症様式と、晩期の進行性脳萎縮が特異であった。

神経病理学的にはDLB common formに分類されるが、臨床経過は、PD/ PDD/ DLBの診断基準のいずれにも厳密には該当せず、いわゆるDLBDの範疇でとらえるのが最も適当と考えられた。晩期に皮質萎縮が進行したのは，神経病理学的には、高度のαシヌクレイノパチーに起因させるのが素直と考えたが、さらなる症例の蓄積が必要である。

E.結論

当院で病理学的に確定診断されたレビー小体病21例を報告した. 本年PDD 1例, DLBD 1例が追加された. 一例は典型例だが, 他一例は LBPAF で発症, DLB が続発, PD が最後に出現し，αシヌクレイン沈着が、前頭側頭型の進行性皮質脳萎縮をきたした点が、特異であった。

F.健康危険情報

なし

G.研究発表

1.論文発表 なし

2.学会発表

足立正、齊藤祐子、関恒慶、五十川孝志、今福一郎、角田幸夫、村山繁雄：進行性の前頭側頭型萎縮を伴った、びまん性ビー小体病の一例。第89回関東臨床神経病理懇話会、2008年3月、東京、http://www.knp.gr.jp/

H.知的財産権の出願・登録状況 なし

1.特許取得 なし

2.実用新案登録 なし

3.その他 なし

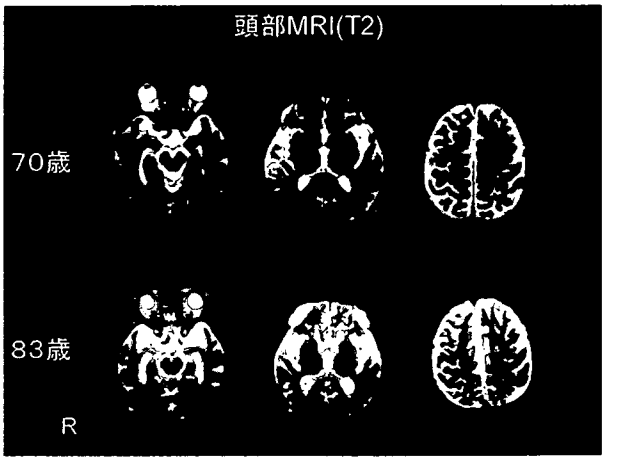

図 1. 進行性の前頭・側頭葉萎縮。MRI T2 強調画像

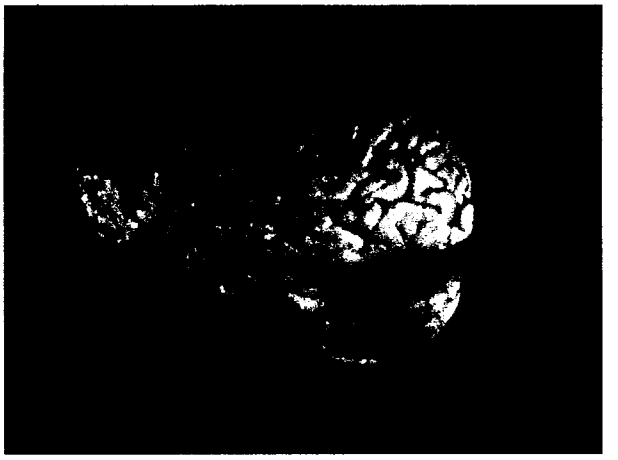

図 2.前頭側頭優位の脳萎縮が著明である。

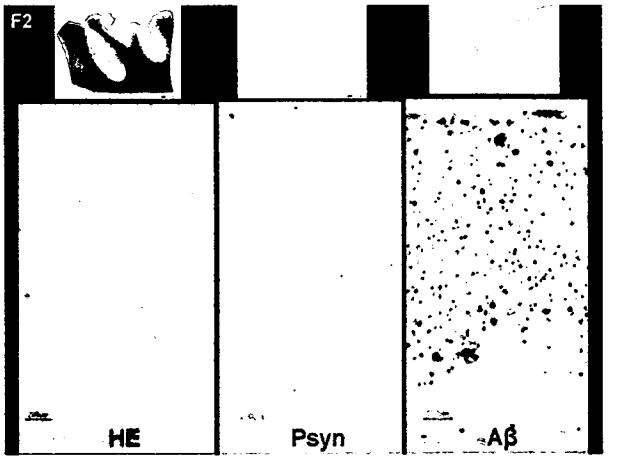

図 3. 前頭葉皮質の H.E.、抗リン酸化αシヌクレイン抗体免疫染色 (Psyn)、アミロイドβ蛋白 (Ab) 免疫染色像。αシヌクレイン沈着は皮質全般にびまん性かつ高度である。

