptoms according to the clinical criteria in this study. Six out of 105 patients were clinically diagnosed as LBD including Parkinson's disease, Parkinson's disease with dementia and DLB.

### Neuropathologic diagnosis

The neuropathologic diagnoses consisted of Alzheimer disease ( $n = 15$ ), dementia with grains ( $n = 11$ ), NFT-predominant form of dementia ( $n = 8$ ), Parkinson's disease ( $n = 2$ ), Parkinson's disease with dementia ( $n = 2$ ), and DLB ( $n = 1$ ), as well as one case each of dentatorubral-pallidolusian atrophy, neuronal hyaline inclusion body disease, frontotemporal lobar degeneration with transac-tion response (TAR) DNA-binding protein-43 kDa-immunoreactive inclusions, and progressive multifocal leukoencephalopathy. Patients with combined pathologies, included Alzheimer's disease plus DLB ( $n = 2$ ), dementia with grains plus NFT-predominant form of dementia ( $n = 3$ ), and one patient each of diffuse NFTs with calcification (DNTC)<sup>44</sup> plus DLB and dementia with grains plus

Alzheimer's disease. The remaining patients did not fulfil the clinical and/or pathological criteria for neurodegenerative diseases.

Eight out of 105 patients ( $8/105 = 7.6\%$ ) were clinically and neuropathologically diagnosed as having LBD, including Parkinson's disease (2 patients), Parkinson's disease with dementia (2 patients) and DLB (4 patients).

### Incidence, distribution and extent of LBAS

#### BBAR staging

Based on clinical and neuropathologic analyses, the BBAR LB stages were as follows: stage 0 = 66 cases, stage 0.5 = 6 cases, stage 1 = 21 cases, stage 2 = 4 cases, stage 3 = 2 cases, stage 4 = 3 cases and stage 5 = 3 cases. All of the stage 5 cases had DLB, with an LB score corresponding to the value for the neocortical form (DLBN).

#### LBAS in CNS and peripheral nervous system

We identified 39 (37.1%) out of the 105 individuals with  $\alpha$ -synuclein immunopositive LBAS in the CNS or peripheral nervous system (Table 3). Therefore, we focused on these 39 cases in the present study. Here, LBAS was identified by using  $\alpha$ -synuclein immunohistochemistry. In LBAS, LBs were confirmed with HE stains and  $\alpha$ -synuclein immunohistochemistry. Out of the 39 cases, 33 showed LBAS in the olfactory bulb, 15 in the enteric nerve plexus, 23 in the sympathetic ganglia, and 16 in the pericardial nerve fibers of the left ventricle (Tables 3 and 4).

#### Olfactory mucosa

The olfactory epithelium is a pseudostratified columnar epithelium lying deep within the recess of the superior nasal cavity; it is composed of a mixture of multipotential stem cells (basal cells), supporting cells and olfactory receptor neurons (Fig. 2). Mature neurons are reported to give rise to fine and unmyelinated axons that ascend through the cribriform plate to synapse at glomeruli in the olfactory bulb.<sup>20,45</sup>

LBAS were found in the olfactory mucosa of seven (17.9%) out of 39 cases (Tables 3 and 4). These seven also had LBAS in the olfactory bulb. LBAS was present in the lamina propria mucosa of the seven cases (Fig. 4a–c). In addition, one case showed LBAS in a bundle of axons in the cribriform plate (Fig. 4d). None of the cases showed LBAS in the olfactory epithelial paraneuron. We summarized the demographic results of these seven individuals with LBAS in the olfactory mucosa in Table 5. Neither phosphorylated tau-positive deposits nor amyloid  $\beta$  immunopositive deposits were detected in the olfactory mucosa.

