11. Kobayashi S, Murayama S	A Japanese family with early-onset ataxia with motor and sensory neuropathy.	J Neurol Sci	in press 別刷未		
12. Maeda S, Sahara N, Saito Y, Murayama M, Yuji Y, Kim H, Miyasaka T, Murayama S, Ikai A, Takashima A	Granular Tau Oligomers as Intermediates of Tau Filaments.	Biochemistry	in press 別刷未		
Mohri I, Kadoyama K, Kanekiyo T, Sato Y, Kagitani-Shimono K, Saito Y, Suzuki K, Kudo T, Takeda M, Urade Y, Murayama S, Taniike M	: Prostaglandin D_2 receptor, DP_1 is selectively up-regulated in microglia and astrocytes within senile plaques from human patients and a mouse model of Alzheimer's disease.	J Neuropath Exp Neurol	in press 別刷未		
Kanekiyo T, Ban T, Aritake K, Huang Z-L, Qu W-M, Okazaki I, Mohri I, Murayama S, Ozono K, Taniike M, Goto Y, Urade Y	Lipocalin-type prostaglandin D synthase/ß-trace is a major amyloid ß-chaperone in human cerebrospinal fluid.	PNAS	in press 別刷未		
徳丸阿耶	認知症　：動的神経病理としての MRI	臨床検査	50	1090-1098	2006
大場洋　徳丸阿耶ら	パーキンソン病の画像診断―ＭＲＩ	Clinical Neuroscience	25	55-58	2006
Tokumaru AM, Sakata I, Terada H. et al	Optic tract hyperintensity on T2 weighted images among patients with pituitary macroadenoma :correlation with visual impairment	AJNR	27	250-254	2006
石井賢二	アルツハイマー病の早期診断に有効なのはアミロイドイメージングか、脳代謝・血流画像か　アミロイドイメージングであるとの立場から.	Cognition and Dementia	6(1)	68-73	2007
Kimura Y, Naganawa M, Yamaguchi J, Uchiyama A, Oda K, Ishii K,	MAP-based Kinetic Analysis for Voxel-by-Voxel Compartment Model	NeuroImage	29(4)	1203-1211	2006

Ishiwata K	Estimation: Detailed Imaging of the Cerebral Glucose Metabolism using FDG.				
Itokawa K, Tamura N, Kawai N, Shimazu K, Ishii K	Parkinsonism in Type I Gaucher's Disease.	Internal Medicine	45(20)	1165-1167	2006
Saito Y, Ishii K, Yagi K, and H Mizusawa.	Cerebral networks for spontaneous and synchronized singing and speaking.	NeuroReport	17(18)	1893-1897	2006
Ishiwata, K., Oda, K., Sakata, M., Kimura, Y., Kawamura, K., Oda, K., Sasaki, T., Naganawa, M., Chihara, K., Okubo, Y., Ishii, K.	A feasibility study of [11C]SA4503-PET for evaluating sigma1 receptor occupancy by neuroleptics: the binding of haloperidol to sigma1 and dopamine D2-like receptors.	Ann Nucl Med	20(10)	569-573	2006
藤原佳典、西真理子、渡辺直紀、李相侖、井上かず子、吉田裕人、佐久間尚子、呉田陽一、石井賢二、内田勇人、角野文彦、新開省二.	都市部高齢者による世代間交流型ヘルスプロモーションプログラム"REPRINTS"の1年間の歩みと短期的効果.	日本公衆衛生学会誌	53(9)	702-714	2006
石井賢二.	脳疾患における PET の現状と展望.	映像情報 Medical	38(11)	1044-1053	2006
石井賢二.	動的神経病理としての PET.	臨床検査	50(10)	1099-1105	2006
Gerloff C, Bushara K, Sailer A, Wassermann EM, Chen R, Matsuoka T, Waldvogel D, Wittenberg GF, Ishii K, Cohen LG, and Hallett M.	Multimodal imaging of brain reorganization in motor areas of the contralesional hemisphere of well recovered patients after capsular stroke.	Brain	129(3)	791-808	2006
Tanaka Y, Nariai T, Nagaoka T, Akimoto H, Ishiwata K, Ishii K, Matsushima Y and Ohno K.	Quantitative evaluation of cerebral hemodynamics in patients with moyamoya disease by dynamic susceptibility contrast magnetic resonance imaging. - Comparison with positron emission tomography-.	J Cereb Bllod Flow Metab	26(2)	291-311	2006
金丸和富	動的神経病理としての髄液バイオマーカー	臨床検査	50	1107-1110	2006