III. 研究成果の刊行に関する一覧表

研究業績録

（添付３）

英文単行本

著　者　名	論　文　題　名	書　名	(編集者名)	発行社名	(発行地名)	出版西暦年	頁
なし							初め頁-終り頁

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著者名	論文題名	雑誌名	巻	頁	出版西暦年
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徳丸阿耶, 齊藤佑子, 村山繁雄, 金丸和富	高齢者の中枢神経感染症	感染症	37	100, 113-115	2007
徳丸　阿耶	プロトン密度強調画像の特徴はなんですか。どのような病変に有用ですか。	小児内科	39	37-39	2007
徳丸　阿耶　拡散強調画像の特徴はなんですか。どのような病変に有用ですか。	拡散強調画像の特徴はなんですか。どのような病変に有用ですか。	小児内科	39	40-43	2007
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別刷

SHORT REPORT

Selective loss of Purkinje cells in a patient with anti-glutamic acid decarboxylase antibody-associated cerebellar ataxia

Kazuyuki Ishida, Hiroshi Mitoma, Yoshiaki Wada, Teruaki Oka, Junji Shibahara, Yuko Saito, Shigeo Murayama, Hidehiro Mizusawa

J Neurol Neurosurg Psychiatry 2007;**78**:190–192. doi: 10.1136/jnnp.2006.091116

Anti-glutamic acid decarboxylase antibody is associated with the development of progressive cerebellar ataxia and slowly progressive insulin-dependent diabetes mellitus. Previously, the neurophysiological characteristics of IgG in the cerebrospinal fluid of a patient with anti-glutamic acid decarboxylase antibody-associated progressive cerebellar ataxia and slowly progressive insulin-dependent diabetes mellitus were reported. Using a voltage-gated whole-cell recording technique, it was observed that the IgG in the cerebrospinal fluid of the patient selectively suppressed the inhibitory postsynaptic currents in the Purkinje cells. The patient died from aspiration pneumonia. Postmortem examination showed almost complete depletion of the Purkinje cells with Bergmann gliosis. Therefore, the main cause of cerebellar ataxia observed in this case may be attributed to the near-complete depletion of the Purkinje cells. In this paper, the pathomechanisms underlying Purkinje cell damage are discussed.

Glutamic acid decarboxylase (GAD) is a catalytic enzyme that converts glutamic acid to γ-aminobutyric acid, a major inhibitory neurotransmitter. A disease group that is characterised by the presence of a circulating autoantibody against GAD (anti-GAD antibody) includes the following: slowly progressive insulin-dependent diabetes mellitus (SPIDDM), stiff-person syndrome (SPS) and progressive cerebellar ataxia (PCA).[1–3] Anti-GAD antibody is one of the serological diagnostic markers of these diseases. Honnorat *et al*[4] reported a significant link between the anti-GAD antibody and cerebellar ataxia after screening 9000 serum samples. In addition, autoimmune mechanisms against GAD are presumed to be the causative agents of these diseases.[5] Here, we report the autopsy findings of PCA with anti-GAD antibody and discuss the pathomechanism of this rare disease.

CASE REPORT

We previously reported part of the clinical course of a patient with PCA and SPIDDM, and showed the neurophysiological characteristics of IgG in the cerebrospinal fluid.[6] In September 1996, a 66-year-old woman developed cerebellar ataxia of the limbs and trunk. In April 1997, she had sudden onset of hyperglycaemia, and was subsequently diagnosed with anti-GAD-associated SPIDDM. In May 1997, she was bedridden due to severe cerebellar ataxia; other symptoms such as extrapyramidal or pyramidal tracts were not observed. The patient was diagnosed with anti-GAD antibody-associated PCA, and received four rounds of plasma exchange and immunosuppressive treatment. After treatment, the patient showed slight improvement in cerebellar ataxia.