**Table 3** The distribution of  $\alpha$ -synuclein deposits in various anatomical regions of 39 cases with Lewy body disease

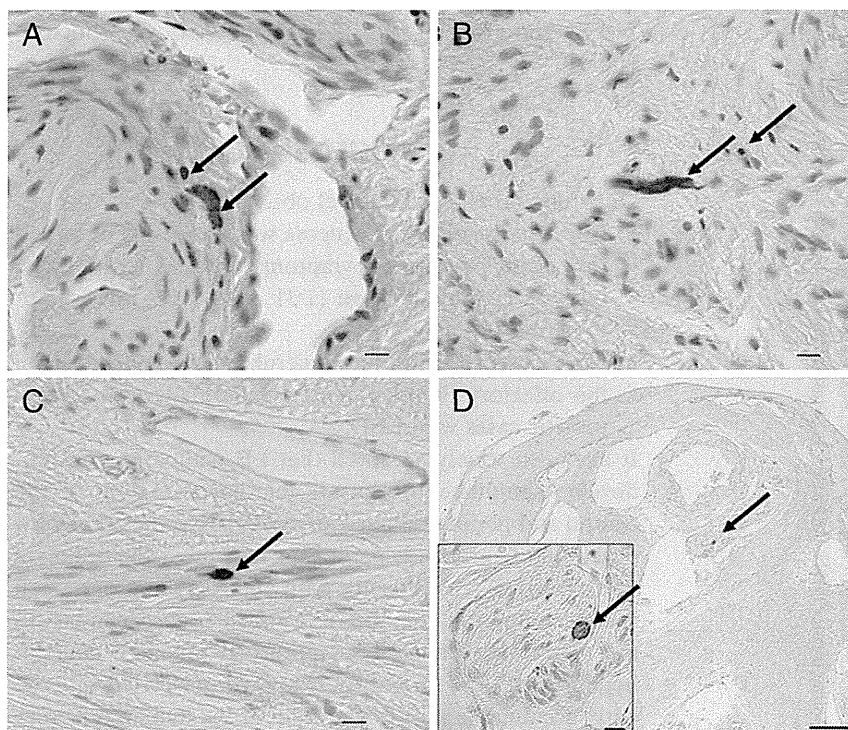
Age at death/gender	Parietal lobe	Frontal lobe	Temporal lobe	Cingulate gyrus	Entorhinal cortex	Amygdala	Olfactory bulb	Nucleus basalis of Meynert	Substantia nigra	Locus coeruleus	Dorsal motor nucleus of the vagus	Spinal Cord	Gastrointestinal system	Olfactory Mucosa	Sympathetic ganglion	Adrenal gland	Pericardial nerve	Skin	BBAR LB stage	NFT stage	SP stage
104/F																			5	4	C
70/F																			5	4	C
86/F																			5	6	C
84/M																			4	2	A
79/F																			4	2	A
80/F																			4	2	A
81/M																			3	2	A
88/M																			3	3	A
79/M																			2	1	A
68/F																			2	2	B
79/F																			2	6	C
77/F																			2	6	C
78/M																			1	2	A
75/M																			1	2	A
89/F																			1	3	C
93/F																			1	4	C
86/M																			1	4	C
81/M																			1	2	A
90/F																			1	2	A
86/M																			1	2	A
97/F																			1	2	A
78/M																			1	1	A
92/M																			1	3	C
94/M																			1	4	A
85/M																			1	3	A
81/F																			1	5	C
96/F																			1	2	0
87/F																			1	3	A
101/F																			1	4	A
69/F																			1	4	0
83/F																			1	3	A
72/M																			1	1	A
77/M																			1	2	A
83/M																			0.5	4	C
71/M																			0.5	2	A
89/M																			0.5	2	A
85/F																			0.5	3	A
85/F																			0.5	2	A
96/F																			0.5	3	A

