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Reduced striatal tyrosine hydroxylase in incidental Lewy body disease

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Abstract

Incidental Lewy body disease (ILBD) is the term used when Lewy bodies are found in the nervous system of subjects without clinically documented parkinsonism or dementia. The prevalence of ILBD in the elderly population has been estimated at between 3.8 and 30%, depending on subject age and anatomical site of sampling. It has been speculated that ILBD represents the preclinical stage of Parkinson's disease (PD) and/or dementia with Lewy bodies (DLB). Studies of ILBD could potentially identify early diagnostic signs of these disorders. At present, however, it is impossible to know whether ILBD is a precursor to PD or DLB or is just a benign finding of normal aging. We hypothesized that, if ILBD represents an early stage of PD or DLB, it should be associated with depletion of striatal dopaminergic markers. Eleven subjects with ILBD and 27 control subjects were studied. The ILBD subjects ranged in age from 74 to 96 years (mean 86.5) while the control subjects' age ranged from 75 to 102 years (mean 86.7). Controls and subjects did not differ in terms of age,

postmortem interval, gender distribution, medical history conditions, brain weight, neuritic plaque density or Braak neurofibrillary stage. Quantitative ELISA measurement of striatal tyrosine hydroxylase (TH), the principal enzyme for dopamine synthesis, showed a 49.8% ($P = 0.01$) reduction in ILBD cases, as compared with control cases. The finding suggests that ILBD is not a benign condition but is likely a precursor to PD and/or DLB.

Keywords

Striatum; Dopamine; Parkinson's disease; Lewy bodies; Pathogenesis; Aging

Introduction

Incidental Lewy body disease (ILBD) may be defined as the presence of Lewy bodies in the absence of clinically documented parkinsonism or dementia. The prevalence of ILBD in the elderly population increases with age. An early prevalence estimate, based on paraffin-sections stained with hematoxylin and eosin, found ILBD to be present in 3.8% of people in the 6th decade, rising to 12.8% by the 9th decade [20]. Subsequent estimates, performed using α -synuclein immunohistochemistry to detect Lewy bodies and neurites, have found ILBD in 16% [42], 17% [8], 26% [34] and 30% [26] of aged subjects. The differences between studies may be due to differing immunohistochemical methods, differing subject ages and differing sampling sites. Although Parkinson's disease (PD) is generally considered a disease of the central nervous system (CNS), it has long been known that Lewy bodies may also be found in the peripheral autonomic nervous system in PD subjects [15,18,36,37,46,49]. Recent studies using immunohistochemical staining for α -synuclein have found that the peripheral autonomic nervous system is a common site of affectation in ILBD.

It has been speculated that ILBD represents the preclinical stage of PD and/or dementia with Lewy bodies (DLB). If this were true, studies of ILBD could identify early clinical or pathological signs that could be used to diagnose PD and/or DLB much earlier than is presently possible. Due to the involvement of the olfactory bulb and peripheral autonomic nervous system in ILBD, such signs might consist of a constellation of autonomic and olfactory dysfunction. Both olfactory dysfunction and constipation have already been reported to be statistically associated with ILBD [2,41]. However, at present, it is impossible to know whether ILBD is a precursor to PD or DLB or is just a non-progressive benign finding of normal aging. We hypothesized that, as depletion of the dopaminergic nigrostriatal system is a defining and pathogenic feature of PD and DLB, its presence in ILBD would suggest that ILBD is not a benign disorder but a probable early stage of one or the other.

