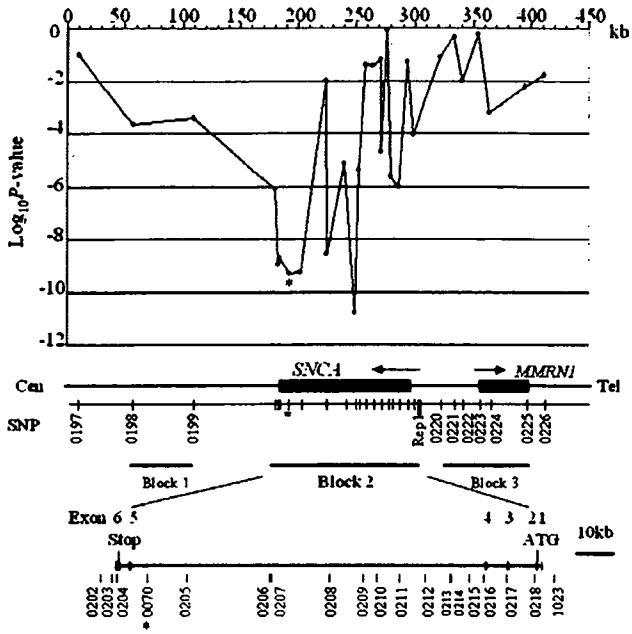

Figure 2. Genomic structure and SNPs of the susceptibility region for sporadic PD and case–control association studies (882 cases and 938 controls). Log *P*-values (allele 1 versus allele 2) are plotted against the physical location of the SNPs. The region includes two genes: *SNCA* and *MMRN1*; transcription orientation is indicated by horizontal arrows. Physical locations of SNPs are shown as axial bars with our experimental ID number. The originally screened SNP0070 is indicated by an asterisk. The location of Rep1, a well-known repeat polymorphism in the *SNCA* promoter region, is indicated by a thick bar. SNPs in block 2 are nominated in an expanded map with the exon–intron structure of *SNCA*. SNPs in group 1 are shown in red. Note that *P*-values are prominently low at the group 1 SNPs located in the 3′ region of *SNCA*. *P*-values in the region around Rep1 are far from significant when compared with those in group 1.

was observed in controls. The mean tended to decrease in the order of CC, CT and TT (Fig. 3) ($P = 0.33$ for CC versus CT, $P = 0.59$ for CT versus TT and $P = 0.54$ for CC versus TT).

These results indicate the possibility that expression of *SNCA* mRNA in the brain tends to be positively correlated with the number of PD-associated allele.

DISCUSSION

To identify susceptibility genes for PD, we performed an extensive candidate gene approach by screening 268 SNPs in 121 genes and identified a prominent association with SNP0070 (rs7684318) in the *SNCA* gene (Table 1). LD mapping localized the entire *SNCA* gene within a single LD block (Figs 1 and 2). Within this block, six SNPs including SNP0070 were in a tight LD group and most strongly associated with PD (Fig. 2; Table 2). The major allele of each SNP in group 1 was positively associated with PD, more strongly in the recessive model than in the dominant model (Table 4). Our genetic analyses establish *SNCA* as a definite susceptibility gene for PD and identify multiple SNPs in group 1 as susceptibility SNPs. Recently, Mueller *et al.* (23) reported that multiple regions of *SNCA* are associated with PD in the German population. Associated SNPs identified by Mueller *et al.* included rs356165 ($P = 1.5 \times 10^{-4}$), which corresponds to SNP0204 in our study, indicating that this SNP has a similar association in Caucasians. Pals *et al.* (24) previously reported no association of the haplotype containing rs356165 with PD in Belgian samples. This contradictory finding may be, at least in part, due to a small sample size (175 cases and 186 controls), as mentioned by the authors.

SNCA/α-synuclein was originally identified in the electric organ of the Pacific electric ray (25). SNCA is a presynaptic protein that is highly and broadly expressed in the brain, but its normal function remains unknown (21). It is a major component of Lewy bodies, the pathological hallmark of PD (20), and the aggregation of SNCA protein is thought to play a crucial role in the loss of dopaminergic neurons (21,26).

SNCA was also the first gene identified as a causative gene in familial PD. Three missense mutations in *SNCA* were reported in families with AD inheritance (6,27,28). These mutations are thought to increase the aggregation of SNCA protein. Point mutations in *SNCA* have not been identified in sporadic PD (27,29), and no SNPs have been found in the coding region, suggesting that disease-related amino acid changes in SNCA are unlikely in sporadic PD.

Genes' overdosage is a potential mechanism for the influence of *SNCA* in PD. Triplication of the *SNCA* locus has been seen in an AD PD family (30), and doubling of *SNCA* gene dosage by triplication has been shown to result in the doubling of mRNA and protein expression in blood and brain (31). Duplication of *SNCA* has also been identified as a cause of familial PD (32,33). Clinical features of patients with *SNCA* duplication resemble those of sporadic cases and are much milder than those with triplication. Taken together, these observations indicate a correlation between increased SNCA protein levels and disease risk. Identification of one or more polymorphisms related to *SNCA* expression level might reveal strong susceptibility indicators for sporadic PD. Many studies have focussed on a mixed repeat microsatellite polymorphism called Rep1 (34), because of its location in the *SNCA* promoter region. However, their significance is uncertain, possibly because of the small number of samples (35–37). Our study demonstrates that the *P*-values of SNPs around Rep1 (0218, 1023 and 0220) are less significant than that of the SNPs in group 1 (Fig. 2). In addition, we genotyped our samples for Rep1. Pairwise *D′*-values showed that Rep1 was not in block 2, but on the boundary (Supplementary Material, Table S2). *P*-value of Rep1 was 7.5×10^{-7} (Supplementary Material, Table S3), which might be explained by its intermediate correlation with the strongest susceptibility SNPs (group 1, $P = 2.0 \times 10^{-9}$–1.7×10^{-11}). Our findings suggest that *P*-value of Rep1 depends on its LD strength with SNPs in group 1. LD strength may be modified by the unstableness of microsatellite markers (38) and may vary among races (39). Taken together, these findings may also partly explain the contradictory findings of previous Rep1 association studies.

To investigate the relationship between the SNPs in group 1 and the *SNCA* expression levels, we analyzed *SNCA* mRNA expression in autopsied frontal cortices (Fig. 3). *SNCA* expression levels tended to be positively correlated with the number of the PD-associated allele, supporting the popular hypothesis that increased SNCA leads to the disease.