Grade 0 = blank, grade 1 = light grey, grade 2 = light blue, grade 3 = blue, grade 4 = navy blue. The number in each cell indicates a score based on the semiquantitative scoring system of Lewy-related pathology. 0 = neither Lewy neurites nor bodies detected by using anti-phosphorylated  $\alpha$ -synuclein antibody. 1 = sparse phosphorylated  $\alpha$ -synuclein immunopositive dots or neurites, neither Lewy bodies nor phosphorylated  $\alpha$ -synuclein immunopositive intracytoplasmic aggregations. 2 = one to three Lewy bodies or phosphorylated  $\alpha$ -synuclein immunopositive intracytoplasmic aggregations in a low-power field ( $\times 10$ ). 3 = more than four Lewy bodies and scattered Lewy neurites in a low-power field ( $\times 10$ ). 4 = numerous LBs and neurites with severe immunoreactivity for phosphorylated  $\alpha$ -synuclein in the neuropil or background. Individuals of BBAR LB stages 3–5, with clinical Parkinsonism and neuropathologically numerous LBASs in the CNS, showed high incidence (75%, 6/8 individuals) of LBASs in the olfactory mucosa. In contrast, individuals of BBAR LB stages 1–3 without Parkinsonism showed extremely low incidence of Lewy body-related  $\alpha$ -synucleinopathy (LBAS) (3%, 1/31) in the olfactory mucosa. LBAS was found in the olfactory mucosa mostly in advanced BBAR LB stages 3–5. BBAR LB Brain Bank for Aging Research Lewy body staging, NFT stage, Braak's stages for neurofibrillary tangles; SP stage, Braak's stages for senile plaques.

**Table 4** Regional frequency of Lewy body-related  $\alpha$ -synucleinopathy (LBAS) in various anatomical regions

The BBAR LB stage	Olfactory epithelium	Olfactory mucosa	Olfactory bulb	Spinal cord	GI tract	Sympathetic ganglia	Adrenal gland	Pericardial nerve	Skin
0.5	0/6	0/6	2/6	0/6	0/6	2/6	0/6	1/6	0/6
1	0/21	1/21	19/21	7/21	6/27	10/21	1/21	6/21	1/21
2	0/4	0/4	4/4	3/4	1/4	3/4	0/4	2/4	0/4
3	0/2	1/2	2/2	2/2	2/2	2/2	2/2	1/2	2/2
4	0/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3
5	0/3	2/3	3/3	3/3	3/3	3/3	1/3	3/3	0/3
<b>All</b>	<b>0/39</b>	<b>7/39</b>	<b>33/39</b>	<b>18/39</b>	<b>15/39</b>	<b>23/39</b>	<b>7/39</b>	<b>16/39</b>	<b>5/39</b>

BBAR LB Brain Bank for Aging Research Lewy body staging.



**Fig. 4** Photomicrographs show  $\alpha$ -synuclein immunopositive deposits (arrows indicate Lewy neurites) in the axonal bundle of the lamina propria (a–c) and cribriform plate (d). The inset in figure (d) shows a higher magnification image of  $\alpha$ -synuclein immunopositive deposits in the axonal bundle of the cribriform plate. Immunohistochemistry using monoclonal antibody against phosphorylated  $\alpha$ -synuclein (pSyn#64). Photomicrographs (a, b, c and d) were obtained from cases 4, 5, 7 and 3, respectively, in Table 5. (a–c), scale bar = 10  $\mu$ m; (d) scale bar = 100  $\mu$ m (inset, 10  $\mu$ m).

### Correlations between $\alpha$ -synuclein immunopositive LBs or LNs in the olfactory mucosa and CNS

Alpha-synuclein immunopositive LBs or LNs in the olfactory mucosa were detected in seven cases, including three with DLB, three with Parkinson's disease or Parkinson's disease with dementia, and one with incidental LBD (Tables 3–5). LBAS in the olfactory mucosa was compared with those in other locations of the CNS (Table 3). Individuals of BBAR LB stages 3–5, clinical and neuropathological diagnosis of LBD, showed a high incidence (75%, 6/8 individuals) of  $\alpha$ -synuclein immunopositive LBAS in the olfactory mucosa (Table 6, Fig. 5). Six individuals with Parkinson's disease also showed a high incidence of  $\alpha$ -synuclein accumulation (66%, 4/6 individuals) in the olfactory mucosa. In contrast, individuals of BBAR LB

stages 0.5–2 (here we classified them into asymptomatic group) showed a low incidence of LBAS (3%, 1/31) in the olfactory mucosa.