Materials and methods

Human subjects

The study took place at Sun Health Research Institute (SHRI), which is part of a non-profit, community-owned and operated health care provider located in the Sun Cities retirement communities of northwest metropolitan Phoenix, Arizona. Sun Health Research Institute and the Mayo Clinic Arizona are the principal member institutes of the Arizona Parkinson's Disease Consortium. Brain necropsies were performed on elderly subjects who had volunteered for the SHRI Brain Donation Program. The Brain Donation Program has been approved by the Institutional Review Board of Sun Health Research Institute. Subjects were chosen by searching the Brain Donation Program Database. Subjects with ILBD were defined as those that had Lewy bodies on neuropathological examination but did not have a clinical diagnosis of parkinsonism or dementia. Control subjects were chosen based on the absence of Lewy bodies, parkinsonism and dementia. Additionally, both ILBD and control subjects were chosen

on the basis of availability of fresh-frozen striatum for biochemical analysis. Subjects were clinically characterized by review of medical records, self-report and interviews with spouses and/or caregivers. As part of the Brain Donation Program's standard protocol, private medical records of 2 years are obtained from the subjects' private physicians, both at the time of enrollment (for those prospectively enrolled) and at the time of death. Subjects who sign up prospectively (before death) fill out a questionnaire in which they (or their spouses or caregivers) are asked to report any significant neurological conditions. Additionally, at the time of death, a telephone interview is conducted with the spouse or caregiver, in which they are asked whether the subject was mentally capable, demented or had Alzheimer's disease. If they were demented or had Alzheimer's disease, they are asked as to when that diagnosis was first given. They are also asked if the subject had any shaking or jerking movements and if so, to describe those and state as to when they were first observed. They are also asked to state all of the subject's known medical diagnoses. Since 1997, a subset of subjects has received standardized cognitive and neuromotor assessments, including the Mini Mental State Examination (MMSE) and Unified Parkinson's Disease Rating Scale (UPDRS).

Tissue processing and analysis

The cerebrum was cut in the coronal plane into 1-cm-thick slices while the brainstem was sliced axially at 1-cm intervals. The entire brain was then divided sagittally into left and right halves. The slices from the right half were frozen between slabs of dry ice while the slices from the left half were fixed by immersion into buffered 4% formaldehyde for 48 h at 4°C. Following cryoprotection in ethylene glycol and glycerol, selected cerebral and midbrain blocks were cut into sections of 40 µm thickness on a sliding freezing microtome. Additionally, a standard set of brainstem, cerebellar and cerebral blocks were embedded in paraffin and cut at 6 µm. Free-floating 40-µm sections were stained with H&E, thioflavine S, Campbell-Switzer and Gallyas methods. Paraffin sections were stained with H&E and a selected set was stained immunohistochemically for α-synuclein (clone LB509, Chemicon), after pretreatment with 90% formic acid. Additionally, selected 40-µm sections were stained immunohistochemically for α-synuclein, using a separate antiserum raised against α-synuclein phosphorylated at serine 129 [19]. The immunoperoxidase method employed an avidin-biotin-horseradish peroxidase complex (ABC); the method has been generally described in a prior publication [5]. Neuritic plaques and neurofibrillary tangles were staged according to CERAD, Braak and NIA-Reagan criteria [1,9,35]. Lewy bodies and Lewy-related neurites (Fig. 1) were identified with H&E, thioflavine S and immunohistochemical methods and staged according to the Dementia with Lewy Bodies Consortium and criteria set forth by Braak et al. [11,33].

Frozen putamen from the level of the globus pallidus pars interna was dissected out and analyzed quantitatively for TH using an ELISA. This site has been reported to show the earliest loss of striatal dopamine in PD [6]. Samples were solubilized in four volumes of extraction buffer composed of 50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 1% Triton X 100, 0.2% SDS and protease inhibitors (Roche Biochemicals, Indianapolis, IN, USA), and centrifuged at 13,000 rpm for 15 min; the supernatant was used to measure TH protein levels. Prior to analyses, samples were diluted to contain equal amounts of protein (10 µg/100 µl). The TH ELISA consisted of an affinity-purified rabbit antibody to tyrosine hydroxylase (Covance Inc, Berkeley, CA, USA) to coat the plates (final concentration 0.5 µg/ml) as capture reagent, and a mouse monoclonal antibody to TH (Sigma-Aldrich, St. Louis, MO, USA) as detection reagent (1:6,000 dilution, final concentration). The amounts of bound antibody-complexes were detected using a horseradish peroxidase-labeled anti-mouse immunoglobulin, reaction with ELISA substrate (R and D Systems, Minneapolis, MN, USA), and measurement of the absorbance in each well at 450 nm wavelength. The concentration of TH in each sample was determined in comparison to standard curves constructed using known amounts of recombinant TH (kindly provided by Dr. Paul Fitzpatrick, Texas A&M University, TX, USA). A number