Table 2. Association analysis in *SNCA* and surrounding region

SNPs			LD block	Genotype							
ID (rs ID)	Alleles 12	Location	(group)	Case 11/12/22	(Total)	Control 11/12/22	(Total)	MAF Case/control	Allele 1 versus allele 2 *P*-value	OR (95% CI)	HWE Case/control
0197 (rs3733450)	TC		1	38/286/549	(873)	33/280/619	(932)	0.21/0.19	0.10	1.15 (0.97–1.36)	1.00/0.93
0198 (rs1390280)	AG		1	366/384/118	(868)	316/454/162	(932)	0.36/0.42	2.1×10^{-4}	1.29 (1.13–1.46)	0.32/1.00
0199 (rs3733449)	CT		1	117/375/374	(866)	154/451/322	(927)	0.35/0.41	3.7×10^{-4}	1.28 (1.11–1.48)	0.16/0.91
0202 (rs356221)	TA	3′-flanking	2	73/369/431	(873)	123/449/360	(932)	0.30/0.37	7.2×10^{-7}	1.42 (1.25–1.63)	0.69/0.40
0203 (rs3857053)	TC	3′-flanking	2 (1)	380/406/87	(873)	293/476/164	(933)	0.33/0.43	1.1×10^{-9}	1.53 (1.33–1.73)	0.18/0.24
0204 (rs356165)	GA	3′-UTR	2 (1)	379/399/89	(867)	289/482/159	(930)	0.33/0.43	2.0×10^{-9}	1.52 (1.33–1.74)	0.32/0.09
0070[a] (rs7684318)	CT	Intron 4	2 (1)	385/394/89	(868)	295/472/165	(932)	0.33/0.43	5.0×10^{-10}	1.54 (1.35–1.75)	0.47/0.35
0205 (rs3775424)	CT	Intron 4	2 (1)	87/406/376	(869)	166/477/288	(931)	0.33/0.43	5.4×10^{-10}	1.52 (1.34–1.75)	0.16/0.22
0206 (rs3775426)	CT	Intron 4	2 (2)	56/350/456	(862)	53/324/555	(932)	0.27/0.23	0.0098	1.22 (1.05–1.41)	0.35/0.59
0207 (rs3796661)	CT	Intron 4	2 (1)	91/367/382	(840)	154/482/296	(932)	0.33/0.42	2.7×10^{-9}	1.52 (1.31–1.76)	0.90/0.08
0208 (rs3775435)	GA	Intron 4	2 (3)	157/434/272	(863)	115/439/375	(929)	0.43/0.36	7.3×10^{-6}	1.36 (1.18–1.56)	0.53/0.48
0209 (rs2737029)	TC	Intron 4	2 (1)	84/377/402	(863)	156/480/297	(933)	0.32/0.42	1.7×10^{-11}	1.60 (1.40–1.83)	0.81/0.12
0210 (rs3775442)	TC	Intron 4	2 (3)	158/438/274	(870)	114/440/378	(932)	0.43/0.36	4.2×10^{-6}	1.37 (1.19–1.58)	0.50/0.46
0211 (rs3756055)	GA	Intron 4	2 (2)	50/339/481	(870)	49/319/565	(933)	0.25/0.22	0.042	1.17 (1.00–1.37)	0.38/0.72
0212 (rs3775446)	TG	Intron 4	2 (2)	50/340/480	(870)	49/317/565	(931)	0.25/0.22	0.034	1.19 (1.01–1.38)	0.36/0.67
0213 (rs3756056)	CT	Intron 4	2 (2)	50/340/482	(872)	48/323/557	(928)	0.25/0.23	0.062	1.16 (0.99–1.34)	0.37/0.97
0214 (rs894278)	GT	Intron 4	2 (3)	156/438/275	(869)	117/441/375	(933)	0.43/0.36	1.9×10^{-5}	1.34 (1.18–1.52)	0.46/0.52
0215 (rs1812923)	CA	Intron 4	2	74/383/413	(870)	92/392/447	(931)	0.31/0.31	0.79	1.01 (0.89–1.16)	0.30/0.71
0216 (rs2298728)	AG	Intron 4	2 (3)	163/432/274	(869)	117/435/380	(932)	0.44/0.36	2.2×10^{-6}	1.38 (1.22–1.56)	0.80/0.72
0217 (rs3796667)	AT	Intron 3	2 (3)	159/430/271	(860)	114/428/383	(925)	0.44/0.36	9.2×10^{-7}	1.41 (1.23–1.61)	0.66/0.80
0218 (rs2035268)	TG	Intron 2	2 (2)	475/339/54	(868)	556/326/51	(933)	0.26/0.23	0.049	1.16 (0.99–1.37)	0.59/0.79
1023 (rs1023777)	CT	5′-flanking	2	66/318/464	(848)	86/433/411	(930)	0.27/0.33	9.3×10^{-5}	1.33 (1.15–1.55)	0.31/0.08
0220 (rs2736994)	GA		3	542/263/22	(827)	529/292/33	(854)	0.19/0.21	0.081	1.16 (0.98–1.38)	0.17/0.41
0221 (rs11097239)	CA		3	245/437/182	(864)	272/431/226	(929)	0.46/0.48	0.48	1.05 (0.92–1.19)	0.67/0.04
0222 (rs1899389)	AG		3	592/245/29	(866)	586/297/46	(929)	0.18/0.21	0.009	1.25 (1.05–1.46)	0.64/0.34
0223 (rs2289515)	AT		3	180/436/238	(854)	221/423/267	(911)	0.47/0.48	0.6	1.03 (0.90–1.18)	0.49/0.04
0224 (rs3775464)	GA		3	109/414/346	(869)	95/385/449	(929)	0.36/0.31	5.9×10^{-4}	1.28 (1.11–1.46)	0.43/0.40
0225 (rs1246270)	GA		3	372/394/84	(850)	474/372/81	(927)	0.33/0.29	0.0061	1.21 (1.05–1.40)	0.19/0.56
0226 (rs3822098)	CT		3	50/300/514	(864)	59/376/494	(929)	0.23/0.27	0.017	1.21 (1.04–1.40)	0.54/0.30

MAF, minor allele frequency. When the odds ratio (OR) is less than 1, an inverted score is indicated.
[a]Originally screened SNP.

Table 3. Haplotype association analysis using representative SNPs in block 2

Haplotypes	Representative SNP (group)						Haplotype frequency		*P*-value
	202	0070 (1)	0206 (2)	0214 (3)	0215	1023	Case	Control	
1	A	C	T	G	A	T	0.39	0.33	4.4×10^{-5}
2	T	T	T	T	A	C	0.24	0.3	5.0×10^{-6}
3	A	C	C	T	C	T	0.24	0.21	0.071
4	A	T	T	T	C	T	0.03	0.06	3.3×10^{-4}
5	T	T	T	T	C	T	0.02	0.03	0.083
6	T	T	T	G	A	T	0.01	0.02	0.62

Table 4. Association of the SNPs in group 1 of block 2

SNP	Allele	Genotype						Dominant model (MM + Mm versus mm)		Recessive model (MM versus Mm + mm)	
		Case			Control						
	M/m	MM	Mm	mm	MM	Mm	mm	*P*-value	Odds ratio (95% CI)	*P*-value	Odds ratio (95%CI)
0203	T/C	380	406	87	293	476	164	3.0×10^{-6}	1.95 (1.45–2.52)	1.0×10^{-7}	1.68 (1.41–2.07)
0204	G/A	379	399	89	289	482	159	2.7×10^{-5}	1.81 (1.36–2.38)	3.0×10^{-8}	1.72 (1.43–2.13)
0070[a]	C/T	385	394	89	295	472	165	5.7×10^{-6}	1.90 (1.44–2.53)	2.8×10^{-8}	1.71 (1.42–2.06)
0205	T/C	376	406	87	288	477	166	1.8×10^{-6}	1.98 (1.45–2.61)	6.0×10^{-8}	1.69 (1.40–2.05)
0207	T/C	382	367	91	296	482	154	5.3×10^{-4}	1.66 (1.25–2.16)	3.0×10^{-9}	1.78 (1.47–2.16)
0209	C/T	402	377	84	297	480	156	1.4×10^{-5}	1.89 (1.41–2.51)	1.5×10^{-10}	1.86 (1.55–2.27)

M and m are major allele and minor allele, respectively. CI, confidence interval.
[a]Originally screened SNP.

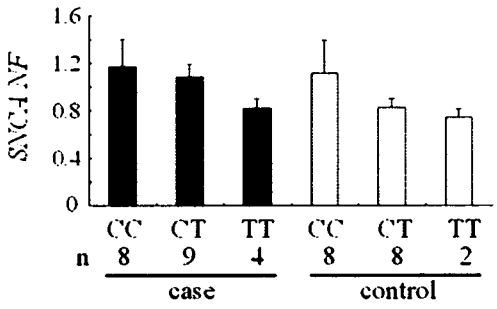

Figure 3. *In vivo* expression of *SNCA* mRNA in relation to susceptibility genotypes. SNP0070 (C/T) is used as a representative of group 1. *SNCA* expression levels in autopsied frontal cortices of cases (solid bar; 8 CC, 9 CT and 4 TT) and controls (open bar; 8 CC, 8 CT and 2 TT). Relative *SNCA* mRNA levels (normalized to *neurofilament L, NF*) are indicated. In cases, mean ± SEM of CC, CT and TT were 1.17 ± 0.23, 1.08 ± 0.11 and 0.82 ± 0.08, respectively. In controls, mean ± SEM of CC, CT and TT were 1.11 ± 0.28, 0.83 ± 0.07 and 0.75 ± 0.07, respectively.