### Olfactory bulb

There is neural connectivity among olfactory receptor neurons and nuclei in the olfactory bulbs.<sup>45</sup> Hence, we analyzed the frequency of LBAS in the glomeruli, tufted cells, mitral cells and granular cells between LBAS-positive and LBAS-negative groups in the olfactory epithelium.

In individuals of BBAR LB stages 3–5 (symptomatic stage), LBAS was frequently observed in the glomeruli (8/8 cases, 100%), granular cells (8/8, 100%) and tufted cells (7/8, 87.5%). In contrast, there were low numbers of cases with LBAS in the mitral cells (2/8, 25%). Asymptomatic stage cases of LBD, corresponding to BBAR stage 0.5–2,

**Table 5** Clinical and neuropathological demography of seven individuals with Lewy body-related  $\alpha$ -synucleinopathy (LBAS) identified in the olfactory mucosa

No.	Age at death	Clinically diagnosed as LBD	Cause of death	Neuropathologic diagnosis	BBAR LB stage	LBAS in the olfactory mucosa			NFT stage	SP stage
						Olfactory epithelium	Lamina propria mucosa	Cribriform plate		
1	104/F	None	CHF, MI, Dementia	DLBN, AD	5	0	1	0	1	C
2	70/F	DLB	DLB, pneumonia	DLBN, AD	5	0	1	0	1	C
3	84/M	DLB	Prostate carcinoma, DLB	DLBL	4	0	1	1	2	A
4	79/F	PDD	PDD	PDDN	4	0	1	0	2	A
5	80/F	PD	Pneumonia, PD	PDDL	4	0	1	0	2	A
6	88/M	PD	Pneumonia, PD	PD	3	0	1	0	2	A
7	86/M	None	Pneumonia	AD, Incidental LBD	1	0	1	0	1	C

AD, Alzheimer's disease; BBAR LB stage, Lewy body staging system of the Braim Bank for Aging Research; CHF, congestive heart failure; DLB, dementia with Lewy bodies; DLBL, dementia with Lewy bodies and a Lewy body score corresponding to the limbic form; DLBN, dementia with Lewy bodies and a Lewy body score corresponding to the neocortical form; F, female; LB, Lewy body; LBD, Lewy body disease; M, male; MI, acute myocardial infarction; NFT stage, Braak's stages for neurofibrillary tangles; PD, Parkinson's disease; PDDL, Parkinson's disease with dementia and a Lewy body score corresponding to the limbic form; PDDN, Parkinson's disease with dementia and a Lewy body score corresponding to the neocortical form; SP stage, Braak's stages for senile plaques.

**Table 6** Incidence of LBAS in the olfactory mucosa in cases with symptomatic LBD (BBAR stage 3–5)

Clinical and neuropathologic diagnosis of LBD	LBAS in OM		Total
	Present	Absent	
Symptomatic (BBAR 3–5)	6*	2	8
Asymptomatic (BBAR 0.5–2)	1	30	31

\* $P < 0.05$ . BBAR, Brain Bank for Aging Research; LBAS, Lewy body-related alpha-synucleinopathy; LBD, Lewy body disease; OM, olfactory mucosa.

showed high incidence of LBAS in the glomeruli (23/31, 74.1%) and granular cells (22/31, 70.9%) of the olfactory bulb (Fig. 5).

### DISCUSSION

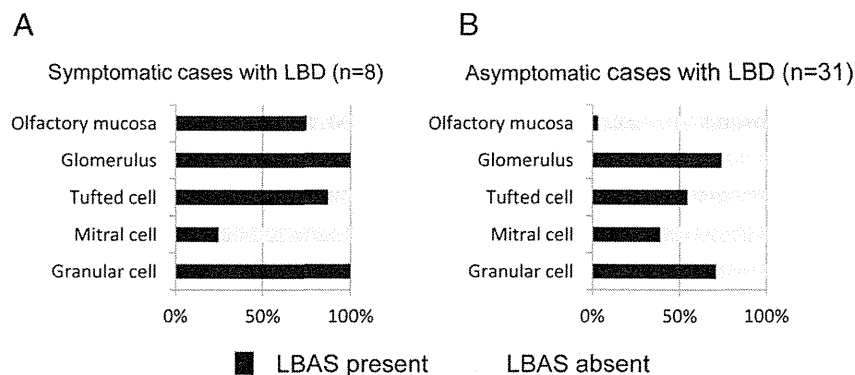
Our study provides two novel observations.