In December 2000, the patient experienced painful spasms and rigidity in the trunk that mimicked symptoms of SPS. Diazepam and baclofen were effective in ameliorating the severe pain associated with the spasms and rigidity. The painful spasms subsided spontaneously within 2 months. The patient died of aspiration pneumonia in October 2001.

During the 5-year clinical course, repeated neuroradiological examinations showed no significant cerebellar atrophy. Using a voltage-gated whole-cell recording technique, we observed that the IgG in the cerebrospinal fluid of the patient, selectively suppressed the inhibitory postsynaptic currents in the Purkinje cells.[6 7]

Postmortem examination

Postmortem examination was performed 22 h after death. The brain weighed 1150 g. The brain and the entire spinal cord were fixed in formalin and prepared for a morphological examination. Macroscopically, there was no atrophy of the cerebrum, brain stem, cerebellum (fig 1A) and spinal cord. The representative areas were examined by routine and immunohistochemical staining, as reported previously.[8] In short, 6-μm thick serial sections were stained with haematoxylin and eosin, Klüver–Barrera and Bodian silver staining. For the immunohistochemical study, 6-μm dewaxed and microwave-irradiated sections were stained using a Ventana 20NX automatic stainer (Ventana, Tucson, Arizona, USA). Microscopical examination showed almost complete depletion of the Purkinje cells and diffuse proliferation of the Bergmann glia (fig 1B). The number of remaining Purkinje cells was no more than one per cerebellar folium. Bodian staining showed multiple empty baskets (fig 1C). There was no specific inflammatory response, and the other structures of the central nervous system, including the cerebral cortex, white matter, basal ganglia, brain stem and spinal cord, did not show marked pathological changes. The pancreas showed a definite and marked decrease in the islets in the tail (fig 1D), and lymphocytic infiltration in the islets situated in the pancreatic body.

DISCUSSION

The selective loss of both Purkinje cells and pancreatic islets was a characteristic finding in this case. The selective degeneration of the Purkinje cells partially mimics the pathological changes observed in paraneoplastic cerebellar ataxia associated with anti-mGluR1 or anti-Yo antibody; however, the exclusive pathological changes related to the Purkinje cells constitute a unique feature of this case.[9 10] On the other hand, the lymphocytic infiltration in the pancreas and the selective decrease in the pancreatic islets corresponded with the pathological findings of autoimmune insulin-dependent diabetes mellitus.[11] Therefore, the main causes of cerebellar ataxia and diabetes mellitus seem to be related to the depletion of the Purkinje cells and the decrease in the pancreatic islets,

Abbreviations: GAD, glutamic acid decarboxylase; PCA, progressive cerebellar ataxia; SPIDDM, slowly progressive insulin-dependent diabetes mellitus; SPS, stiff-person syndrome