of the protein extracts of putamen that had been analyzed by ELISA were also assessed qualitatively by standard Western blot methodology. Membranes were probed with the TH antibody used for ELISA capture, which was detected using an HRP-labeled anti-rabbit immunoglobulin and reaction with Supersignal Dura chemiluminescent substrate (Pierce Chemical Co, Rockland, IL, USA) followed by imaging on an Fluorochem 8900 imaging system (Alpha Innotech, San Leandro, CA, USA).

Statistical analysis

Statistical analyses consisted of unpaired, two-tailed *t*-tests for comparisons of continuous variables. The Mann–Whitney *U*-test was used for comparisons of discontinuous variables. Semi-quantitative scores for neuritic plaques (none, sparse, moderate, frequent), neurofibrillary tangles (Braak stages I–VI) and Lewy bodies (DLB Consortium stages brainstem predominant, limbic and neocortical; Braak stages I–VI) were converted to numerical equivalents (0, 1, 2, 3 for plaques; 0–6 for tangles; 0–3 for DLB Consortium Lewy body score; 0–6 for Braak Lewy body stage) prior to analysis. Prevalence of medical history conditions were compared using Chi-square tests while MMSE and UPDRS scores were compared using unpaired, two-tailed *t*-tests.

Results and discussion

Using the search criteria described, 11 subjects with ILBD and 27 control subjects were identified. The ILBD subjects ranged in age from 74 to 96 years (mean 86.5; SD = 5.9) while the control subjects ranged from 75 to 102 years (mean 86.7; SD = 7.4). Controls and ILBD cases did not differ in terms of age, postmortem interval, gender distribution, brain weight, neuritic plaque density or Braak neurofibrillary stage (Table 1). A subset of subjects had received standardized cognitive (seven ILBD and seven control subjects) and neuromotor (six ILBD and six control subjects) assessments. The groups did not differ significantly in their MMSE or UPDRS (UPDRS: Part III-Motor Scores). The mean MMSE scores were 27.0 (SD 2.2) and 28.1 (SD 1.9) for ILBD and control cases, respectively, while the mean UPDRS scores were 13.7 (SD 12.0) and 20.4 SD (7.2), respectively.

Six ILBD cases were rated as brainstem predominant and five were rated as limbic or transitional, using the DLB Consortium system. Lewy body stage according to the Braak system ranged from 2 to 4, with 6 cases rated as stage 2 or 3 and 5 rated as stage 4 (see Table 3).

Review of medical records in all 11 ILBD cases and 25 control cases (two control cases did not have medical records available) showed that the average time between the last physician's note and death was 5.4 months (SD 8.4). The most common medical history conditions in ILBD cases were supraventricular arrhythmia, hypertension, falls, dysphagia and depression (Table 2). On comparison with the control group, however, none of these were significantly different on Chi-square analysis.

Quantitative measurement of striatal TH by ELISA (Fig. 2) showed a significant reduction in ILBD cases to 49.8% of control values (ILBD mean = 17.5 ng/mg protein, SD = 14.1; control mean = 35.1 ng/mg protein, SD = 20.0; *P* = 0.01) (Table 3).