- 1 LBAS in the olfactory mucosa was frequently observed (6/8 cases, 75%) in the symptomatic patients with LBD, but was a rare condition (1/31 cases, 3.2%) in asymptomatic LBD patients.
- 2 LBAS was seen in the glomeruli and granular cells in the olfactory bulbs of most symptomatic and asymptomatic cases with LBD.

It has been widely accepted that LB pathology does not develop simultaneously in all anatomical regions of the central and peripheral nervous systems. Hawkes *et al.* proposed that neurotropic pathogens may enter the brain via two routes: (i) a nasal route, with anterograde progression into the temporal lobe; and (ii) a gastric route secondary to the swallowing of nasal secretions in saliva (a dual hit hypothesis).<sup>46</sup> The former route may be associated with the early accumulation of  $\alpha$ -synuclein in the human olfactory bulb and cause olfactory dysfunction in sporadic Parkinson's disease. In the present study, there was rare observation of LBAS in the olfactory mucosa in the asymptomatic cases of LBD. Further analysis is important to clarify the possibility of propagation of  $\alpha$ -synuclein in the nervous systems.

In the present study, LBAS was frequently observed in the olfactory mucosa (6/8 cases, 75%) in the individuals with clinical LBD. In contrast, LBAS in the olfactory mucosa was a rare observation in asymptomatic patients. It is also important that all seven cases with LBAS in the olfactory mucosa had LBAS in the cerebral cortex and brainstem. Our results have similarities with a previous report concerning Alzheimer's disease.<sup>20</sup> Detection of LBAS in the olfactory mucosa could be hindered by two problems: technical difficulty in obtaining enough nerve fibers and rapid turnover of olfactory receptor neurons.<sup>47,48</sup> In fact, a recent study reported that a biopsy study revealed no  $\alpha$ -synuclein immunopositive deposits in the olfactory





74.1%), tufted cells (17/31, 54.8%) and granular cells (22/31, 70.9%). The number of cases with LBAS in the mitral cells (12/31, 38.7%) is low. LBAS pathology of the olfactory mucosa is present in only one case (1/31, 3.2%).

mucosa of patients of Parkinson's disease.<sup>21</sup> Our previous study indicated a high incidence of  $\alpha$ -synuclein immunopositive LBs or neurites in aging human olfactory bulbs, and suggested that they extend from the periphery (the second olfactory structure) to the anterior olfactory nucleus (the tertiary olfactory structure).<sup>11</sup> The present study, using 6  $\mu$ m-thick paraffin embedded sections, revealed that LBAS was most frequently observed in the glomeruli which were composed of axon terminals of olfactory epithelial cells and dendrites of mitral and tufted cells<sup>45</sup> as well as in the glomerular cells which were most numerous in the periphery of the olfactory bulb (Fig. 5). We consider that high incidence of LBAS in glomeruli may represent affected terminal axons of olfactory epithelial neurons. In contrast to our result, a previous study, employing 50  $\mu$ m-thick floating sections, reported high frequency of LBAS in mitral cells and the internal plexiform layer in individuals with Parkinson's disease but no LBAS in age-matched controls.<sup>49</sup> Further studies are necessary to identify the most vulnerable subset in the periphery of the olfactory bulb.

In conclusion, presence of LBAS in the olfactory mucosa and olfactory glomeruli further supports the importance of olfactory system as an entry zone of LBD. Future studies of LB pathology involving the olfactory system are indicated to understand the pathomechanism of  $\alpha$ -synuclein accumulation in individuals with LBD.