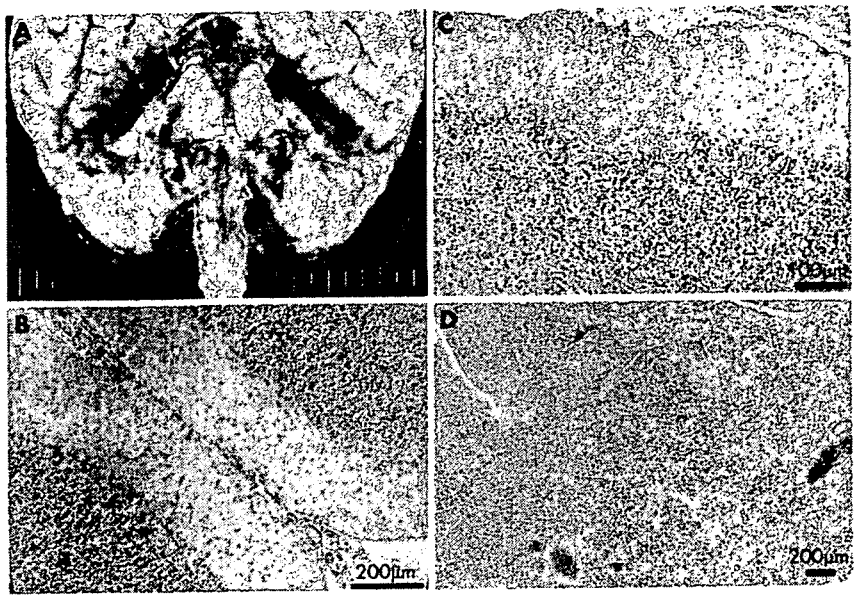

Figure 1 (A) Macroscopic appearance of the brain stem and cerebellum. There are no atrophic changes in the cerebellum and brain stem. (B) Haematoxylin and eosin staining of the cerebellar cortex. There is severe depletion of Purkinje cells and proliferation of Bergmann glia. (C) Bodian staining of the cerebellar cortex. Multiple empty baskets can be observed. (D) Pancreatic tail (haematoxylin and eosin staining). There is a selective decrease in the pancreatic islets.

respectively. To our knowledge, this is the first autopsy report of PCA associated with anti-GAD antibody.

Immunohistochemical staining using anti-GAD and anti-calbindin antibodies failed to react with the patient's specimen; this indicated a complete loss of antigenicity in the patient's specimen, due to postmortem delay and excessive fixation. Therefore, it became difficult to analyse the morphological changes in the other GAD-containing neurones, such as the cerebellar basket cells and the spinal Renshaw cells. However, the existence of multiple empty baskets suggested that, in contrast to the Purkinje cells that were lost, the basket cells were relatively preserved.[12]

We inferred two possible pathomechanisms to explain the Purkinje cell damage: indirect and direct immune-mediated mechanisms. The indirect mechanism might be associated with excitotoxicity of the Purkinje cells by the selective suppression of inhibitory postsynaptic currents and the attenuation of inhibition of excitatory postsynaptic currents by the anti-GAD antibody.[6 7 13] The direct mechanism might be mediated by cytotoxic reactions against the Purkinje cells caused by the invading leucocytes, as observed in the pancreatic islets. However, it is presently unclear whether the mechanisms that are more likely to have caused the Purkinje cell damage are indirectly or directly immune-mediated.

The patient experienced painful muscle spasms that mimic symptoms of SPS. The muscle spasms observed in SPS are considered to occur as a result of the dysfunction of the Renshaw cells that are γ-aminobutyric acid inhibitory interneurones in the spinal cord.[14] Various pathological changes are observed in the spinal cord of patients with SPS; however, lymphocytic cuffing and a decrease in the number of anterior horn neurones are considered to be representative of SPS.[15] In contrast, the pathological changes observed in our patient were unremarkable; this suggests that the Renshaw cells were not severely damaged. This may explain the transient nature of the muscular spasms in this case.

Based on the quantitative analysis of the brain autopsy of a patient with SPS and without cerebellar ataxia, Warich-Kirches *et al*[16] reported diminished cell density of the inhibitory neurones in the cerebellar cortex. Combining their case results with ours might show the phenotypic overlap of the anti-GAD autoimmunity-associated neurological diseases.

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Competing interests: None declared.

Informed consent was obtained from the family of the patient for the publication of her details in this paper.

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Received 17 February 2006
Revised 13 October 2006
Accepted 25 October 2006
Published Online First 17 November 2006

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Journal of the Neurological Sciences 254 (2007) 44–48

Journal of the Neurological Sciences

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A Japanese family with early-onset ataxia with motor and sensory neuropathy

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Received 16 July 2006; received in revised form 8 December 2006; accepted 18 December 2006
Available online 29 January 2007

Abstract

We report the case of a Japanese family with hereditary ataxia with peripheral neuropathy. Three affected siblings from this family exhibited very similar clinical features: teenage-onset, slowly progressive ataxia, followed by distal weakness, which developed after the age of 30 years. Magnetic resonance imaging studies showed marked atrophy in the cerebellar hemisphere and vermis, and a sural nerve biopsy revealed a marked reduction in the number of both myelinated and unmyelinated fibers. All patients exhibited hyperglutamatemia, but serum levels of albumin and lipid were normal. The clinicopathological and biochemical features of these cases suggest that they form a distinct entity of autosomal recessive hereditary ataxia with peripheral neuropathy.