著者	題名	雑誌	巻	ページ	年
小久保康昌	紀伊半島のALSとパーキンソン痴呆複合	臨床検査	50	1171-1174	2006
小久保康昌	神経スイート病　　　神経病理	神経内科	61	401-402	2006
小久保康昌	紀伊半島南部に多発する筋萎縮性側索硬化症	生命の彩 会報			2006
Kokubo Y, Ito K, Fukunaga T, Matsubara H, Kuzuhara S	The prevalence of pigmentary retinopathy in the amyotrophic lateral sclerosis / parkinsonism-dementia complex focus in the Kii peninsula of Japan	Opthalmology	113	2111.e1-2	2006
Basri R, Ybe I, Soma H, Takei A, Nishimura H, Machino Y, Kokubo Y, Kosugi M, Okada R, Yukitake M, Tachibana H, Kuroda Y, Kuzuhara S, Sasaki H	Four novel mutations of the spastin gene in Japanese family with spastic paraplegia	J Hum Genet.	51	711-5	2006
Sato M, Kokubo Y, Kuzuhara S	A case of idiopathic musical hallucination with increasing repeitoire	JNNP		203-204	2007
Mimuro M, Kokubo Y, Kuzuhara S	Similar topographical distribution of neurofibrillary tangles in amyotrophic lateral scleosis and parkinsonism-demntia complex in people living in the Kii peninsula of Japan suggestsa single tauopathy	Acta neuropathologica in press			2007
新堂晃大、小久保康昌、谷口　彰、久瀬真奈美、葛原茂樹	球後視神経炎として15年間経過観察されていたAZOOR (acute zonal occult outer retinopathy)の一例	臨床神経 in press			2007
Kuroda R, Satoh J, Yananura T, Anezaki T, Terada T, Yamazaki K, Obi T, Mizoguchi K	A novel compound heterozygous mutation in the DAP12 gene in a patient with Nasu-Hakola disease	J Neurol Sci	252	88-91	2007
國本雅也、上坂義和、宇羽野恵、山下裕子、松本英之、久賀敦	救急場面における脳波検査の意義.	日本神経救急学会雑誌	19(1)	11-15	2006
笹原鉄平、中原康雄、上坂義和、國本雅也、田山二朗	Bi-level Positive Airway Pressure(BiPAP)により夜間呼吸不全が改善した多系統萎縮症の63歳女性例.	神経治療学	23(2)	151-155	2006
黒野裕子、上坂義和、國本雅也、今福一郎	延髄外側症候群急性期の嚥下障害における疑核の関与〜MRI画像からの検討〜.	臨床神経学	46(7)	461-466	2006

Matsumoto L, Yamamoto T, Higashihara M, Sugimoto I, Kowa H, Shibahara J, Nakamura K, Shimizu J, Ugawa Y, Goto J, Dalmau J, Tsuji S.	Severe Hypokinesis Caused by Paraneoplastic Anti-Ma2 Encephalitis Associated with Bilateral Intratubular Germ-Cell Neoplasia.	Movement disorders			Published online, 31 Jun 2007
松本ルミネ、宇川義一	脊髄神経根磁気刺激の末梢神経への応用	神経内科	64	454-459	2006

研究成果の刊行に関する一覧表
(2006 年 4 月 1 日－2007 年 3 月 31 日迄)

書籍

編著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
村山繁雄	軽度認知障害の背景病理解明のためのクリニカルパス	村山繁雄	アルツハイマー病診断 － 早期発見・早期介入のために	真興出版	東京	2006.1	15-18
齊藤祐子	ブレインバンクの貢献	齊藤祐子	アルツハイマー病診断 － 早期発見・早期介入のために	真興出版	東京	2006.1	139-150
村山繁雄・齊藤祐子	PDD（認知症を伴うパーキンソン病）とDLB（レヴィー小体型認知症）の臨床と病理		Annual Review 神経 2007	中外医学社	東京	2007	167-174
徳丸阿耶	アルツハイマー病	土屋一洋	カンファランス形式頭部画像診断演習	真興交易出版	東京	2006.7	184-185
徳丸阿耶	前頭葉側頭型痴呆、Pick 病	土屋一洋	カンファランス形式頭部画像診断演習	秀潤社	東京	2006.9	186-187
徳丸阿耶	Binswanger 病	土屋一洋	カンファランス形式頭部画像診断演習	秀潤社	東京	2006.9	188-189
徳丸阿耶	進行性核上性麻痺 PSP	土屋一洋	カンファランス形式頭部画像診断演習	秀潤社	東京	2006.9	190-191
徳丸阿耶	多系統萎縮症 MSA	土屋一洋	カンファランス形式頭部画像診断演習	秀潤社	東京	2006.9	192-104
徳丸阿耶	晩発性皮質小脳萎縮小　LCCA	土屋一洋	カンファランス形式頭部画像診断演習	秀潤社	東京	2006.9	194-195
徳丸阿耶	Sepinocerebellar ataxia type 3	土屋一洋	カンファランス形式頭部画像診断演習	秀潤社	東京	2006.9	196-197

	Machado-Joseph病						
石井賢二	アルツハイマー病診断：画像（PET）	村山繁雄	アルツハイマー病診断	真興交易出版	東京	2006.7	15-18
金丸和富	髄液バイオマーカー	村山繁雄	アルツハイマー病診断	真興交易出版	東京	2006.7	121-126
小久保康昌	認知症を伴う筋萎縮性側索硬化症	村山繁雄	アルツハイマー病診断	真興交易出版	東京	2006.7	214-226
國本雅也	痙攣.	小林祥泰,水澤英洋	神経疾患最新の治療2006-2008	南江堂	東京	2006	57-59
國本雅也	睡眠障害.	田村晃, 松谷雅生,清水輝夫	EBMに基づく脳神経疾患の基本治療指針.	MEDICAL VIEW	東京	2006	528-530
國本雅也	脳波検査等, 神経・筋検査.	谷 昌尚,寺島裕夫	臨床手技の完全解説 2006年版	医学通信社	東京	2006	142-150
國本雅也	脊髄・脊椎疾患, 平山病, 後縦靭帯骨化症, 脊髄腫瘍, 脊髄血管障害.	北村 聖,楠 進	臨床病態学1脳・神経系疾患	NOUVELLE HIROKAWA	東京	2006	153-161
國本雅也	G.末梢神経疾患5.自律神経ニューロパチーに治療法はあるか.	岡本幸市,棚橋紀夫,水澤英洋	EBM 神経疾患の治療2007-2008	中外医学社	東京	2006	352-355