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#### REFERENCES

- Baba M, Nakajo S, Tu PH *et al.* Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. *Am J Pathol* 1998; **152**: 879–884.
- Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M. alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. *Proc Natl Acad Sci U S A* 1998; **95**: 6469–6473.
- Goedert M, Spillantini MG, Davies SW. Filamentous nerve cell inclusions in neurodegenerative diseases. *Curr Opin Neurobiol* 1998; **8**: 619–632.
- Ogunnyi A, Akang EE, Gureje O *et al.* Dementia with Lewy bodies in a Nigerian: a case report. *Int Psychogeriatr* 2002; **14**: 211–218.
- Takao M, Ghetti B, Yoshida H *et al.* Early-onset dementia with Lewy bodies. *Brain Pathol* 2004; **14**: 137–147.
- Braak H, Ghebremedhin E, Rub U, Bratzke H, Del Tredici K. Stages in the development of Parkinson's disease-related pathology. *Cell Tissue Res* 2004; **318**: 121–134.
- Braak H, Sastre M, Bohl JR, de Vos RA, Del Tredici K. Parkinson's disease: lesions in dorsal horn layer I,

- involvement of parasympathetic and sympathetic pre- and postganglionic neurons. *Acta Neuropathol* 2007; **113**: 421–429.
8. Braak H, Braak E. Pathoanatomy of Parkinson's disease. *J Neurol* 2000; **247** (Suppl 2): I13–I10.
  9. 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.
  10. Del Tredici K, Rub U, De Vos RA, Bohl JR, Braak H. Where does Parkinson disease pathology begin in the brain? *J Neuropathol Exp Neurol* 2002; **61**: 413–426.
  11. Sengoku R, Saito Y, Ikemura M *et al*. Incidence and extent of Lewy body-related alpha-synucleinopathy in aging human olfactory bulb. *J Neuropathol Exp Neurol* 2008; **67**: 1072–1083.
  12. Ponsen MM, Stoffers D, Booij J, van Eck-Smit BL, Wolters ECh, Berendse HW. Idiopathic hyposmia as a preclinical sign of Parkinson's disease. *Ann Neurol* 2004; **56**: 173–181.
  13. Haehner A, Boesveldt S, Berendse HW *et al*. Prevalence of smell loss in Parkinson's disease – a multicenter study. *Parkinsonism Relat Disord* 2009; **15**: 490–494.
  14. Daniel SE, Hawkes CH. Preliminary diagnosis of Parkinson's disease by olfactory bulb pathology. *Lancet* 1992; **340** (8812): 186.
  15. Pearce RK, Hawkes CH, Daniel SE. The anterior olfactory nucleus in Parkinson's disease. *Mov Disord* 1995; **10**: 283–287.
  16. Beach TG, White CL 3rd, Hladik CL *et al*. Olfactory bulb alpha-synucleinopathy has high specificity and sensitivity for Lewy body disorders. *Acta Neuropathol* 2009; **117**: 169–174.
  17. Parkkinen L, Silveira-Moriyama L, Holton JL, Lees AJ, Revesz T. Can olfactory bulb biopsy be justified for the diagnosis of Parkinson's disease? Comments on 'olfactory bulb alpha-synucleinopathy has specificity and sensitivity for Lewy body disorders'. *Acta Neuropathol* 2009; **117** (2): 213–214.
  18. Jellinger KA. Olfactory bulb alpha-synucleinopathy has specificity and sensitivity for Lewy body disorders. *Acta Neuropathol* 2009; **117** (2): 215–216.
  19. Duda JE, Shah U, Arnold SE, Lee VM, Trojanowski JQ. The expression of alpha-, beta-, and gamma-synucleins in olfactory mucosa from patients with and without neurodegenerative diseases. *Exp Neurol* 1999; **160**: 515–522.
  20. Arnold SE, Lee EB, Moberg PJ *et al*. Olfactory epithelium amyloid-beta and paired helical filament-tau pathology in Alzheimer disease. *Ann Neurol* 2010; **67**: 462–469.
  21. Witt M, Bormann K, Gudziol V *et al*. Biopsies of olfactory epithelium in patients with Parkinson's disease. *Mov Disord* 2009; **24**: 906–914.
  22. Fumimura Y, Ikemura M, Saito Y *et al*. Analysis of the adrenal gland is useful for evaluating pathology of the peripheral autonomic nervous system in Lewy body disease. *J Neuropathol Exp Neurol* 2007; **66**: 354–362.
  23. Saito Y, Ruberu NN, Sawabe M *et al*. Staging of argyrophilic grains: an age-associated tauopathy. *J Neuropathol Exp Neurol* 2004; **63**: 911–918.
  24. Saito Y, Ruberu NN, Sawabe M *et al*. Lewy body-related alpha-synucleinopathy in aging. *J Neuropathol Exp Neurol* 2004; **63**: 742–749.
  25. Saito Y, Kawashima A, Ruberu NN *et al*. Accumulation of phosphorylated alpha-synuclein in aging human brain. *J Neuropathol Exp Neurol* 2003; **62**: 644–654.
  26. Ikemura M, Saito Y, Sengoku R *et al*. Lewy body pathology involves cutaneous nerves. *J Neuropathol Exp Neurol* 2008; **67**: 945–953.
  27. 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**: 1113–1124.
  28. 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**: 189–198.
  29. Hasegawa K, Inoue K, Moriya K. An investigation of dementia rating scale for the elderly. *Seishin Igaku* 1974; **16**: 965–969.
  30. Hosokawa T, Yamada Y, Isagoda A, Nakamura R. Psychometric equivalence of the Hasegawa Dementia Scale-Revised with the Mini-Mental State Examination in stroke patients. *Percept Mot Skills* 1994; **79**: 664–666.
  31. Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. *Gerontologist* 1969; **9**: 179–186.
  32. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology* 1993; **43**: 2412–2414.
  33. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984; **34**: 939–944.
  34. McKeith IG, Dickson DW, Lowe J *et al*. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology* 2005; **65**: 1863–1872.