The PD-associated alleles may positively correlate with the basal transcription level of *SNCA* and/or the induction of *SNCA* expression by certain stimulators, for example, oxidative stress.

Other possible functional effects of associated SNPs include alternative splicing, which may result in a protein isoform that aggregates more readily. The C-terminal region of SNCA is rich in acidic amino acid residues, and its truncation promotes aggregation *in vitro* (40,41). The known splice variant *SNCA112* lacks exon 5, which encodes 28 amino acids (10 of which are acidic) in frame. Thus, SNCA112 may also promote aggregation. We investigated *SNCA112* mRNA expression in frontal cortices using splice variant-specific primers, but observed little difference among the three genotypes (data not shown).

In summary, our study establishes *SNCA* as a susceptibility gene for sporadic PD. Focussed investigations of SNCA function will further enhance our understanding of how genetic factors contribute to the complex etiology of PD.

MATERIALS AND METHODS

Subjects

We recruited 882 unrelated sporadic PD patients (age, 64.9 ± 9.8; male/female ratio, 0.79) and 938 unrelated controls (age, 45.3 ± 16.3; male/female ratio, 1.10). The diagnosis of idiopathic PD was based on the presence of two or more of the cardinal features of PD (tremor, rigidity, bradykinesia and postural instability), according to the criteria for sporadic PD (42). Patients were evaluated by the certified neurologists specializing in PD. The average age of onset was 57.4 ± 10.9 years. Forty-two patients showed early onset of PD (<40 years) and 51 patients had a positive family history of PD. Patients who carried *parkin* mutations were excluded. All patients and controls were of Japanese ancestry. Informed consent was obtained from each individual, and approval for the study was obtained from the University Ethical Committees.

SNP genotyping

Genomic DNA was extracted from whole blood using FlexGene (Qiagen). SNP information was obtained from the dbSNP (http://www/ncbi.nlm.nih.gov/SNP/), JSNP (http://snp.ims.u-tokyo.ac.jp/) (43) and Celera Discovery System

(http://myscience.appliedbiosystems.com/) databases. We genotyped SNPs using the Invader assay (Third Wave Technologies), TaqMan (Applied Biosystems) or direct sequencing using an ABI3730 capillary sequencer (Applied Biosystems). Rep1 genotyping and allele designations followed those described previously (35). The Rep1 region was amplified using FAM5′-CCTGGCATATTTGATTGCAA-3′ and 5′-GACTGGCCCAAGATTAACCA-3′ as primers and analyzed using ABI3730 capillary sequencer.

Statistical analysis

SNPAlyze software (DYNACOM, Japan) was used for the case–control study (χ^2-test), calculation of odds ratio and its 95% CI (Bootstrap method), haplotype analysis (Expectation–Maximization algorithm) and pairwise LD analysis (Lewontin's coefficient D' and standardized coefficient r).

Real-time RT–PCR

Autopsied frontal cortices were obtained from the Brain Bank for Aging Research (Tokyo Metropolitan Geriatric Hospital/Tokyo Metropolitan Institute of Gerontology) and from the Department of Neurology, Juntendo University School of Medicine. The samples contained 21 cases [age, 82.6 ± 7.1 (SD) years; 11 males and 10 females] with Lewy body pathology defined by the third Consensus Guideline for Dementia with Lewy Bodies (44), comprising PD with and without dementia and dementia with Lewy bodies, and 18 control subjects (age, 81.2 ± 5.2; 12 males and six females) without parkinsonism or dementia and without neurodegenerative pathological changes. Total RNA was extracted from tissues using RNeasy (Qiagen), and cDNA was prepared using Superscript reverse transcriptase (Invitrogen). Real-time RT–PCR was carried out on ABI PRISM 7900 sequence detection system (Applied Biosystems) using SYBR Premix Ex Taq (TAKARA, Japan). First-strand cDNA was amplified using primers specific for *SNCA* (forward: 5′-GCAGAAGCA GCAGGAAAGAC-3′; reverse: 5′-CTGGGCTACTGCTGTC ACAC-3′; product size: 159 bp) and *NF* (*neurofilament L*, forward: 5′-AGAACGCTGAGGAATGGTTC-3′; reverse: 5′-CTGGTGAAACTGAGTCGGGT-3′; product size: 391 bp). A single band of the expected size was amplified from cDNA samples, but not from RNA samples. For quantification, we used a relative standard curve method. Standard curves of *SNCA* and *NF* were generated from the amplification of diluted series of cDNA from cortices. *SNCA* expression levels were normalized to those of *NF*. One of the experimental samples was used as the calibrator. Each of the normalized *SNCA* values was divided by the calibrator normalized *SNCA* value to generate the relative expression levels. The values were determined in triplicate. Reproducibility of the results was confirmed by repeating cDNA synthesis and real-time PCR twice for seven samples, and similar results were obtained.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

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Conflict of Interest statement. None declared.

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Short communication

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

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Abstract

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

Keywords: Lewy body; α-synuclein; Distal axonopathy

1. Introduction

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

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

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

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

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

2. Case report and methods

2.1. Case report

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

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

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

2.2. MIBG myocardial scintigraphy

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

2.3. Neuropathology

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

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

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

3. Results

3.1. MIBG myocardial scintigraphy

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

3.2. Neuropathology

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

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

4. Discussion

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

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

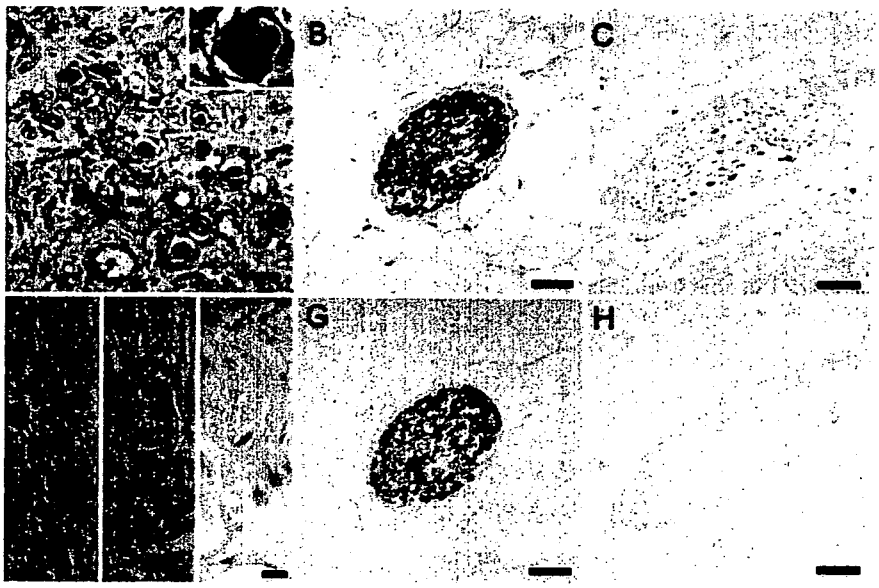

Fig. 1. Pathology of the sympathetic nervous system of a case of Parkinson disease A: a sympathetic ganglion showing a Nageotte's residual nodule (arrows) with Lewy bodies (LBs) (arrowhead) (hematoxylin and eosin staining, bar=50 μm). Inset: a typical LB in the sympathetic ganglion (bar=10 μm). B and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