The major finding of this report is that ILBD cases have significant reductions of striatal TH concentrations as compared with age-similar normal elderly subjects. As TH is a dopaminergic marker, the results indicate that the nigrostriatal dopaminergic system is significantly depleted in these individuals, suggesting that ILBD is not a benign condition but is likely a precursor to PD and/or DLB. This result contrasts with that of an earlier study, which found no difference in striatal dopamine levels between control and ILBD cases [16]. It is possible that the

difference between ILBD cases and controls in this study may have been obscured by the long PMIs (means were 14 and 21 h for controls and cases, respectively) for the 12 cases they studied. Although the authors found no correlation between PMI and dopamine levels for their subjects, a larger study of 30 cases [13] reported a significant inverse correlation between PMI and striatal dopamine concentrations (correlation coefficient -0.53 , $P < 0.01$). Alternatively, as we measured TH rather than dopamine, it is possible that a decreased TH protein level might be compensated for, in the ILBD striatum, by increased TH functional activity, decreased dopamine catabolism, or other mechanisms. It has generally been established, through both human and primate studies, that parkinsonian symptoms occur when striatal dopaminergic markers are 80% depleted compared to age-similar normal subjects [7,23]. The finding that ILBD subjects have approximately 50% reductions of striatal TH is therefore consistent with their lack of clinically detectable parkinsonian signs. The results for TH support previous studies showing that substantia nigra pigmented neuron numbers are depleted in ILBD, falling between control and PD subjects [17].

It has been reported that striatal pathology may differentiate Parkinson's disease with dementia from dementia with Lewy bodies [27]. We stained the striatum of all cases with the Campbell–Switzer method for amyloid and found 7 of 27 cases were positive in the control subjects while 6 of 11 ILBD cases were positive. The difference did not reach the significance level on a Chi-square test ($P = 0.09$), but is suggestive of a greater likelihood for striatal amyloid deposition in ILBD.

Study of ILBD subjects may allow detection of non-parkinsonian clinical features that would be predictive of the development of PD and/or DLB. It has been suggested, for example, that olfactory dysfunction, depression and autonomic nervous system dysfunction may be predictive for subsequent PD [3,22,29]. Olfactory bulb and peripheral autonomic nervous system synucleinopathy, are very common if not universal findings in PD [10–12,14,37,39, 46,48,49]. Olfactory dysfunction and olfactory bulb synucleinopathy have been reported to be present in ILBD cases and hence smell tests and/or olfactory bulb biopsy may be useful for early diagnosis in PD and/or DLB.

Review of the private medical records of the ILBD and control cases did not reveal significant disease associations. As there are multiple reports of synucleinopathy of the cardiac sympathetic innervation in PD and DLB [4,21,25,32,43,45,47], an increased prevalence of cardiac arrhythmias in ILBD might have been predicted. Similarly, depression has been suggested to be a common early symptom in PD [24,31]. However, although supraventricular arrhythmias and depression were common in our ILBD cases, they were also common in our control cases. Hypertension, which has been reported to be less common in PD [38,44] was common in our ILBD cases but was somewhat more common in the control cases. Dysphagia has also been reported to be a potential early marker for PD [28,30,40] but this was present to a similar degree in both our ILBD and control subjects. Falls are usually considered a later-stage feature of PD [50,51], so it was surprising to see this as a common finding in ILBD cases. Falling was, however, also common in the control cases, suggesting that this is age-related. The lack of differences between ILBD subjects and controls in our study may be due to their advanced ages or to the small sample size, but it appears from this study that there are no large medical history differences between the groups. Larger studies, with the inclusion of standardized ante-mortem clinical assessments, are needed to fully determine whether there are any specific disease associations with ILBD.