35. Gallyas F. Silver staining of Alzheimer's neurofibrillary changes by means of physical development. *Acta Morphol Acad Sci Hung* 1971; **19**: 1–8.
36. Fujiwara H, Hasegawa M, Dohmae N *et al.* alpha-Synuclein is phosphorylated in synucleinopathy lesions. *Nat Cell Biol* 2002; **4**: 160–164.
37. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol* 1991; **82**: 239–259.
38. Braak H, Alafuzoff I, Arzberger T, Kretschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol* 2006; **112**: 389–404.
39. Murayama S, Saito Y. Neuropathological diagnostic criteria for Alzheimer's disease. *Neuropathology* 2004; **24**: 254–260.
40. Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. 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.
41. Hyman BT, Trojanowski JQ. Consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. *J Neuropathol Exp Neurol* 1997; **56**: 1095–1097.
42. Jellinger KA. Dementia with grains (argyrophilic grain disease). *Brain Pathol* 1998; **8**: 377–386.
43. Jellinger KA, Bancher C. Senile dementia with tangles (tangle predominant form of senile dementia). *Brain Pathol* 1998; **8**: 367–376.
44. Kosaka K. Diffuse neurofibrillary tangles with calcification: a new presenile dementia. *J Neurol Neurosurg Psychiatry* 1994; **57**: 594–596.
45. Hawkes CH, Doty RL. *The Neurology of Olfaction*. Cambridge, UK: Cambridge University Press, 2009; 16–21.
46. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: a dual-hit hypothesis. *Neuropathol Appl Neurobiol* 2007; **33**: 599–614.
47. Graziadei PP, Okano M. Neuronal degeneration and regeneration in the olfactory epithelium of pigeon following transection of the first cranial nerve. *Acta Anat (Basel)* 1979; **104**: 220–236.
48. Graziadei PP, Monti Graziadei AG. Regeneration in the olfactory system of vertebrates. *Am J Otolaryngol* 1983; **4**: 228–233.
49. Ubeda-Bañon I, Saiz-Sanchez D, de la Rosa-Prieto C, Argandoña-Palacios L, Garcia-Muñozguren S, Martínez-Marcos A. alpha-Synucleinopathy in the human olfactory system in Parkinson's disease: involvement of calcium-binding protein- and substance P-positive cells. *Acta Neuropathol* 2010; **119**: 723–735.