付録　　重要別刷

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.
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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 intra-cytoplasmic 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
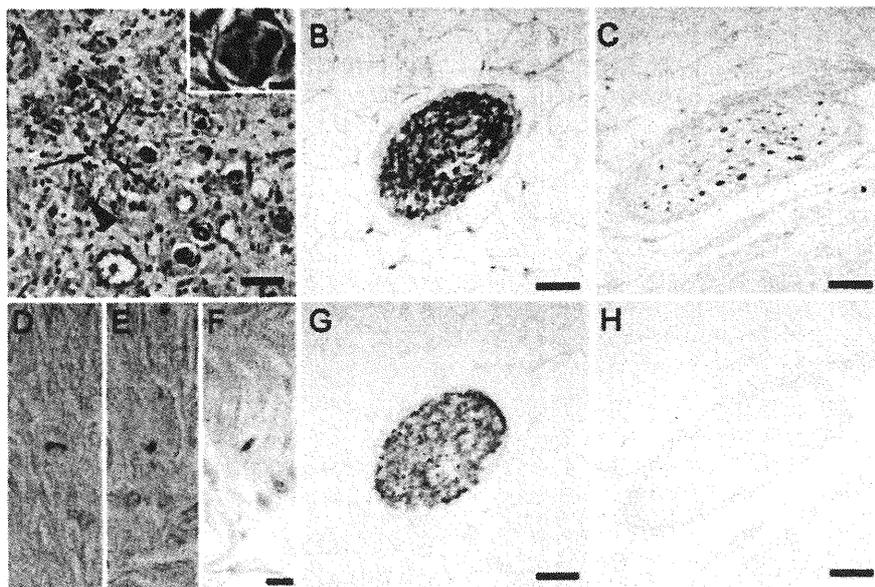

Fig. 1. Pathology of the sympathetic nervous system of a case of Parkinson disease A: a sympathetic ganglion showing a Nageotte's residual nodule (arrows) with Lewy bodies (LBs) (arrowhead) (hematoxylin and eosin staining, bar=50 μm). Inset: a typical LB in the sympathetic ganglion (bar=10 μm). B and 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.

Acknowledgements

References

[1] Orimo S, Ozawa E, Nakade S, Sugimoto T, Mizusawa H. ^{123}I-metaiodobenzylguanidine myocardial scintigraphy in Parkinson's disease. J Neurol Neurosurg Psychiatry 1999;67:189–94.

[2] Taki J, Nakajima K, Kwang EH, Matsunari I, Komai K, Yoshita M, et al. Peripheral sympathetic dysfunction in patients with Parkinson's disease without autonomic failure is heart selective and disease specific. Eur J Nucl Med 2000;27:566–73.

[3] Braune S, Reinhardt M, Schnitzer R, Riedel A, Lücking CH. Cardiac uptake of ^{123}I MIBG separates Parkinson's disease from multiple system atrophy. Neurology 1999;53:1020–5.

[4] Yoshita M. Differentiation of idiopathic Parkinson's disease from striatonigral degeneration and progressive supranuclear palsy using iodine-123meta-iodobenzylguanidine myocardial scintigraphy. J Neurol Sci 1998;155:60–7.

[5] Yoshita M, Taki J, Yamada M. A clinical role for ^{123}I MIBG myocardial scintigraphy in the distinction between dementia of Alzheimer's-type and dementia with Lewy bodies. J Neurol Neurosurg Psychiatry 2001;71:583–8.

[6] Orimo S, Ozawa E, Oka T, Nakade S, Tsuchiya K, Yoshimoto M, et al. Different histopathology accounting for a decrease in myocardial MIBG uptake in PD and MSA. Neurology 2001;57:1140–1.

[7] Amino T, Orimo S, Itoh Y, Takahashi A, Uchichara T, Mizusawa H. Profound cardiac sympathetic denervation occurs in Parkinson disease. Brain Pathol 2005;15:29–34.

[8] Orimo S, Amino T, Itoh Y, Takahashi A, Kojo T, Uchichara T, et al. Cardiac sympathetic denervation precedes neuronal loss in the sympathetic ganglia in Lewy body disease. Acta Neuropathol 2005; 109:583–8.

[9] McKeith I.G., Galasko D, Kosaka K, Perry EK, Dickson DW, Hansen LA, et al. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. Neurology 1996;47: 1113–24.

[10] Saito Y, Kawashima A, Ruberu NN, Fujiwara H, Koyama S, Sawabe M, et al. Accumulation of phosphorylated α-synuclein in aging human brain. J Neuropathol Exp Neurol 2003;62:644–54.

[11] Wakabayashi K, Takahashi H. Neuropathology of autonomic nervous system in Parkinson's disease. Eur Neurol 1997;38(Suppl 2):2–7.

[12] Iwanaga K, Wakabayashi K, Yoshimoto M, Tomita I, Satoh H, Takashima H, et al. Lewy body-type degeneration in cardiac plexus in Parkinson's and incidental Lewy body diseases. Neurology 1999;52: 1269–71.

Multiple candidate gene analysis identifies α-synuclein as a susceptibility gene for sporadic Parkinson's disease

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Parkinson's disease (PD), one of the most common human neurodegenerative diseases, is characterized by the loss of dopaminergic neurons in the substantia nigra of the midbrain. PD is a complex disorder with multiple genetic and environmental factors influencing disease risk. To identify susceptible genes for sporadic PD, we performed case–control association studies of 268 single nucleotide polymorphisms (SNPs) in 121 candidate genes. In two independent case–control populations, we found that a SNP in α-synuclein (SNCA), rs7684318, showed the strongest association with PD ($P = 5.0 \times 10^{-10}$). Linkage disequilibrium (LD) analysis using 29 SNPs in a region around rs7684318 revealed that the entire SNCA gene lies within a single LD block ($D' > 0.9$) spanning ~120 kb. A tight LD group ($r^2 > 0.85$) of six SNPs, including rs7684318, associated most strongly with PD ($P = 2.0 \times 10^{-9}$–1.7×10^{-11}). Haplotype association analysis did not show lower P-values than any single SNP within this group. SNCA is a major component of Lewy bodies, the pathological hallmark of PD. Aggregation of SNCA is thought to play a crucial role in PD. SNCA expression levels tended to be positively correlated with the number of the associated allele in autopsied frontal cortices. These findings establish SNCA as a definite susceptibility gene for sporadic PD.