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

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

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

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

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ORIGINAL ARTICLE

Analysis of the Adrenal Gland Is Useful for Evaluating Pathology of the Peripheral Autonomic Nervous System in Lewy Body Disease

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Abstract

Lewy body disease is defined as Lewy body-related neuronal degeneration involving the nigrostriatal system, limbic-neocortical system, and peripheral autonomic nervous system (PANS). We investigated whether the adrenal gland, which is evolutionarily related to sympathetic ganglia and is routinely examined in general autopsy, could be used to assess pathology of the PANS in Lewy body disease. Brains, spinal cords, and adrenal glands from 783 consecutive autopsy cases from a general geriatric hospital were examined immunohistochemically with antiphosphorylated α-synuclein antibodies and routine staining. Parkinson disease (PD) with dementia and dementia with Lewy bodies (DLB) were defined using 1996 Consensus Guidelines for DLB and the secondary Lewy body-related α-synucleinopathy or amygdala variants using previously established criteria. Lewy body-related α-synucleinopathy was found in 207 (26.4%) of 783 cases, with 1 case solely in the adrenal gland. In all 18 PD cases with or without dementia and in 33 of 38 DLB cases, the adrenal gland was involved, but it was spared in all cases of amygdala variants. Our results indicate that the adrenal gland can provide useful information for evaluation of the PANS in Lewy body disease.

Key Words: Alzheimer disease, Amygdala variant, Autonomic failure, Dementia with Lewy bodies, Parkinson disease, Sympathetic ganglion, α-Synucleinopathy.

INTRODUCTION

Lewy body disease was originally defined pathologically as degeneration of the central nervous system associated with Lewy bodies (1, 2) and includes Parkinson disease (PD) and dementia with Lewy bodies (DLB). Subsequently, clinical and pathologic studies indicated that progressive autonomic failure of the Lewy body type presented with Lewy body-related pathology in the peripheral autonomic nervous system, as well as in the central nervous system (3). Clinical and pathologic studies confirmed that DLB always accompanies Lewy body-related pathology in the peripheral autonomic nervous system (4). Thus, it is more practical to use the term "Lewy body disease" to designate disorders involving both the central nervous system and the peripheral autonomic nervous system, which clinically present with various combinations of parkinsonism, cognitive decline, or autonomic failure (5).

Clinical evaluation of the involvement of the peripheral autonomic nervous system in Lewy body disease has been improved by the adoption of [^{123}I]metaiodobenzylguanidine (MIBG) cardiac scintigraphy (6), which shows low uptake of ^{123}I in PD and progressive autonomic failure (7, 8). Histologically, this low uptake corresponds to a decrease in the number of tyrosine hydroxylase-immunoreactive axons (9) associated with α-synucleinopathy in the epicardium of the anterior wall of the left ventricles of the heart (10) seen on postmortem examination. MIBG cardiac scintigraphy reportedly has 100% specificity and sensitivity for the differential diagnosis of DLB and Alzheimer disease (AD) (11). Thus, evaluation of the peripheral autonomic nervous system is now a standard for confirmation of the pathologic diagnosis of Lewy body disease.

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The sympathetic ganglia are the most widely used specimen for the evaluation of the peripheral autonomic nervous system in Lewy body disease (12). However, these ganglia and the epicardium of the anterior wall of the left ventricle of the heart are not a routine site for investigation in general autopsy. In contrast, the adrenal gland is always included in routine autopsy examinations and is a good candidate for examination of the peripheral autonomic nervous system because it is evolutionarily related to sympathetic ganglia and includes autonomic nerves and ganglia in the capsular fatty tissue. Several previous studies indicated that the adrenal gland might be involved in PD (13). However, the exact incidence of adrenal gland involvement in Lewy body disease is not well established.

We recently reported a staging paradigm for Lewy body-related α-synucleinopathy (LBAS) in consecutive autopsy cases roughly representing a general cohort of the elderly (14, 15). Employing the same strategy in the present study, we provide evidence that evaluation of the peripheral autonomic nervous system in Lewy body disease is possible through the examination of archival paraffin blocks of adrenal glands. Our studies also suggest that adrenal involvement may be associated with orthostatic hypotension in Lewy body disease.

MATERIALS AND METHODS

Tissue Source

For the present study, we used 783 consecutive autopsy brains, spinal cords, and adrenal glands obtained from the Tokyo Metropolitan Geriatric Hospital (TMGH). This hospital provides community-based medical service to the aged population 24 hours/day in cooperation with local general practitioners. The number of patients requiring emergency admission to the hospital reaches almost 5,000 per year. The hospital holds 711 beds in its ward and is directly run by the Tokyo Metropolitan Government to promote the health and welfare of an aged population of nearly 1 million residents of the Tokyo metropolitan area. In the present study, 452 of the 783 examined cases overlapped cases used in a previous study (15). The patient ages ranged from 48 to 104 years (80.68 ± 8.8 years, mean ± SD) at the time of death, and the male to female ratio was 455:328. The postmortem interval ranged from 52 minutes to 88 hours (13.16 ± 6:36 hours). Tissue samples were collected after informed consent was obtained from relatives of the deceased according to the Article 18 of the Cadavers Autopsy and Preservation Act in Japan.

Neuropathology

Routine Staining

All brains and spinal cords were examined as described previously (15). Briefly, 6-μm-thick sections of the representative anatomical areas were stained with hematoxylin and eosin using the Klüver-Barrera method and further examined by means of modified methenamine (16) and Gallyas-Braak silver (17) staining to detect senile changes, Congo red staining to detect amyloid deposition, and elastica Masson trichrome staining to detect vascular changes. In addition, the bilateral adrenal glands were fixed in 10% buffered formalin and embedded in paraffin and then 3-μm-thick serial sections were obtained for hematoxylin and eosin staining.

Immunohistochemistry

A Ventana NX20 autoimmunostainer (Ventana, Tucson, AZ) was used (18) with the following antibodies: anti-phosphorylated tau (ptau) (AT8, monoclonal; Innogenetics, Temse, Belgium), anti-β amyloid (11–28, 12B2, monoclonal; IBL, Maebashi, Japan), anti-phosphorylated α-synuclein (psyn#64 [14] and Pser129 polyclonal [19]), anti-α-synuclein (LB509, amino acids 115–122 [20], monoclonal), anti-ubiquitin (polyclonal, Sigma-Aldrich, St. Louis, MO), anti-phosphorylated neurofilament (SMI31, monoclonal; Sternberger Immunochemicals, Bethesda, MA) and anti-tyrosine hydroxylase (anti-TH, monoclonal; Calbiochem-Novabiochem Corporation, Darmstadt, Germany).

TABLE 1. Lewy Body (LB) Stages in the Central Nervous System (14, 15)

Stage	Substantia Nigra and Locus Ceruleus: Loss of Pigmentation	LB				Dementia	Parkinsonism	Diagnosis
		Nigrostriatal	Limbic-Neocortical	Spinal Cord	LB Score			
0	−	−	−	−	0			
0.5	−	+/−	+/−	+/−	0			
I	−	+/−	+/−	+/−	0			Incidental LB disease
II	+	+	+/−	+/−	0–10	−*	−*	Subclinical LB disease
III	+	+	+	+	0–10	−	+	PD
IV	+	+	+	+	3–6	+	+	PDDT
							+/−	DLBT†
V	+	+	+	+	7–10	+	+	PDDN
							+/−	DLBN†

*, No dementia or parkinsonism associated with Lewy body-related α-synucleinopathy.

†, Differential diagnosis of PDD and DLB was based on the "1-year rule" according to the Consensus Guidelines (21).