# Prion-like Properties of Pathological TDP-43 Aggregates from Diseased Brains

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## SUMMARY

TDP-43 is the major component protein of ubiquitin-positive inclusions in brains of patients with frontotemporal lobar degeneration (FTLD-TDP) or amyotrophic lateral sclerosis (ALS). Here, we report the characterization of prion-like properties of aggregated TDP-43 prepared from diseased brains. When insoluble TDP-43 from ALS or FTLD-TDP brains was introduced as seeds into SH-SY5Y cells expressing TDP-43, phosphorylated and ubiquitinated TDP-43 was aggregated in a self-templating manner. Immunoblot analyses revealed that the C-terminal fragments of insoluble TDP-43 characteristic of each disease type acted as seeds, inducing seed-dependent aggregation of TDP-43 in these cells. The seeding ability of insoluble TDP-43 was unaffected by proteinase treatment but was abrogated by formic acid. One subtype of TDP-43 aggregate was resistant to boiling treatment. The insoluble fraction from cells harboring TDP-43 aggregates could also trigger intracellular TDP-43 aggregation. These results indicate that insoluble TDP-43 has prion-like properties that may play a role in the progression of TDP-43 proteinopathy.

## INTRODUCTION

Frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) are well-known neurodegenerative disorders. FTLD is the second most common form of cortical dementia in the population below the age of 65 years (Snowden et al., 2002). ALS is the most common form of motor neuron disease

and is characterized by progressive weakness and muscular wasting, and death within a few years. Ubiquitin-positive inclusions composed of misfolded proteins in neuronal and glial cells are common neuropathological features of most neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), FTLD, and ALS. Recently, TAR DNA-binding protein of 43 kDa (TDP-43) was identified as the major component of inclusions found in the brains of patients with ALS and FTLD (FTLD-U or FTLD-TDP) (Arai et al., 2006; Neumann et al., 2006). TDP-43, a 414-amino-acid protein expressed in nuclei, belongs to the heterogeneous ribonucleoprotein family, members of which are involved in repression of gene transcription, regulation of exon splicing, and nuclear body functions (Buratti and Baralle, 2009; Buratti et al., 2001). TDP-43 is thought to be essential for early embryonic development, because homozygous disruption of the TDP-43 gene (*TARDBP*) causes early embryonic lethality (Sephton et al., 2010; Wu et al., 2010). Interestingly, affected neurons containing cytoplasmic TDP-43 inclusions show depletion of normal nuclear TDP-43 (Arai et al., 2006; Neumann et al., 2006). Patients with these diseases show autosomal-dominant missense mutations in the *TARDBP* gene, mostly located in the C-terminal glycine-rich region (Pesiridis et al., 2009), and pathological TDP-43 is hyperphosphorylated, ubiquitinated, and abnormally cleaved to generate aggregation-prone C-terminal fragments (CTFs) (Arai et al., 2010; Hasegawa et al., 2008, 2011). Thus, loss of normal function of nuclear TDP-43 due to cytoplasmic mislocalization, and toxic gain of function due to cytoplasmic TDP-43 aggregation are potential disease mechanisms (Arai et al., 2006; Neumann et al., 2006).

Aberrant protein aggregates in affected neurons are well-known hallmarks of neurodegenerative diseases, but the mechanisms involved remain unclear. Recent reports suggest that prion-like propagation of protein aggregates composed of tau or  $\alpha$ -synuclein may be involved in progression of neurodegenerative diseases such as AD or PD. This is consistent with findings that tau- or  $\alpha$ -synuclein pathology spreads in a