INTRODUCTION

Sporadic Parkinson's disease (PD) (OMIM no. 168600) is the second most common neurodegenerative disease following Alzheimer's disease. PD is late onset and progressive, affecting 1–2% of persons older than 65 years. Clinical features of PD include resting tremor, bradykinesia, rigidity and postural instability. The disease is pathologically characterized by the loss of dopaminergic neurons in the substantia nigra and the presence of intracellular inclusions known as Lewy bodies. Various medical managements are available for PD, including drugs (l-dopa, dopamine agonists, anti-cholinergic drugs, etc.) and surgery (thalamotomy, pallidotomy, deep brain stimulation, etc.) (1). These treatments improve PD symptoms, but do little to deter disease progression. Identifying risk factors for PD can be helpful in delaying disease onset and slowing its progression.

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PD is a complex common disease, caused by multiple genetic and environmental factors (2). The contribution of genetic factors to sporadic PD is indicated by several findings. First, ~10% of patients with PD have a positive family history (3). Secondly, a recent large-scale survey in Iceland showed that the risk ratio for PD was increased in related individuals (6.7 for siblings, 3.2 for offspring and 2.7 for nephews and nieces of patients with PD) (4). Thirdly, a twin study using [^{18}F]dopa PET showed that the concordance rate for PD, including subclinical cases, is approximately three times higher in monozygotic twins (55%) than in dizygotic twins (18%) (5).

Causal genes for Mendelian-inherited PD have been reported, including *α-synuclein* [4q21, autosomal dominant (AD)] (6), *parkin* [6q25.2–27, autosomal recessive (AR)] (7), *UCH-L1* (4p14, AD) (8), *PINK1* (1p36, AR) (9), *DJ-1* (1p36, AR) (10), *LRRK2/dardarin* (12q12, AD) (11,12) and *NR4A2/Nurr1* (2q22–23, AD) (13).

Many case–control association studies using single nucleotide polymorphisms (SNPs) in candidate genes have been reported, but few consistent findings have been obtained (2). This is due, in part, to limited numbers of available samples, target genes and/or genetic markers. Since 2001, genome-wide, non-parametric linkage analysis of PD families has revealed significant linkage in multiple chromosomal regions (14–17), leading to the identification of *tau* (18) and *FGF20* (19) as susceptibility genes.

To date, polymorphisms that influence PD as strongly as *APOE-ε4* influences Alzheimer's disease have not been identified. Through extensive candidate gene association studies, we have established *α-synuclein* (*SNCA*) as a definite susceptibility gene for sporadic PD.

RESULTS

Screening of SNPs in candidate genes for PD

We selected candidate genes from the literature describing genetic, pathological and biochemical findings in PD, as well as genes that participate in the proposed mechanisms for PD. Finally, we picked up 121 genes relevant to familial PD, Lewy bodies, dopaminergic neurons, cytokines and trophic factors, mitochondrial functions, oxidative stress, proteasome function, autophagy, endoplasmic reticulum-associated degradation (ERAD) and toxins. One to seven SNPs per gene (268 SNPs total) were selected from the dbSNP, JSNP and Celera Discovery System databases.

In the initial screen, we genotyped 190 patients and 190 controls (Supplementary Material, Table S1). To avoid false negatives, we set the α-value at 0.05 in the first screen. From 268 SNPs, 22 SNPs in 16 genes showed association with PD (*P* < 0.05) in genotype frequency, allele frequency, dominant model or recessive model. We genotyped the 22 qualifying SNPs in a replication panel of 692 patients and 748 controls and tested again for association. This independent test revealed that SNP0070 (rs7684318 C/T) was prominently associated with PD (*P* = 5.0 × 10^{-10} for allele frequency) (Table 1). We corrected the α-value to 0.00019 after Bonferroni's correction (tests for 268 SNPs). The remaining 21 SNPs did not show *P*-values lower than

0.00019 (data not shown). SNP0070 is located in intron 4 of the *α-synuclein* (*SNCA*) gene on chromosome 4q21. SNCA is a primary component of intracellular inclusions called Lewy bodies, which are considered to be the pathological hallmark of PD (20). Aggregation of SNCA is thought to play a crucial role in the pathogenesis of PD (21). The allele C frequency of SNP0070 was higher in PD (0.67) than in controls (0.57) (Table 1). The association of SNP0070 was significant in genotype frequency, allele frequency, dominant model and recessive model. Of the two disease models, allele C of SNP0070 was more significantly associated in the recessive model than in the dominant model (Table 1).

Linkage disequilibrium (LD) mapping and search for susceptibility SNPs

We performed LD mapping in a 430 kb region around SNP0070. This region contains two genes: *SNCA* and *MMRN1*. Using SNP0070 and 28 additional SNPs in this region, we genotyped 134 control subjects and constructed an LD map based on pairwise D' and r^2 (Fig. 1) (Supplementary Material, Table S2). Three LD blocks were observed on the basis of D' ($D' > 0.9$). The entire *SNCA* gene was included in a block containing SNP0070 (block 2). The *MMRN1* gene was in another LD block, indicating that *MMRN1* does not correlate with the SNP0070 association (Fig. 2).