LB, Lewy body; DLBN, dementia with Lewy bodies, with a Lewy body score corresponding to the value for the neocortical form; DLBT, dementia with Lewy bodies, with a Lewy body score corresponding to the value for the transitional form; PDDN, Parkinson disease with dementia, with a Lewy body score corresponding to the value for the neocortical form; PDDT, Parkinson disease with dementia, with a Lewy body score corresponding to the value for the transitional form.

Lewy Body-Related Pathology

Central Nervous System

The medulla oblongata at the level of the dorsal motor nucleus of the vagus, the upper pons at the level of the locus ceruleus, and the midbrain (including the substantia nigra, the amygdala, and the anterior hippocampus from all cases) were immunohistochemically stained with anti-phosphorylated α-synuclein antibodies. When positive results were obtained in any case, the anterior cingulate gyrus, the entorhinal cortex, the second frontal and temporal gyri, and the supramarginal gyrus were immunohistochemically examined using anti-ubiquitin antibody to provide Lewy body scores (21), and the results were confirmed using anti-α-synuclein and anti-phosphorylated α-synuclein antibodies. The basal nucleus of Meynert (22), CA2-3 of the posterior hippocampus (23), and several (at least upper, middle, and lower) levels of the thoracic spinal cord were also examined with the anti-phosphorylated α-synuclein antibodies. The Lewy body stage (Table 1) was determined for all the cases examined, as reported previously (14, 15). In this study, we added Stage 0.5 as Lewy neurites alone, or diffuse or fine granular cytoplasmic staining lacking any focal aggregates, in sections immunohistochemically stained with anti-phosphorylated α-synuclein antibodies, following the revised Consensus Guidelines for DLB (22). PD with dementia was differentiated from DLB using the definition in the Consensus Guidelines: "dementia appears more than 12 months after the onset of parkinsonism" (21). In this study, we subcategorized our Stages I and II into primary and secondary α-synucleinopathy, based on our previous results (14, 15). Primary α-synucleinopathy (24) showed accentuation in the brainstem and spread to the spinal cord and was further subdivided into brainstem, transitional, and neocortical forms, according to the Lewy body score (21). Secondary α-synucleinopathy preferentially involved the amygdala and was termed the amygdala variant (25) in both Stage I (IA) and Stage II (IIA) (26).

The Adrenal Glands

The adrenal glands from all 783 cases were studied with hematoxylin and eosin staining and immunohistochemistry using monoclonal and polyclonal anti-phosphorylated α-synuclein antibodies. The immunoreactive structures were screened in the parenchyma as well as in the autonomic nerves or ganglia in the capsular fatty tissue.

Evaluation of Pathology Related to Other Disorders Presenting With Dementia or Parkinsonism

All 783 cases were evaluated with modified methenamine (16) and Gallyas-Braak silver (17) stainings as well as immunohistochemically using anti-phosphorylated tau

TABLE 2. Lewy Body-Related α-Synucleinopathy in the Central Nervous System and Adrenal Glands

LB Stage*	Type of Distribution/Diagnosis	PA	Dementia	Number of Cases	LBAS in the Adrenal Gland	Ratio (%)
0				577	1	0.2
0.5				36	1	2.8
I				85	14	16.5
	B			41	6	14.6
	T			35	8	22.9
	A			9	0	0
II				29	20	69
	B			5	4	80
	T			19	14	73.7
	N			2	2	100
	A			3	0	0
III	PD	+	−	4	4	100
IV				27	25	92.6
	PDDT	+	+	10	10	100
	DLBT			17	15	88.2
		+	+	7	7	100
		−	+	10	8	80
V				25	22	88
	PDDN	+	+	4	4	100
	DLBN			21	18	85.7
		+	+	7	7	100
		−	+	14	11	78.6
Total				783	87	11.1

*Lewy body stage (14, 15).

LB, Lewy body; PA, parkinsonism; LBAS, Lewy body-related α-synucleinopathy; B, brainstem; T, transitional; N, neocortical; A, amygdala variant; PD, Parkinson disease without dementia; PDDT, Parkinson disease with dementia, with a Lewy body score corresponding to the value for the transitional form; DLBT, dementia with Lewy bodies, with a Lewy body score corresponding to the value for the transitional form; PDDN, Parkinson disease with dementia, with a Lewy body score corresponding to the value for the neocortical form; DLBN, dementia with Lewy bodies, with a Lewy body score corresponding to the value for the neocortical form.

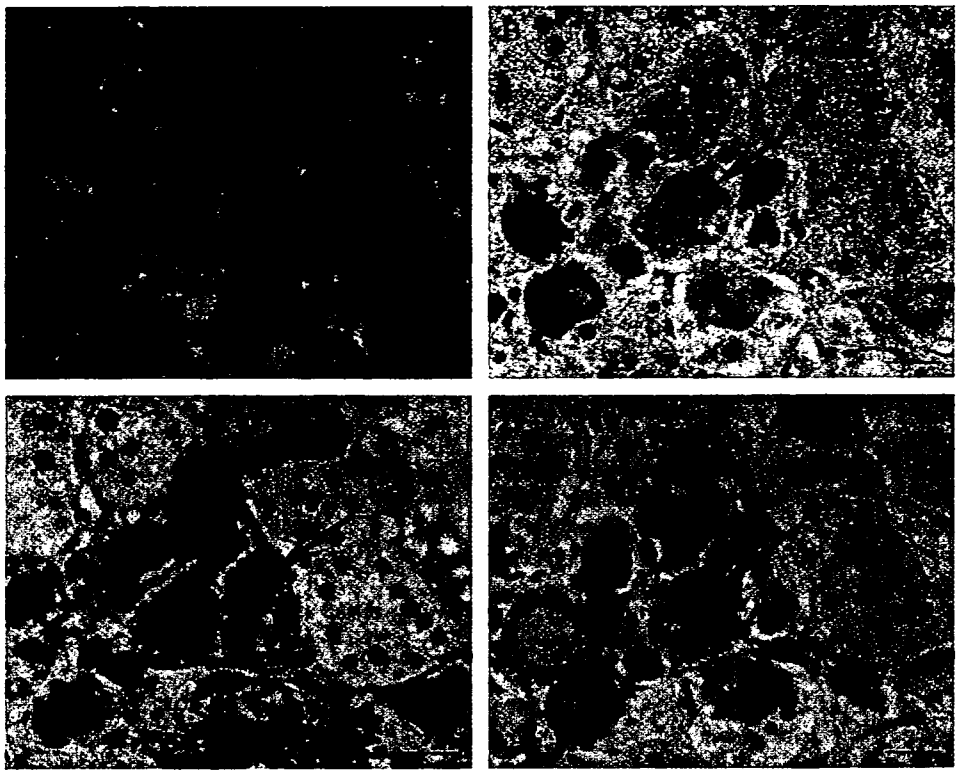

FIGURE 1. Lewy body-related α-synucleinopathy in the adrenal medulla. **(A)** Lewy bodies (arrow) are identified in a hematoxylin and eosin-stained section. **(B)** Anti-phosphorylated α-synuclein (Pser129) antibody clearly visualizes Lewy body-related inclusions, some of which show a central halo and a peripheral rim (arrow). This section is adjacent to that shown in **(A)**. **(C)** Anti-tyrosine hydroxylase immunohistochemistry demonstrates a positively stained cytoplasm of the ganglion cells (arrowhead) and Lewy bodies (arrow), in addition to adrenal medullary cells (double arrows). This section is adjacent to that shown in panel **(A)**. **(D)** Anti-phosphorylated neurofilament (SMI31) antibody intensely stained the periphery of some Lewy bodies (arrow). This section is a serial section of that shown in **(B)**. Scale bars = **(A–D)** 25 μm.

(AT8) and anti-β amyloid antibodies. Neurofibrillary tangles were classified into 7 stages as defined by Braak and Braak (27). Senile plaques were also stratified according to Braak and Braak (27) because this Braak stage was the only available stage for parenchymal deposition of β amyloid. Argyrophilic grains were classified into 4 stages as we have previously described (28).