Keywords: Cerebellar atrophy; Peripheral neuropathy; Autosomal recessive inheritance; Hyperglutamatemia

1. Introduction

Hereditary ataxia is sometimes associated with peripheral neuropathy, and it appears to comprise a heterogeneous group of diseases. The most common of these diseases among the Caucasian population is Friedreich's ataxia (FA), which is caused by mutation of the *frataxin* gene [1]. However, no patients with FA have been reported in the Japanese population. Early-onset ataxia with ocular motor apraxia and hypoalbuminemia (EAOH), which is caused by a mutation of the *aprataxin* gene, forms a homogeneous group within the Japanese population who suffer from hereditary ataxia associated with peripheral neuropathy [2,3]. Various types of hereditary ataxia with peripheral neuropathy have been reported [4,5], but their clinical entity remains unclear. Here we report the familial cases of patients with early-onset ataxia with severe motor and sensory neuropathy and hyperglutamatemia.

2. Patients and clinical evaluation

Three patients (two males and one female), who are siblings (Fig. 1), were diagnosed with ataxia and motor sensory neuropathy, based on clinical, neuroradiological, and neuropathological findings. Their parents were cousins who died in their 90s and had no signs of the disease. Patients IV-3 and IV-6 were admitted to Tokyo University Hospital when they were 63 and 52 years old, respectively. Patient IV-4 was examined by one of the authors at a nursing

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doi:10.1016/j.jns.2006.12.014

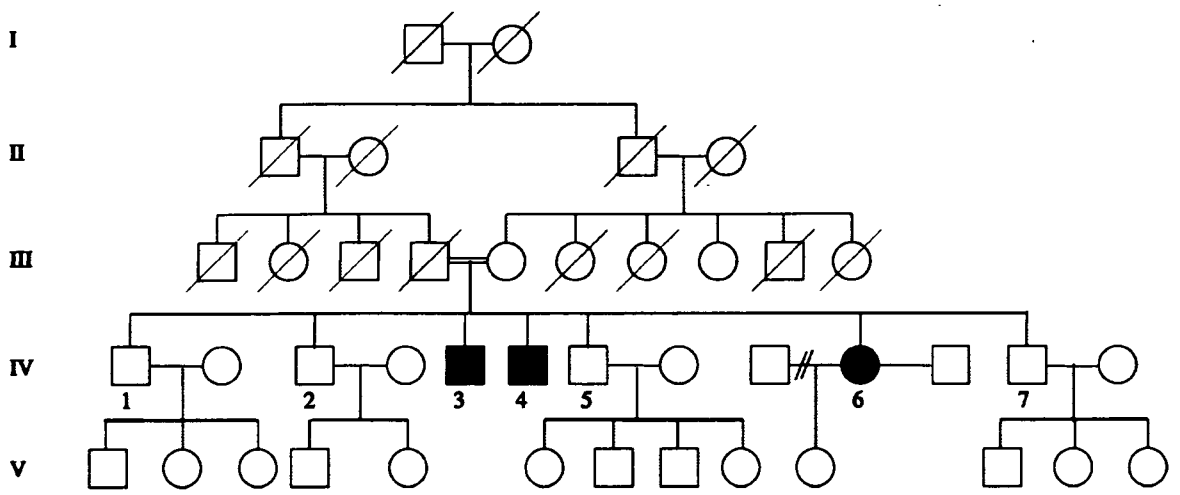

Fig. 1. Pedigree of the family. Squares and circles indicate males and females, respectively. Filled symbols represent affected individuals.

house when he was 61 years old. Three siblings of these patients (IV-1, IV-2, and IV-7) were also examined and no positive neurological signs were detected.

The clinical histories of the three affected siblings resemble each other closely. The first symptom of the disease, gait instability, was noted at age 15, 17, and 18 years in patients IV-3, IV-4, and IV-6, respectively. In all patients, limb and truncal ataxia progressed mainly in their 20s, and muscular weakness of the upper and lower distal extremities progressed mainly in their 30s and 40s. These patients were confined to a wheelchair at the age of 47 years (patient IV-3), 44 years (patient IV-4), and 39 years (patient IV-6). Patients IV-3 and IV-4 were both diagnosed as mildly diabetic at the age of 52 years. At age 55 years, patient IV-3 was diagnosed with reflux esophagitis, and patient IV-4 developed cholelithiasis at the age of 58 years.