To search for the most strongly associated SNP(s) in the region, we next performed association studies with these 29 SNPs (Fig. 2; Table 2). We found significant associations for SNPs in block 2, but not in blocks 1 and 3. Block 2, thought to be a susceptibility block for PD, was further analyzed on the basis of r^2-values. Of the 19 SNPs in block 2, 16 belonged to three groups with high pairwise r^2 (>0.85) and the remaining three did not belong to any group (Fig. 1; Table 2) (Supplementary Material, Table S2). Six SNPs in group 1, including originally screened SNP0070 and five additional SNPs (0203, 0204, 0205, 0207 and 0209), showed prominent association with PD (*P* = 2.0 × 10^{-9}–1.7 × 10^{-11}, allele 1 versus allele 2) (Fig. 2; Table 2). Population attributable risk (PAR) (22) of SNP0070 was 42.5% in the dominant model and 18.5% in the recessive model.

We next performed haplotype analysis using six representative SNPs in block 2 (Table 3). Six common haplotypes (>1% of PD and controls) covered >90% of the population haplotypes in both PD and controls. The major haplotypes 1 and 2 showed significant associations; however, their *P*-values were not lower than that of any single SNP in group 1. Therefore, the presence of hidden SNP(s) with a lower *P*-value than group 1 seemed unlikely, as was the possibility that the haplotype(s) is implicated in PD susceptibility. These findings establish the six SNPs in group 1 as the strongest susceptibility SNPs. All showed stronger associations in the recessive model than in the dominant model, similar to the originally screened SNP0070 (Table 4).

Taken together, our genetic analyses indicate that *SNCA* is a definite susceptibility gene for sporadic PD and that multiple SNPs in group 1 are susceptibility SNPs, likely in a recessive model.

Table 1. Association of SNP0070 in SNC4 between cases and controls

| | Genotype | | | | Allele | | | P-value (χ^2-test) | | | |
	CC	CT	TT	Total	C	T	Total	Genotype	Allele	Dominant[a] model	Recessive[b] model
First screen											
Case	87 (0.46)	87 (0.46)	14 (0.07)	188	261 (0.69)	115 (0.31)	376	3.4×10^{-4}	1.8×10^{-4}	1.8×10^{-4}	1.1×10^{-2}
Control	62 (0.33)	85 (0.46)	39 (0.21)	186	209 (0.56)	163 (0.44)	372				
Replication											
Case	298 (0.44)	307 (0.45)	75 (0.11)	680	903 (0.66)	457 (0.34)	1360	1.3×10^{-6}	4.2×10^{-7}	1.5×10^{-5}	9.0×10^{-7}
Control	233 (0.31)	387 (0.52)	126 (0.17)	746	853 (0.57)	639 (0.43)	1492				
Total											
Case	385 (0.44)	394 (0.45)	89 (0.10)	868	1164 (0.67)	572 (0.33)	1736	2.7×10^{-9}	5.0×10^{-10}	5.7×10^{-6}	2.8×10^{-8}
Control	295 (0.32)	472 (0.51)	165 (0.18)	932	1062 (0.57)	802 (0.43)	1864				

Frequencies of genotypes and alleles are in parentheses.
[a]Genotype CC+CT versus TT.
[b]Genotype CC versus CT+TT.
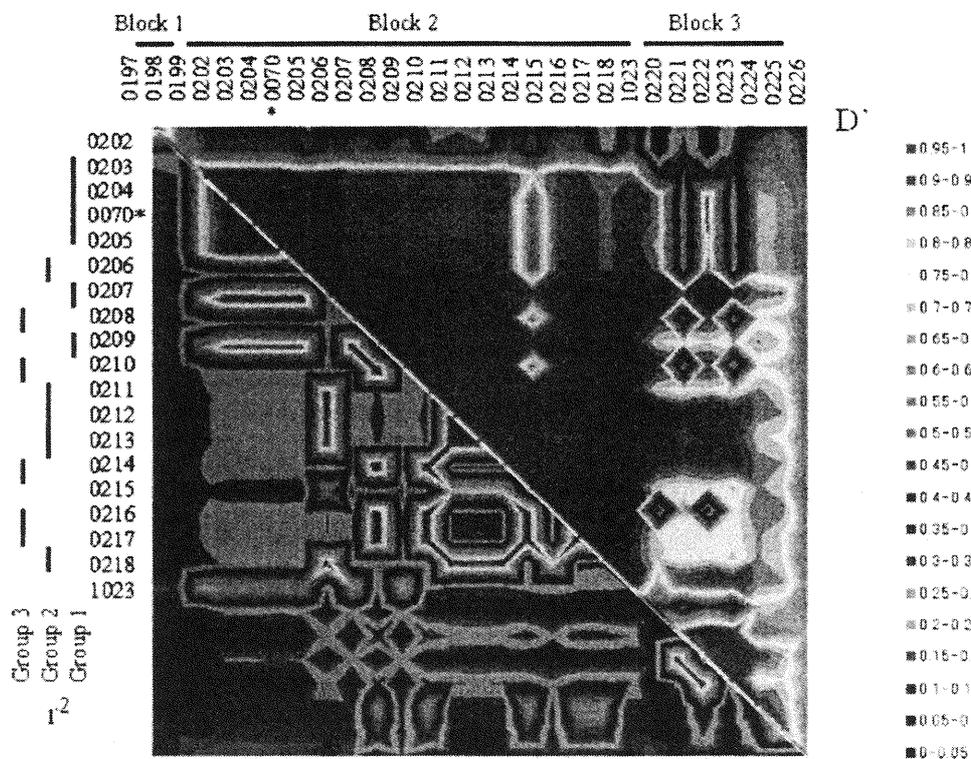
Figure 1. LD structure of the susceptibility region for sporadic PD. Pairwise LD between SNPs, as measured by D' in 134 controls, is graphically indicated. The region spanning 430 kb around the originally screened SNP0070(*) was divided into three LD blocks ($D' > 0.9$) (upper right). On the basis of r^2, SNPs in block 2, including SNP0070, were further divided into three groups ($r^2 > 0.85$) and three solitary SNPs (lower left). The scale is nominal.