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References

1. 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. [PubMed: 9330978]
2. Abbott RD, Petrovitch H, White LR, Masaki KH, Tanner CM, Curb JD, Grandinetti A, Blanchette PL, Popper JS, Ross GW. Frequency of bowel movements and the future risk of Parkinson's disease. *Neurology* 2001;57:456–462. [PubMed: 11502913]
3. Adler CH. Nonmotor complications in Parkinson's disease. *Mov Disord* 2005;20:S23–S29. [PubMed: 15822106]
4. Amino T, Orimo S, Itoh Y, Takahashi A, Uchihara T, Mizusawa H. Profound cardiac sympathetic denervation occurs in Parkinson disease. *Brain Pathol* 2005;15:29–34. [PubMed: 15779234]
5. Beach TG, Tago H, Nagai T, Kimura H, McGeer PL, McGeer EG. Perfusion-Wxation of the human brain for immunohistochemistry: comparison with immersion-fixation. *J Neurosci Methods* 1987;19:183–192. [PubMed: 2437408]
6. Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K, Seitelberger F. Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. *J Neurol Sci* 1973;20:415–455. [PubMed: 4272516]
7. Bezard E, Dovero S, Prunier C, Ravenscroft P, Chalon S, Guilloteau D, Crossman AR, Bioulac B, Brotchie JM, Gross CE. Relationship between the appearance of symptoms and the level of nigrostriatal degeneration in a progressive 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned macaque model of Parkinson's disease. *J Neurosci* 2001;21:6853–6861. [PubMed: 11517273]
8. Bloch A, Probst A, Bissig H, Adams H, Tolnay M. Alpha-synuclein pathology of the spinal and peripheral autonomic nervous system in neurologically unimpaired elderly subjects. *Neuropathol Appl Neurobiol* 2006;32:284–295. [PubMed: 16640647]
9. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol (Berl)* 1991;82:239–259. [PubMed: 1759558]
10. Braak H, Del Tredici K, Bratzke H, Hamm-Clement J, Sandmann-Keil D, Rub U. Staging of the intracerebral inclusion body pathology associated with idiopathic Parkinson's disease (preclinical and clinical stages). *J Neurol* 2002;249:III/1–III/5.
11. 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. [PubMed: 12498954]
12. 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. [PubMed: 15338272]
13. Carlsson A, Winblad B. Influence of age and time interval between death and autopsy on dopamine and 3-methoxytyramine levels in human basal ganglia. *J Neural Transm* 1976;38:271–276. [PubMed: 956813]
14. Daniel SE, Hawkes CH. Preliminary diagnosis of Parkinson's disease by olfactory bulb pathology. *Lancet* 1992;340:186. [PubMed: 1352606]
15. den Hartog Jager WA, Bethlem J. The distribution of Lewy bodies in the central and autonomic nervous systems in idiopathic paralysis agitans. *J Neurol Neurosurg Psychiatry* 1960;23:283–290. [PubMed: 13711997]
16. Dexter DT, Sian J, Rose S, Hindmarsh JG, Mann VM, Cooper JM, Wells FR, Daniel SE, Lees AJ, Schapira AH. Indices of oxidative stress and mitochondrial function in individuals with incidental Lewy body disease. *Ann Neurol* 1994;35:38–44. [PubMed: 8285590]
17. Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain* 1991;114:2283–2301. [PubMed: 1933245]
18. Forno LS, Norville RL. Ultrastructure of Lewy bodies in the stellate ganglion. *Acta Neuropathol (Berl)* 1976;34:183–197. [PubMed: 178142]

19. Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, Masliah E, Goldberg MS, Shen J, Takio K, Iwatsubo T. Alpha-Synuclein is phosphorylated in synucleinopathy lesions. *Nat Cell Biol* 2002;4:160–164. [PubMed: 11813001]
20. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *J Neurol Neurosurg Psychiatry* 1988;51:745–752. [PubMed: 2841426]
21. Goldstein DS, Holmes C, Li ST, Bruce S, Metman LV, Cannon RO III. Cardiac sympathetic denervation in Parkinson disease. *Ann Intern Med* 2000;133:338–347. [PubMed: 10979878]
22. Hawkes C. Olfaction in neurodegenerative disorder. *Adv Otorhinolaryngol* 2006;63:133–151. [PubMed: 16733338]
23. Hornykiewicz O. Parkinson's disease and the adaptive capacity of the nigrostriatal dopamine system: possible neurochemical mechanisms. *Adv Neurol* 1993;60:140–147. [PubMed: 8420131]
24. Ishihara L, Brayne C. A systematic review of depression and mental illness preceding Parkinson's disease. *Acta Neurol Scand* 2006;113:211–220. [PubMed: 16542159]
25. Iwanaga K, Wakabayashi K, Yoshimoto M, Tomita I, Satoh H, Takashima H, Satoh A, Seto M, Tsujihata M, Takahashi H. Lewy body-type degeneration in cardiac plexus in Parkinson's and incidental Lewy body diseases. *Neurology* 1999;52:1269–1271. [PubMed: 10214756]
26. Jellinger KA. Lewy body-related alpha-synucleinopathy in the aged human brain. *J Neural Transm* 2004;111:1219–1235. [PubMed: 15480835]
27. Jellinger KA, Attems J. Does striatal pathology distinguish Parkinson disease with dementia and dementia with Lewy bodies? *Acta Neuropathol (Berl)* 2006;112:253–260. [PubMed: 16804711]
28. Johnston BT, Li Q, Castell JA, Castell DO. Swallowing and esophageal function in Parkinson's disease. *Am J Gastroenterol* 1995;90:1741–1746. [PubMed: 7572887]
29. Katzenschlager R, Lees AJ. Olfaction and Parkinson's syndromes: its role in differential diagnosis. *Curr Opin Neurol* 2004;17:417–423. [PubMed: 15247536]
30. Kovari E, Burkhardt K, Lobrinus JA, Bouras C. Lewy body dysphagia. *Acta Neuropathol (Berl)* 2007;114:295–298. [PubMed: 17576582]
31. Lemke MR, Fuchs G, Gemende I, Herting B, Oehlwein C, Reichmann H, Rieke J, Volkmann J. Depression and Parkinson's disease. *J Neurol* 2004;251:VI/24–VI/27.
32. Li ST, Dendi R, Holmes C, Goldstein DS. Progressive loss of cardiac sympathetic innervation in Parkinson's disease. *Ann Neurol* 2002;52:220–223. [PubMed: 12210793]
33. McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, Cummings J, Duda JE, Lippa C, Perry EK, Aarsland D, Arai H, Ballard CG, Boeve B, Burn DJ, Costa D, Del Ser T, Dubois B, Galasko D, Gauthier S, Goetz CG, Gomez-Tortosa E, Halliday G, Hansen LA, Hardy J, Iwatsubo T, Kalaria RN, Kaufer D, Kenny RA, Korczyn A, Kosaka K, Lee VM, Lees A, Litvan I, Londo E, Lopez OL, Minoshima S, Mizuno Y, Molina JA, Mukaetova-Ladinska EB, Pasquier F, Perry RH, Schulz JB, Trojanowski JQ, Yamada M. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology* 2005;65:1863–1872. [PubMed: 16237129]
34. Minguez-Castellanos A, Chamorro CE, Escamilla-Sevilla F, Ortega-Moreno A, Rebollo AC, Gomez-Rio M, Concha A, Munoz DG. Do alpha-synuclein aggregates in autonomic plexuses predate Lewy body disorders?: a cohort study. *Neurology* 2007;68:2012–2018. [PubMed: 17548551]
35. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G, Berg L. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 1991;41:479–486. [PubMed: 2011243]
36. Nakajima R, Takahashi K, Nakamura H, Otomo E, Kameyama M. A quantitative study on the intermediolateral cells of the thoracic cord in degenerative diseases of the nervous system (author's transl). *Rinsho Shinkeigaku* 1981;21:581–586. [PubMed: 7307398]
37. Oyanagi K, Wakabayashi K, Ohama E, Takeda S, Horikawa Y, Morita T, Ikuta F. Lewy bodies in the lower sacral parasympathetic neurons of a patient with Parkinson's disease. *Acta Neuropathol (Berl)* 1990;80:558–559. [PubMed: 2251914]
38. Paganini-Hill A. Risk factors for Parkinson's disease: the leisure world cohort study. *Neuroepidemiology* 2001;20:118–124. [PubMed: 11359079]
39. Pearce RK, Hawkes CH, Daniel SE. The anterior olfactory nucleus in Parkinson's disease. *Mov Disord* 1995;10:283–287. [PubMed: 7651444]