A neurofibrillary tangle stage equal to or greater than IV and senile plaque stage C were adopted for the diagnosis of AD, as previously reported (29). Diagnoses of "dementia with grains" and the "neurofibrillary tangle-predominant form of dementia" were based on Jellinger's definitions (30, 31). A diagnosis of vascular dementia was based on the National Institute of Neurological Disorders and Stroke (NINDS)-Association Internationale pour la Recherche et l'Enseignement en Neurosciences (AIREN) criteria (32). A diagnosis of progressive supranuclear palsy was based on the NINDS diagnostic criteria (33), skipping the clinical inclusion scheme.

TABLE 3. Distribution of Lewy Body-Related α-Synucleinopathy in the Adrenal Gland Specimens

Region	Lewy Body-Related Pathology (Number)	Frequency (%)
Ganglia in the adrenal medulla	58	66.7
Nerve fascicles in the adrenal cortex	23	26.4
Ganglia in the periadrenal fatty tissue	37 (of 50 cases*)	(74.0)
Nerve fascicles in the periadrenal fatty tissue	81	93.1

*, Ganglia in the periadrenal fatty tissue were identified in 50 of the 87 cases.

Clinical Information

Clinical information, including the presence or absence of parkinsonism and autonomic failure, as well as an assessment of the patient's cognitive state, was obtained from medical charts. The entire collection of medical records, including neuroimages (magnetic resonance imaging, computed tomography, single photon emission computed tomography, and positron emission tomography) of the patients on whom an autopsy was performed, was stored in the TMGH's database. When the previous medical history of another hospital was available, the medical records from that hospital were also obtained with written informed consent from the patient's relatives. Scores from the Mini-Mental State Examination (34) or the Hasegawa Dementia Scale (35) or its revised version (36) and Instrumental Activities of Daily Living scale (37) were used to evaluate cognitive function.

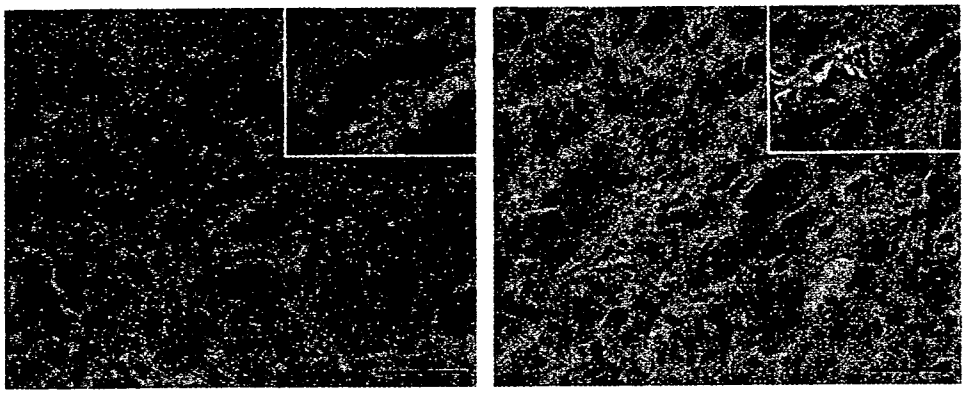

FIGURE 2. Lewy body-related α-synucleinopathy in the adrenal cortex. **(A, B)** Anti-phosphorylated α-synuclein antibody **(A)**, psyn#64, monoclonal; Pser129, polyclonal **(B)**. Both antibodies are raised against the same synthetic peptide and show the same specificity in immunoblots (15, 19). The polyclonal antibody presents less background than the monoclonal one in the peripheral autonomic nervous tissues examined and demonstrates positively stained thick neurites with focal swelling (the left inset). Scale bars = **(A, B)** 50 μm.

The Clinical Dementia Rating Scale (38) was retrospectively determined by 2 independent board-certified neurologists. If the resulting Clinical Dementia Rating Scale scores were in agreement, the score was accepted. If not, the neurologists reconciled their differences after interviews with the patient's attending physicians and caregivers. Locomotor activity was evaluated using the Barthel Index of Activity of Daily Living (39). Information about parkinsonism, tremor (resting), rigidity (cogwheel), bradykinesia, and postural instability was extracted from the records of neurologic examinations, and the presence of more than 2 of these symptoms was interpreted as positive for parkinsonism. To assess autonomic failure, documentation of orthostatic hypotension was retrieved from the charts. There were limitations in clinical assessment in this retrospective manner, compared with prospective clinical studies, but we made efforts to decrease the gap, using the merit of community-based settings. The majority of the cases had long-term follow-up (up to more than 40 years) and both cognitive and motor function parameters were routinely evaluated at each admission to TMGH. The majority of the relatives who approved the autopsy were also medically followed by the TMGH, and we tried to have direct interviews with them to confirm descriptions in clinical charts.

Statistical Analysis

Statistical analysis was performed using the chi-square test or the Fisher exact test for comparisons of categorical data. Statistical significance was set at $p < 0.05$.

RESULTS

Incidence and Distribution of Lewy Body-Related α-Synucleinopathy in the Adrenal Glands

LBAS was found in 207 (26.4%) of 783 cases examined. Among them, 87 cases (11.1%) (Table 2) showed LBAS in the following areas of sections of the adrenal glands: 1) sympathetic ganglion cells in the adrenal medulla (Fig. 1); 2) sympathetic nerve fascicles in the interstitial tissue of the adrenal cortex (Fig. 2); 3) sympathetic ganglia in the fatty tissue surrounding the adrenal capsule (Fig. 3); and 4) nerve fascicles in the fatty tissue surrounding the adrenal capsule (Fig. 4). The above 4 structures were immunoreactive for anti-TH antibody, a marker of the sympathetic nervous system (Figs. 1C and 3C). The regional distribution of LBAS is summarized in Table 3.

So-called "adrenal bodies" (40) were always negative for anti-phosphorylated α-synuclein antibodies (data not shown). SMI31 stained preserved unmyelinated fibers of TH-immunoreactive nerve fascicles in all of the cases with adrenal LBAS, including PD cases, in contrast to a marked decrease in TH-immunoreactive unmyelinated fibers in the pericardium in these cases (data not shown) (9, 10).

Comparison With the Lewy Body Stage in the Central Nervous System

The correlation between the Lewy body stage in the central nervous system and the presence or absence of α-synucleinopathy in the adrenal gland is summarized in Table 2. Lewy bodies were found in one Lewy body Stage 0 case and in one Lewy body Stage 0.5 case. All of the PD cases with or without dementia had LBAS in the adrenal gland.

To elucidate the initial stage of LBAS, the percentage of cases with positive anti-phosphorylated α-synuclein immunoreactivity in the adrenal glands was estimated in each subgroup of Lewy body Stage I and Stage II (Table 2). None of the amygdala variants exhibited anti-phosphorylated α-synuclein immunoreactivity in the adrenal glands. In contrast, nearly 20% of Stage I and approximately 80% of Stage II cases of the primary α-synucleinopathy presented with LBAS in the adrenal glands.

We further analyzed Lewy body Stage IV and Stage V cases (n = 5) that did not present with Lewy body-related pathology in the adrenal glands. These cases had no clinical description of Parkinsonism or orthostatic hypotension. Four of these cases were complicated by AD pathology (changes in senile plaque stage C and an neurofibrillary tangle stage

equal to or greater than Stage III), and the fifth case was complicated by argyrophilic grain Stage III. All 14 DLB cases with a clinical description of parkinsonism and all 8 DLB cases with no such description but with other mild senile changes presented with adrenal LBAS. However, the 7 DLB cases with similar Alzheimer pathology and the 3 DLB cases with argyrophilic grain Stage III contained adrenal Lewy body pathology and did not show easily detectable morphologic differences from the above mentioned 5 cases without the adrenal Lewy body pathology.