SNCA gene expression in relation to susceptibility genotypes

To examine whether the strongest associated SNPs (group 1) affect SNCA gene expression, we further quantified SNCA mRNA in autopsied frontal cortices and compared the values among the genotypes. SNP0070, in which allele C is associated with PD, was used as a representative of group 1.

The relative values of SNCA mRNA for all cases ($n = 21$) and all controls ($n = 18$) were 1.07 ± 0.10 and 0.95 ± 0.13, respectively, showing almost the same level ($P = 0.46$, Student's t-test). When compared among the genotypes in cases, the mean tended to decrease in the order of CC, CT and TT (Fig. 3), although the differences did not reach the significant levels ($P = 0.71$ for CC versus CT, $P = 0.16$ for CT versus TT and $P = 0.32$ for CC versus TT). Similar tendency
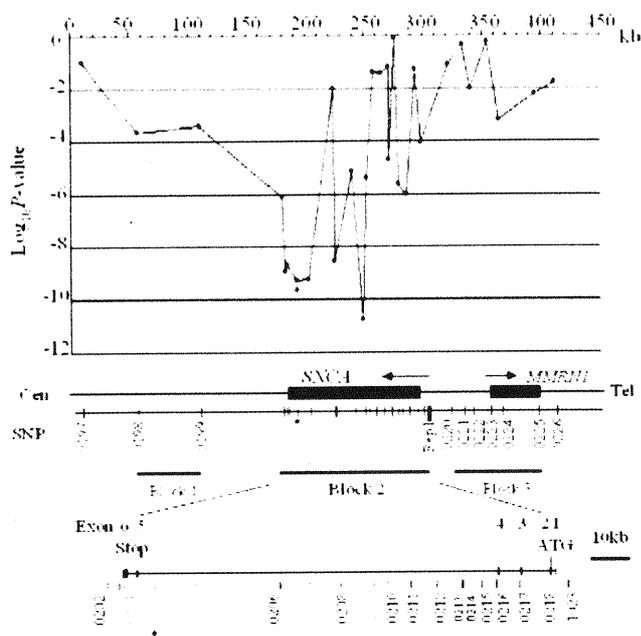
Figure 2. Genomic structure and SNPs of the susceptibility region for sporadic PD and case-control association studies (882 cases and 938 controls). Log P-values (allele 1 versus allele 2) are plotted against the physical location of the SNPs. The region includes two genes: *SNCA* and *MMRN1*; transcription 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, $P = 2.0 \times 10^{-9} - 1.7 \times 10^{-11}$). 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 ID (rs ID)	Alleles 12	Location	LD block (group)	Genotype 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 | | Recessive model | |
| | | Case | | | Control | | | (MM + Mm versus mm) | | (MM versus Mm + mm) | |
	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.
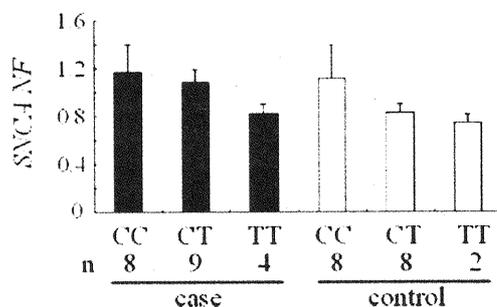
Figure 3. *In vivo* expression of *SNCA* mRNA in relation to susceptibility genotypes. SNP0070 (C/T) is used as a representative of group 1. *SNCA* expression levels in autopsied frontal cortices of cases (solid bar; 8 CC, 9 CT and 4 TT) and controls (open bar; 8 CC, 8 CT and 2 TT). Relative *SNCA* mRNA 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.