40. Potulska A, Friedman A, Krolicki L, Spychala A. Swallowing disorders in Parkinson's disease. *Parkinsonism Relat Disord* 2003;9:349–353. [PubMed: 12853234]
41. Ross GW, Abbott RD, Petrovitch H, Tanner CM, Davis DG, Nelson J, Markesbery WR, Hardman J, Masaki K, Launer L, White LR. Association of olfactory dysfunction with incidental Lewy bodies. *Mov Disord* 2006;21:2062–2067. [PubMed: 16991138]
42. Saito Y, Ruberu NN, Sawabe M, Arai T, Kazama H, Hosoi T, Yamanouchi H, Murayama S. Lewy body-related alpha-synucleinopathy in aging. *J Neuropathol Exp Neurol* 2004;63:742–749. [PubMed: 15290899]
43. Satoh A, Serita T, Seto M, Tomita I, Satoh H, Iwanaga K, Takashima H, Tsujihata M. Loss of 123I-MIBG uptake by the heart in Parkinson's disease: assessment of cardiac sympathetic denervation and diagnostic value. *J Nucl Med* 1999;40:371–375. [PubMed: 10086697]
44. Scigliano G, Musicco M, Soliveri P, Piccolo I, Ronchetti G, Girotti F. Reduced risk factors for vascular disorders in Parkinson disease patients: a case-control study. *Stroke* 2006;37:1184–1188. [PubMed: 16574924]
45. Sharabi Y, Li ST, Dendi R, Holmes C, Goldstein DS. Neurotransmitter specificity of sympathetic denervation in Parkinson's disease. *Neurology* 2003;60:1036–1039. [PubMed: 12654979]
46. Takeda S, Yamazaki K, Miyakawa T, Arai H. Parkinson's disease with involvement of the parasympathetic ganglia. *Acta Neuropathol (Berl)* 1993;86:397–398. [PubMed: 8256591]
47. Taki J, Yoshita M, Yamada M, Tonami N. Significance of 123I-MIBG scintigraphy as a pathophysiological indicator in the assessment of Parkinson's disease and related disorders: it can be a specific marker for Lewy body disease. *Ann Nucl Med* 2004;18:453–461. [PubMed: 15515743]
48. Tsuboi Y, Wszolek ZK, GraV-Radford NR, Cookson N, Dickson DW. Tau pathology in the olfactory bulb correlates with Braak stage, Lewy body pathology and apolipoprotein epsilon4. *Neuropathol Appl Neurobiol* 2003;29:503–510. [PubMed: 14507342]
49. Wakabayashi K, Takahashi H, Takeda S, Ohama E, Ikuta F. Parkinson's disease: the presence of Lewy bodies in Auerbach's and Meissner's plexuses. *Acta Neuropathol (Berl)* 1988;76:217–221. [PubMed: 2850698]
50. Wenning GK, Ebersbach G, Verny M, Chaudhuri KR, Jellinger K, McKee A, Poewe W, Litvan I. Progression of falls in postmortem-confirmed parkinsonian disorders. *Mov Disord* 1999;14:947–950. [PubMed: 10584668]
51. Wood BH, Bilclough JA, Bowron A, Walker RW. Incidence and prediction of falls in Parkinson's disease: a prospective multidisciplinary study. *J Neurol Neurosurg Psychiatry* 2002;72:721–725. [PubMed: 12023412]

Fig 1.