On examination, two of the patients showed a normal range of intelligence (patient IV-3: WAIS-R IQ 84, VIQ 95, PIQ 72; patient IV-4: full score in Mini-Mental Scale Examination). Patient IV-6 showed mild cognitive impairment (WAIS-R IQ 65, VIQ 84, PIQ 50, Raven's progressive color matrices 22/37). In all patients, external eye movements were full, but smooth pursuit was abnormally saccadic. Ocular motor apraxia was not present. Lateral gaze nystagmus was observed in all patients and rebound nystagmus was remarkable in patient IV-6. None of the patients had any other ophthalmologic abnormalities, including retinitis pigmentosa. Speech was slurred and ataxic in all three patients, and their distal extremities exhibited wasting and weakness, especially the lower extremities. The musculature in the proximal extremities was well preserved. All patients had sensory loss of all modalities in a "glove and stocking" distribution. Deep tendon reflexes were all absent. None of the patients exhibited pathological reflexes. Although evaluation of coordination was difficult due to severe distal limb weakness, all of the patients showed large oscillatory movement on the finger-to-nose test, suggesting the presence of limb ataxia. Patient IV-6 had mild scoliosis. None of the patients had cardiopulmonary abnormalities.

3. Laboratory study

Blood cell counts were normal in all three patients. Routine blood chemistry was normal, with the exception of high glycosylated hemoglobin A1c in patient IV-4 (7.7%). Serum alpha-fetoprotein, lactate, pyruvate, cholestanol, plasma vitamin E, very long-chain fatty acid, ceruloplasmin, beta-lipoprotein, leukocyte liposome enzymes, CSF cell count and protein level were within the normal ranges in all three patients. Plasma transferrin isofocusing showed normal property, excluding the diagnosis of congenital disorders of glycosylation. Plasma glutamate levels were higher than normal in all three patients (Table 1). All examined unaffected siblings (IV-1, IV-2, and IV-7) showed normal levels of plasma glutamate (<63 nmol/ml). The results of genetic screening for SCA1, SCA2, MJD, DRPLA, PMP22, and P0, carried out for patients IV-3 and IV-6, were all negative.

4. Neuroimaging study

Brain MRI showed a marked atrophy of the cerebellar hemisphere and vermis in all three patients. There was no clear atrophy in the cerebral cortex and the brainstem (Fig. 2A–C).

5. Electrodiagnostic study

Electrodiagnostic studies were performed in patients IV-3 and IV-6. Motor NCVs were reduced in both patients.

Table 1
Summary of biochemical data

	Patients			Normal range
	IV-3	IV-4	IV-6	
Serum total protein (g/dl)	6.2	6.8	6.9	6.2–8.2
Serum albumin (g/dl)	3.4	3.8	3.6	3.4–5.8
Serum total cholesterol (mg/dl)	136	150	162	130–220
Serum triglyceride (mg/dl)	76	80	92	50–150
Serum β-lipoprotein (mg/dl)	252	271	315	190–500
Plasma glutamate (nmol/ml)	140	158.5	130	12–63