ACKNOWLEDGEMENTS

REFERENCES

1. Rascol, O., Payoux, P., Ory, F., Ferreira, J.J., Brefel-Courbon, C. and Montastruc, J.-L. (2003) Limitations of current Parkinson's disease therapy. *Ann. Neurol.*, **53** (Suppl. 3), S3–S12.
2. Warner, T.T. and Schapira, A.H. (2003) Genetic and environmental factors in the cause of Parkinson's disease. *Ann. Neurol.*, **53** (Suppl. 3), S16–S23.
3. Elbaz, A., Grigoletto, F., Baldereschi, M., Breteler, M.M., Manubens-Bertran, J.M., Lopez-Pousa, S., Dartigues, J.F., Alperovitch, A., Tzourio, C., Rocca, W.A. *et al.* (1999) Familial aggregation of Parkinson's disease: a population-based case–control study in Europe. *Neurology*, **52**, 1876–1882.
4. Sveinbjörnsdóttir, S., Hicks, A.A., Jónsson, T., Pétursson, H., Gugmundsson, G., Frigge, M.L., Kong, A., Gulcher, J.R. and Stefánsson, K. (2000) Familial aggregation of Parkinson's disease in Iceland. *N. Engl. J. Med.*, **343**, 1765–1770.
5. Piccini, P., Burn, D.J., Ceravolo, R., Maraganore, D. and Brooks, D.J. (1999) The role of inheritance in sporadic Parkinson's disease: evidence from a longitudinal study of dopaminergic function in twins. *Ann. Neurol.*, **45**, 577–582.
6. Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R. *et al.* (1997) Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science*, **276**, 2045–2047.
7. Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y. and Shimizu, N. (1998) Mutations in the *parkin* gene cause autosomal recessive juvenile parkinsonism. *Nature*, **392**, 605–608.
8. Leroy, E., Boyer, R., Auburger, G., Leube, B., Ulm, G., Mezey, E., Harta, G., Brownstein, M.J., Jonnalagada, S., Chernova, T. *et al.* (1998) The ubiquitin pathway in Parkinson's disease. *Nature*, **395**, 451–452.
9. Valente, E.M., Abou-Sleiman, P.M., Caputo, V., Muqit, M.M.K., Harvey, K., Gispert, S., Ali, Z., Del Turco, D., Bentivoglio, A.R., Healy, D.G. *et al.* (2004) Hereditary early-onset Parkinson's disease caused by mutations in *PINK1*. *Science*, **304**, 1158–1160.
10. Bonifati, V., Rizzu, P., van Baren, M.J., Schaap, O., Breedveld, G.J., Krieger, E., Dekker, M.C.J., Squitieri, F., Ibanez, P., Joosse, M. *et al.* (2003) Mutations in the *DJ-1* gene associated with autosomal recessive early-onset parkinsonism. *Science*, **299**, 256–259.
11. Paisán-Ruiz, C., Jain, S., Evans, E.W., Gilks, W.P., Simón, J., van der Brug, M., Lopez de Munain, A., Aparicio, S., Gil, A.M., Khan, N. *et al.* (2004) Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron*, **44**, 595–600.
12. Zimprich, A., Biskup, S., Leitner, P., Lichtner, P., Farrer, M., Lincoln, S., Kachergus, J., Hulihan, M., Uitti, R.J., Calne, D.B. *et al.* (2004) Mutations in *LRRK2* cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron*, **44**, 601–607.

13. Le, W.-D., Xu, P., Jankovic, J., Jiang, H., Appel, S.H., Smith, R.G. and Vassilatis, D.K. (2003) Mutations in *NR4A2* associated with familial Parkinson disease. *Nat. Genet.*, **33**, 85–89.

14. DeStefano, A.L., Golbe, L.I., Mark, M.H., Lazzarini, A.M., Maher, N.E., Saint-Hilaire, M., Feldman, R.G., Guttman, M., Watts, R.L., Suchowersky, O. *et al.* (2001) Genome-wide scan for Parkinson's disease: the GenePD Study. *Neurology*, **57**, 1124–1126.

15. Hicks, A.A., Pétursson, H., Jónsson, T., Stefánsson, H., Jóhannsdóttir, H.S., Sainz, J., Frigge, M.L., Kong, A., Gulcher, J.R., Stefánsson, K. *et al.* (2002) A susceptibility gene for late-onset idiopathic Parkinson's disease. *Ann. Neurol.*, **52**, 549–555.

16. Pankratz, N., Nichols, W.C., Uniacke, S.K., Halter, C., Rudolph, A., Shults, C., Conneally, P.M., Foroud, T. and the Parkinson Study Group (2002) Genome screen to identify susceptibility genes for Parkinson disease in a sample without *parkin* mutations. *Am. J. Hum. Genet.*, **71**, 124–135.

17. Scott, W.K., Nance, M.A., Watts, R.L., Hubble, J.P., Koller, W.C., Lyons, K., Pahwa, R., Stern, M.B., Colcher, A., Hiner, B.C. *et al.* (2001) Complete genomic screen in Parkinson disease: evidence for multiple genes. *JAMA*, **286**, 2239–2244.

18. Martin, E.R., Scott, W.K., Nance, M.A., Watts, R.L., Hubble, J.P., Koller, W.C., Lyons, K., Pahwa, R., Stern, M.B., Colcher, A. *et al.* (2001) Association of single-nucleotide polymorphisms of the tau gene with late-onset Parkinson disease. *JAMA*, **286**, 2245–2250.

19. van der Walt, J.M., Noureddine, M.A., Kittappa, R., Hauser, M.A., Scott, W.K., McKay, R., Zhang, F., Stajich, J.M., Fujiwara, K., Scott, B.L. *et al.* (2004) Fibroblast growth factor 20 polymorphisms and haplotypes strongly influence risk of Parkinson disease. *Am. J. Hum. Genet.*, **74**, 1121–1127.

20. Spillantini, M.G., Schmidt, M.L., Lee, V.M.-Y., Trojanowski, J.Q., Jakes, R. and Goedert, M. (1997) α-synuclein in Lewy bodies. *Nature*, **388**, 839–840.

21. Goedert, M. (2001) Alpha-synuclein and neurodegenerative diseases. *Nat. Rev. Neurosci.*, **2**, 492–501.

22. Schildkraut, J.M. (1998) Examining complex genetic interactions. In Haines, J.L. and Pericak-Vance, M.A. (eds), *Approaches to Gene Mapping in Complex Human Diseases*. Wiley-Liss, NY, pp. 379–410.

23. Mueller, J.C., Fuchs, J., Hofer, A., Zimprich, A., Lichtner, P., Illig, T., Berg, D., Wüllner, U., Meitinger, T. and Gasser, T. (2005) Multiple regions of α-synuclein are associated with Parkinson's disease. *Ann. Neurol.*, **57**, 535–541.

24. Pals, P., Lincoln, S., Manning, J., Heckman, M., Skipper, L., Hulihan, M., Van den Broeck, M., De Pooter, T., Cras, P., Crook, J. *et al.* (2004) α-Synuclein promoter confers susceptibility to Parkinson's disease. *Ann. Neurol.*, **56**, 591–595.

25. Maroteaux, L., Campanelli, J.T. and Scheller, R.H. (1988) Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal. *J. Neurosci.*, **8**, 2804–2815.