Brain sections from ILBD cases depicting Lewy bodies and Lewy-related neuritic pathology.

a Paraffin-embedded section stained immunohistochemically for α -synuclein, showing multiple neuritic processes and Lewy bodies in the medullary dorsal motor nucleus of the vagus nerve. **b** A 40- μ m-thick section stained with an immunoperoxidase method for α -synuclein phosphorylated at serine 129, showing multiple neuritic processes and neurons with diffusely-stained cytoplasm in the intermediate reticular zone of the pontine tegmentum. **c** A 40- μ m-thick section stained with thioflavine S, showing a pigmented neuron of the substantia nigra pars compacta with several fluorescent intracytoplasmic Lewy bodies. **d** A 40- μ m-thick section stained with an immunoperoxidase method for α -synuclein phosphorylated at serine 129, showing many positive neuritic processes and a single Lewy body in the entorhinal cortex

Fig 2. Graph showing the concentrations of tyrosine hydroxylase (TH), as measured by ELISA, in the striatum of ILBD and control cases. The concentrations of TH were depleted in ILBD cases to 49.8% of control levels ($P = 0.01$)

Table 1
Demographic and neuropathologic characteristics of study subjects

Diagnosis	Age (years)	Gender	PMI (h)	Brain weight (g)	NP density	Braak NF Stage
ILBD (N = 11)	86.5 (5.9)	6M, 5F	3.4 (3.9)	1,195 (115)	1.0 (0.77)	2.5 (1.2)
Control (N = 27)	86.7 (7.4)	12M, 15F	2.6 (7.4)	1,183 (150)	0.79 (0.78)	2.6 (1.0)

Values shown (except for gender distribution) are means with standard deviations in parentheses. Control and ILBD subjects were not significantly different with respect to any of these measures
PMI Postmortem interval; NP neuritic plaque; NF neurofibrillary

Table 2

Frequent findings of private physicians in incidental Lewy body disease cases and controls

Finding	ILBD	Control
Supraventricular arrhythmias (Afib, SVT, SSS)	5/11 (45%)	12/25 (48%)
Hypertension	5/11 (45%)	15/25 (60%)
Falls	4/11 (36%)	11/25 (44%)
Dysphagia	4/11 (36%)	7/25 (28%)
Depression	4/11 (36%)	8/25 (32%)

Afib Atrial Wbrillation; *SVT* supraventricular tachycardia; *SSS* sick sinus syndrome

Table 3
Lewy body regional distribution and stage in incidental Lewy body disease cases

Case	med	pons	mid	nbM	Amyg	ecx/tr	cing	neocx	DLBst	BKLB	BKNF
7	+	+	—	—	—	—	—	—	Bstem	2	I
1	+	+	+	—	—	—	—	—	Bstem	3	IV
2	+	+	+	+	—	—	—	—	Bstem	3	III
4	+	+	+	+	—	—	—	—	Bstem	3	IV
5	+	+	+	+	—	—	—	—	Bstem	3	I
6	+	+	+	+	+	+	—	—	Limbic	4	II
3	+	+	—	+	+	—	—	—	Limbic	4	IV
8	+	+	+	+	+	+	+	—	Limbic	4	I
9	+	+	+	+	+	+	—	—	Limbic	4	III
10	+	+	+	+	—	+	—	—	Limbic	4	I
11	+	+	+	+	+	+	+	—	Limbic	4	III

DLBst Dementia with Lewy Bodies Consortium stage; *BKLB* Braak Lewy body stage; *BKNF* Braak neurofibrillary stage; *med* medulla; *mid* midbrain; *nbM* nucleus basalis of Meynert; *amyg* amygdala; *ecx/tr* entorhinal cortex and/or transentorhinal cortex; *cing* cingulate gyrus; *neocx* neo-cortex; *Bstem* brainstem predominant stage; *Limbic* limbic or transitional stage