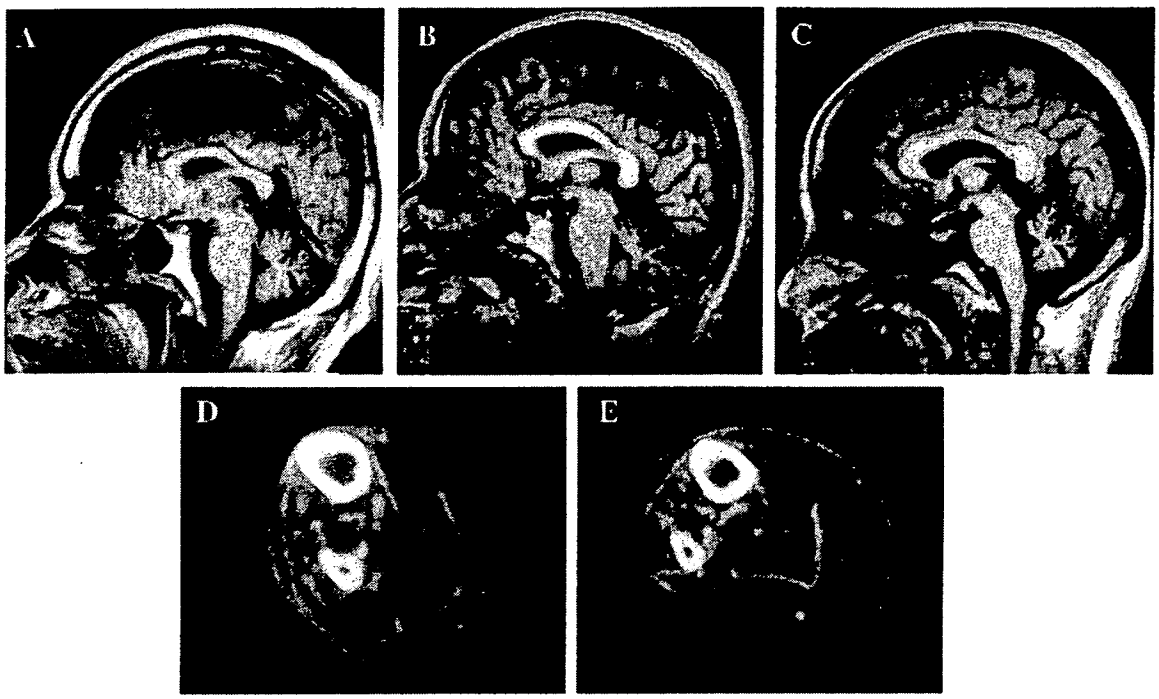

Fig. 2. Brain magnetic resonance imaging (MRI) and muscle computed tomography (CT) images. A–C: Sagittal planes of T1-weighted MRI images in patients IV-3 (A), IV-4 (B), and IV-6 (C). All images show marked atrophy of the cerebellar vermis. D, E: CT scans of calf muscles in patients IV-3 (D) and IV-6 (E). The low-density appearance of the muscles indicates fatty degeneration.

Compound muscle action potentials (CMAP) were markedly low [NCV (m/s)/CMAP (mV) patient IV-3: median nerve 25/0.046, ulnar nerve 26/0.013, tibial nerve — not detected, peroneal nerve — not detected; patient IV-6: median nerve 41/1.77, ulnar nerve 44/1.42, tibial nerve — not detected, peroneal nerve 53/0.32]. Sensory nerve action potentials were not detected in either patient IV-3 or IV-6 at any of the tested nerves. Needle electromyography demonstrated

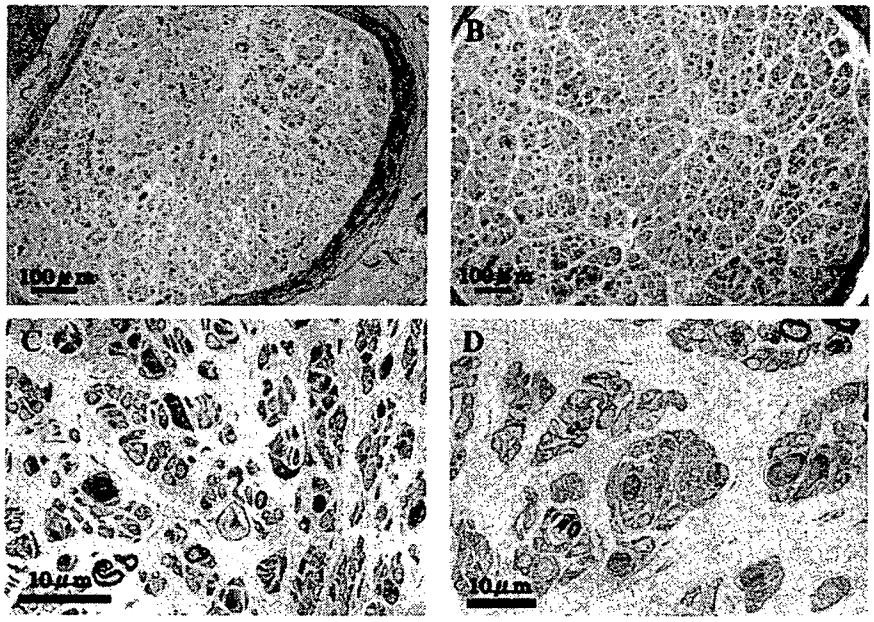

Fig. 3. Sural nerve biopsy specimens. A, B: Light microscopy of transverse semithin sections of the distal sural nerve (A: patient IV-3, magnification ×1200, toluidine blue stain; B: patient IV-6, magnification ×1000, toluidine blue stain). C, D: Electron micrographs of sural nerves (C: patient IV-3, magnification ×25,000; D: patient IV-6, magnification ×33,000).

spontaneous fibrillation potentials in the hand muscles of both of these patients. On volitional muscle contraction, discharges had a high amplitude and long duration in all examined muscles except the tibialis anterior muscle, where no discharge was observed. The quantity of discharges was markedly reduced, especially in the leg muscles. In summary, needle electromyography examination indicated chronic neurogenic change that was more severe in the distal than the proximal muscles.