26. Eriksen, J.L., Dawson, T.M., Dickson, D.W. and Petrucelli, L. (2003) Caught in the act: α-synuclein is the culprit in Parkinson's disease. *Neuron*, **40**, 453–456.

27. Krüger, R., Kuhn, W., Müller, T., Woitalla, D., Graeber, M., Kösel, S., Przuntek, H., Epplen, J.T., Schöls, L. and Riess, O. (1998) Ala30Pro mutation in the gene encoding α-synuclein in Parkinson's disease. *Nat. Genet.*, **18**, 106–108.

28. Zarranz, J.J., Alegre, J., Gómez-Esteban, J.C., Lezcano, E., Ros, R., Ampuero, I., Vidal, L., Hoenicka, J., Rodriguez, O., Atarés, B. *et al.* (2004) The new mutation, E46K, of α-synuclein causes Parkinson and Lewy body dementia. *Ann. Neurol.*, **55**, 164–173.

29. Nagar, S., Juyal, R.C., Chaudhary, S., Behari, M., Gupta, M., Rao, S.N. and Thelma, B.K. (2001) Mutations in the α-synuclein gene in Parkinson's disease among Indians. *Acta Neurol. Scand.*, **103**, 120–122.

30. Singleton, A.B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R. *et al.* (2003) α-Synuclein locus triplication causes Parkinson's disease. *Science*, **302**, 841.

31. Miller, D.W., Hague, S.M., Clarimon, J., Baptista, M., Gwinn-Hardy, K., Cookson, M.R. and Singleton, A.B. (2004) α-synuclein in blood and brain from familial Parkinson disease with *SNCA* locus triplication. *Neurology*, **62**, 1835–1838.

32. Chartier-Harlin, M.-C., Kachergus, J., Roumier, C., Mouroux, V., Douay, X., Lincoln, S., Levecque, C., Larvor, L., Andrieux, J., Hulihan, M. *et al.* (2004) α-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet*, **364**, 1167–1169.

33. Ibáñez, P., Bonnet, A.-M., Débarges, B., Lohmann, E., Tison, F., Pollak, P., Agid, Y., Dürr, A., Brice, A. and French Parkinson's Disease Genetics Study Group (2004) Causal relation between α-synuclein gene duplication and familial Parkinson's disease. *Lancet*, **364**, 1169–1171.

34. Xia, Y., Rohan de Silva, H.A., Rosi, B.L., Yamaoka, L.H., Rimmler, J.B., Pericak-Vance, M.A., Roses, A.D., Chen, X., Masliah, E., DeTeresa, R. *et al.* (1996) Genetic studies in Alzheimer's disease with an NACP/α-synuclein polymorphism. *Ann. Neurol.*, **40**, 207–215.

35. Farrer, M., Maraganore, D.M., Lockhart, P., Singleton, A., Lesnick, T.G., de Andrade, M., West, A., de Silva, R., Hardy, J. and Hernandez, D. (2001) α-synuclein gene haplotypes are associated with Parkinson's disease. *Hum. Mol. Genet.*, **10**, 1847–1851.

36. Parsian, A., Racette, B., Zhang, Z.H., Chakraverty, S., Rundle, M., Goate, A. and Perlmutter, J.S. (1998) Mutation, sequence analysis, and association studies of α-synuclein in Parkinson's disease. *Neurology*, **51**, 1757–1759.

37. Tan, E.-K., Tan, C., Shen, H., Chai, A., Lum, S.-Y., Teoh, M.-L., Yih, Y., Wong, M.-C. and Zhao, Y. (2003) Alpha synuclein promoter and risk of Parkinson's disease: microsatellite and allelic size variability. *Neurosci. Lett.*, **336**, 70–72.

38. Jobling, M.A., Hurles, M. and Tyler-Smith, C. (2004) *Human Evolutionary Genetics*. Garland Science, NY, pp. 45–86.

39. Altshuler, D., Brooks, L.D., Chakravarti, A., Collins, F.S., Daly, M.J., Donnelly, P. and the International HapMap Consortium (2005) A haplotype map of the human genome. *Nature*, **437**, 1299–1320.

40. Murray, I.V.J., Giasson, B.I., Quinn, S.M., Koppaka, V., Axelsen, P.H., Ischiropoulos, H., Trojanowski, J.Q. and Lee, V.M.-Y. (2003) Role of α-synuclein carboxy-terminus on fibril formation *in vitro*. *Biochemistry*, **42**, 8530–8540.

41. Serpell, L.C., Berriman, J., Jakes, R., Goedert, M. and Crowther, R.A. (2000) Fiber diffraction of synthetic α-synuclein filaments shows amyloid-like cross-β conformation. *Proc. Natl Acad. Sci. USA*, **97**, 4897–4902.

42. Bower, J.H., Maraganore, D.M., McDonnell, S.K. and Rocca, W.A. (1999) Incidence and distribution of Parkinsonism in Olmsted County, Minnesota, 1976–1990. *Neurology*, **52**, 1214–1220.

43. Haga, H., Yamada, R., Ohnishi, Y., Nakamura, Y. and Tanaka, T. (2002) Gene-based SNP discovery as part of the Japanese Millennium Genome Project: identification of 190 562 genetic variations in the human genome. *J. Hum. Genet.*, **47**, 605–610.

44. McKeith, I.G., Dickson, D.W., Lowe, J., Emre, M., O'Brien, J.T., Feldman, H., Cummings, J., Duda, J.E., Lippa, C., Perry, E.K. *et al.* (2005) Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology*, **65**, 1863–1872.

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.

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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 [123I]metaiodobenzylguanidine (MIBG) cardiac scintigraphy (6), which shows low uptake of 123I 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 AQ2 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.