Clinicopathologic Correlation With Lewy Body Pathology in the Adrenal Glands

Orthostatic hypotension was clinically described in the medical records for 6 of the 783 cases. Five of these cases showed LBAS in the adrenal glands: one case of PD without clinical description of dementia, one case of PD with dementia with the Lewy score of the transitional form, one case of PD with dementia with the Lewy score of the neocortical form, and 2 cases with DLB transitional form. Of the 2 cases in which Lewy bodies were restricted to the adrenal glands, one case with Lewy body Stage 0.5 clinically presented with syncope-like attack, but there was no definite evidence of orthostatic hypotension.

DISCUSSION

Our studies represent the first demonstration in the literature of the following. 1) LBAS always involved the adrenal gland in PD, with or without dementia. 2) Adrenal glands were always free of LBAS in cases with the amygdala variant. 3) DLB cases that lacked LBAS in the adrenal glands were always complicated by the presence of moderate to severe Alzheimer pathology or argyrophilic grain disease and had no clinical description of parkinsonism. 4) LBAS in the adrenal glands can occur independently of LBAS in the central nervous system. Thus, the immunohistochemical evaluation of adrenal glands with anti-phosphorylated α-synuclein antibodies can be used to evaluate Lewy body pathology involving the peripheral autonomic nervous system.

Lewy bodies and their related structures are present in the adrenal glands of patients with PD or DLB (13, 41). However, the detection ratio was only approximately 30% (41), which differed from the ratio in the sympathetic ganglia (42), in which Lewy bodies were always present in patients with PD or DLB. In the present study we were able to detect Lewy body-related pathology immunohistochemically in adrenal glands or their associated sympathetic tissues with anti-phosphorylated α-synuclein antibodies in

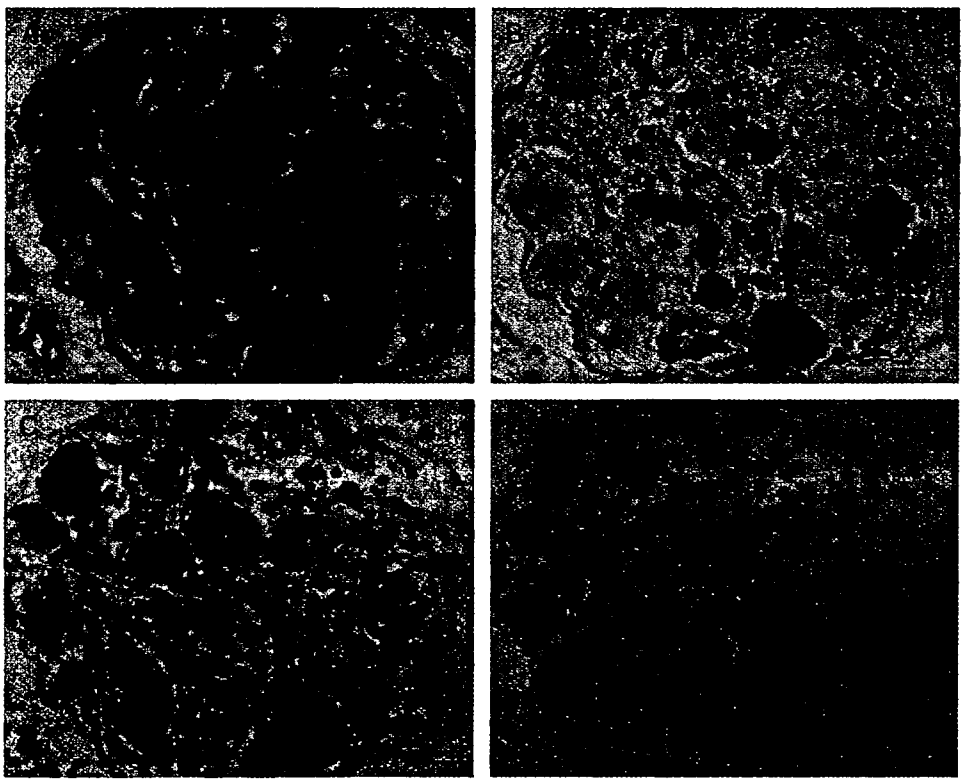

FIGURE 3. Lewy body-related α-synucleinopathy in a sympathetic ganglion from the fatty tissue surrounding the adrenal capsule. **(A)** Lewy bodies (arrow) are visible in a hematoxylin and eosin-stained section. **(B)** Anti-phosphorylated α-synuclein (Pser129) immunostaining visualizes the abundant Lewy body-related α-synucleinopathy (arrow). This image represents a serial section of that shown in **(A)**. **(C)** Anti-tyrosine hydroxylase staining in the neuronal cytoplasm, neurites, and Lewy bodies (arrow). This image is a serial section of that shown in **(B)**. **(D)** Anti-phosphorylated neurofilament antibody (SMI31) reveals axons (arrowheads) and some neuronal perikarya (double arrows). The periphery of some Lewy bodies (arrow) is intensely stained by the antibody. Scale bars = **(A–D)** 25 μm.)

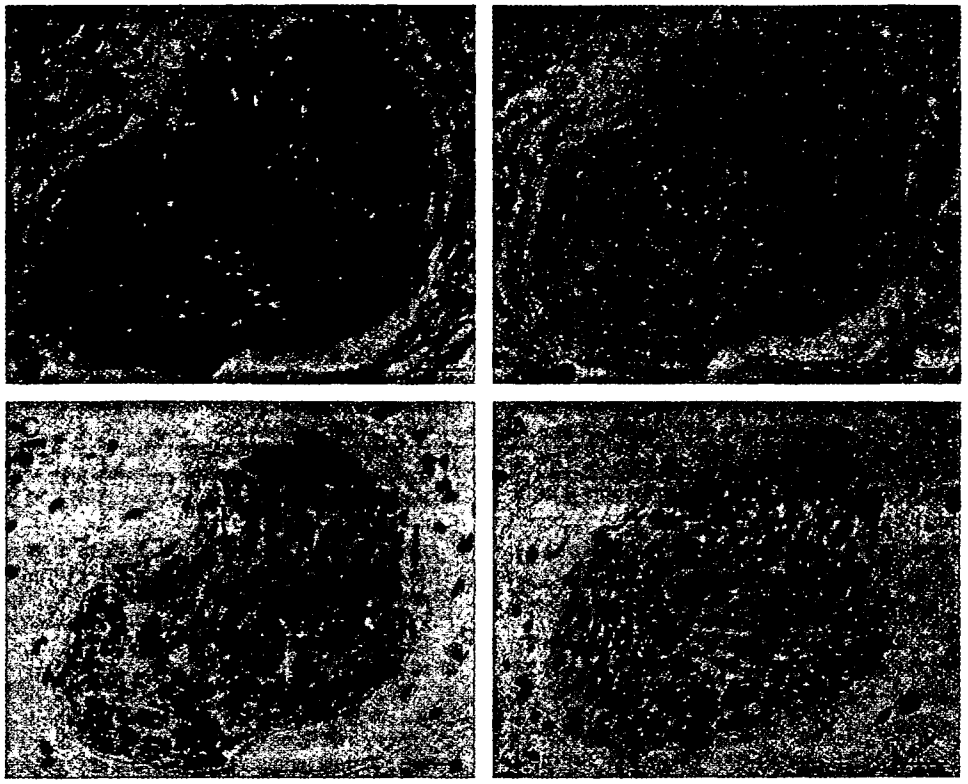

FIGURE 4. Lewy body-related α-synucleinopathy in a nerve fascicle from the fatty tissue surrounding the adrenal capsule. **(A)** An axonal pale body (arrowheads) is detectable with hematoxylin and eosin staining. **(B)** Anti-phosphorylated α-synuclein antibody (psyn#64) stains the pale body (arrowheads). Lewy dots are also visualized by the antibody. This image is a serial section of that shown in **(A)**. **(C)** Anti-tyrosine hydroxylase antibody clearly visualizes the pale body (arrowheads) as well as adjacent axons in the fascicle. This image is a serial section of that shown in **(B)**. **(D)** Anti-phosphorylated neurofilament antibody (SMI31) stains the periphery of the pale body (arrowheads) as well as axons of the nerve fascicle. This image is a serial section of that shown in **(C)**. Scale bars = **(A–D)** 25 μm.

all cases of PD. Because adrenal glands are routine sites of investigation in general autopsy, our results indicate that evaluation of the peripheral autonomic nervous system in Lewy body disease is possible through the examination of archival paraffin blocks of adrenal glands. Pathologic examination in TMGH requires strict removal of fatty tissue from adrenal glands to evaluate their exact weight. When such removal is not done, the detection rate of periadrenal paraganglia was almost 100% (Dr. K. Kawabata, Director, Department of Pathology, Akashi City Hospital, personal communication, 2006). Because the periadrenal retroperitoneal space contains abundant paraganglia and associated sympathetic ganglia and nerves, even the very thin surrounding tissue of the adrenal glands in our series always included useful peripheral sympathetic nervous tissue.

Adrenal glands are frequently affected by autolysis, inflammation, or metastasis, but our study suggests that the organs and the surrounding sympathetic ganglia and nerves are nevertheless useful for assessing morphologic changes in the peripheral autonomic nervous system in PD or DLB.

The amygdala variant of α-synucleinopathy is complicated by either a severe burden of tangles and plaques or by argyrophilic grains in the amygdala. This type of α-synucleinopathy is associated with AD and Down syndrome (25, 26, 43, 44), as well as with other tauopathies (45). We termed this type "secondary" (14, 15). The present study clearly shows that immunopathologic studies of the adrenal glands can distinguish secondary α-synucleinopathy from PD.

Braak et al (46) proposed a staging system for α-synucleinopathy in the brains of a nondemented general cohort and in cases of PD. Our series, with a cohort having a mean age of approximately 80 years, included a high percentage of dementia, as was expected. The differential diagnosis between DLB and PD with dementia is often difficult in such aged cohorts. Thus, Braak et al's staging paradigm could not be applied effectively to our group (15). We always examined the spinal cord, a structure not included in Braak et al's staging, to evaluate the preganglionic sympathetic neurons. Our results show that some DLB cases, whose α-synucleinopathy definitely involved sympathetic preganglionic neurons, did not present with α-synucleinopathy in the adrenal or periadrenal tissues. All of these cases met the morphologic criteria for AD from the elderly cohort (47) or for dementia with grains (28) and lacked a clinical description of parkinsonism. However, many other cases of DLB, complicated by similar changes in AD or dementia with grains and lacking a clinical description of parkinsonism, presented with α-synucleinopathy involving the adrenal or periadrenal tissues. Although

morphologic differences in the Lewy body pathology in the central nervous system at the final stage of the illness may become unclear, the pathologic examination of LBAS in the peripheral autonomic nervous system could delineate those DLB cases complicated by other senile changes and presenting with a limbic-neocortical-dominant distribution of Lewy body pathology, lesser involvement of the brainstem and spinal cord, and a lack of adrenal and periadrenal Lewy body pathology from other DLB cases with a pathology more common to PD (with or without dementia), which always presents with adrenal or periadrenal Lewy body pathology.

Although the number of cases was small, 2 cases presented with Lewy bodies only in the adrenal glands and lacked Lewy bodies in the central nervous system. These cases could possibly represent the earliest stage of Lewy body-related progressive autonomic failure. From the points of disease pathogenesis and hierarchy of the Lewy body disorders, this result indicates that the adrenal gland could be the initial and primary target for these progressive disorders.

In the present study, we retrospectively investigated the correlation between clinical and pathologic presentations of adrenal glands. Although there were definite limitations in our study based on the review of medical records, the observed Lewy body-related pathology involving adrenal tissues did not correspond to any symptomatology of adrenal insufficiency, except for orthostatic hypotension. Also, our studies did not indicate how to detect this adrenal or periadrenal Lewy body-related pathology in clinical testing. Reduced uptake of MIBG in cardiac scintigraphy in Lewy body disease corresponds to decreased TH-immunoreactivity as well as to α-synucleinopathy in unmyelinated fibers from the epicardial fatty tissue of the anterior wall of the left ventricle of the heart (8–10). However, because the TH-immunoreactive unmyelinated fibers in the periadrenal fatty tissue were relatively preserved in this study, MIBG scintigraphy, which is also used for the detection of pheochromocytoma of the adrenal glands, may not be useful for detection of this Lewy body-related pathology in adrenal tissues. Therefore, we are now planning a prospective functional study of cases with Lewy body disease consisting of the tilt test and simultaneous blood sampling to gauge the serum noradrenalin level, as well as the resting adrenalin level, to detect this adrenal pathology clinically.

In conclusion, the immunohistochemical examination of adrenal glands with anti-phosphorylated α-synuclein antibodies can help differentiate the primary and the secondary forms of LBAS, as well as identify where LBAS starts in the human body and how it spreads.

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1. PDD（認知症を伴うParkinson病）とDLB（Lewy小体型認知症）の臨床と病理

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key words　α-synuclein, autonomic failure, substantia nigra, Lewy body, amygdala

略　号

PD: Parkinson disease（Parkinson病）
PDD: Parkinson disease with dementia（認知症を伴うParkinson病）
LB: Lewy body（Lewy小体）
DLB: dementia with Lewy bodies（Lewy小体型認知症）
AD: Alzheimer disease（Alzheimer病）
LBD: Lewy body disease（Lewy小体病）（Lewy body dementia: Lewy小体型認知症ではないことに注意）
SP: senile plaque（老人斑）
NFT: neurofibrillary tangle（神経原線維変化）
α-syn: α-synuclein
psyn: phosphorylated α-synuclein

要　旨

PDDとDLBは，McKeithらにより，認知症で発症するか，Parkinson病（PD）の発症と認知症の出現が1年以内ならDLBとする，いわゆる「1年ルール」で分類された．しかし，高齢者の場合PDの発症時に記憶障害を伴うことが一般的で，臨床的にこの区分は成立しない．神経病理学的にも，PDDは脳幹優位，DLBは辺縁系・新皮質優位の傾向をもち，Alzheimer病（AD）病変の合併はDLBに優位に多いが，両者の病理は大きく重なる．LB病変を，黒質・線条体系，辺縁・新皮質系，節前・節後自律神経系の三系統で評価し，AD病変と合わせ，臨床的Parkinson症状，認知障害，自律神経症状と，症例毎に対応させ検討していくことが，重要である．本来同一疾患であるPDDとDLBを別名でよぶことは混乱を招き，人口に膾炙しているPDに統合することが，今後の学問の発展上有用である．

動　向

本稿は，the 10th International Congress of Parkinsons's Disease and Movement Disorders (October 2006, Kyoto)[1]と，the 4th International Workshop on DLB and PDD (October 2006, Yokohama)[2]の成果を含め，特に後者での議論を中心に，できる限りup to dateの内容になるようにこころがけた．

両学会を通じ，PD（Parkinson disease: 認知症を伴わないParkinson病）/PDD（Parkinson disease with dementia: 認知症を伴うParkinson病）/DLB（dementia with Lewy body: Lewy小体型認知症）という三病名を連記する記述が頻回に用いられた．これらは，Kosakaにより，Lewy小体（LB）病（LBD）と命名された群を指す．本来同一でよばれるべき疾患が，なぜ三つの病名でよばれなければならないか，またなぜLBD，ついでDLBという新しい病名がつくられなければな