6. Muscle CT

Patients IV-3 and IV-6 both exhibited similar findings on the muscle CT. Muscles in the lower extremities showed low density, indicating fat replacement (Fig. 2D and E). In contrast, the truncal, paraspinal, and limb-girdle muscles were well preserved. The forearm muscles were atrophic in patient IV-6.

7. Neuropathological study

Examination of the sural nerves revealed a marked reduction in the numbers of both myelinated and unmyelinated fibers in patients IV-3 and IV-6 (Fig. 3). Proof of demyelination/remyelination was not found in either case. Fiber density was 825/mm^2 in patient IV-3 (normal range: 7500–10,000/mm^2).

8. Discussion

The clinical picture of the cases reported here is a combination of spinocerebellar degeneration (SCD) and hereditary motor sensory neuropathy (HMSN). Healthy consanguineous parents and three affected siblings in this family suggest autosomal recessive inheritance. The involvement of the central nervous system appears to be limited to the cerebellum, except for mild cognitive impairment in one patient. Conduction studies and morphologic studies indicated that peripheral nerve involvement in our patients was axonal neuropathy, which was much more severe than that reported for some autosomal dominant SCDs [6–8]. In all three patients, cerebellar ataxia, which developed in their teenage years, was followed by motor sensory neuropathy in their 30s or 40s.

Differential diagnoses of Refsum disease, Bassen–Kornzweig disease, metachromatic leukodystrophy, and congenital disorders of glycosylation were excluded by laboratory tests. Ataxia-oculomotor apraxia 1 is not likely because of its earlier age at onset and oculomotor apraxia. Ataxia-oculomotor apraxia 2 can be excluded because of normal alpha-fetoprotein level in our patients. Mitochondrial disorders are not likely because of normal serum lactate and pyruvate levels in our patients. Absence of spasticity and retinal involvement makes the diagnosis of autosomal recessive spastic ataxia of Charlevoix–Saguenay unlikely. Cerebrotendinous xanthomatosis is excluded because of the normal level of serum cholestanol and lack of xanthoma formation. Fukuhara et al. reported patients with HMSN associated with cerebellar atrophy (HMSNCA) who showed hypoalbuminemia and hyperlipidemia [9]. Recently, it was demonstrated that an atypical form of FA in Japan, labeled EAOH, was caused by mutation of the *aprataxin* gene [2]. Diagnoses of HMSNCA [9,10] and EAOH [3] are unlikely because of normal serum albumin and total cholesterol levels in our patients. Tanji et al. reported a Japanese family with progressive cerebellar ataxia, distal amyotrophy of Charcot–Marie–Tooth type and hyperglutamatemia [11], which resembles our cases very much. Present cases together with Tanji's cases appear to form a clinical entity distinct from previously reported diseases of ataxia with peripheral neuropathy including FA, EAOH and HMSNCA.

Hyperglutamatemia was observed in all of our patients and in none of the three unaffected siblings. The results indicate involvement of glutamate in the pathogenesis of the present disorder. Glutamate is the most abundant free amino acid in the central nervous system playing a prominent role in synaptic plasticity, learning and memory. Glutamate is also a potent neuronal excitotoxin, implicated in the pathogenesis of cerebral ischemia, and epilepsy [12]. Experimentally, infusions of high concentrations of glutamate elicit neuronal degeneration by, at least in part, enhanced calcium entry in the cytosol through excessive stimulation of the *N*-methyl-D-aspartate (NMDA) receptors. Hyperglutamatemia has also been reported in some neurodegenerative diseases [12–14]. Partial deficiency of the enzyme glutamate dehydrogenase in fibroblasts, leukocytes, and platelets has been reported to occur in various types of ataxia [13,14], and it does not appear to identify a specific type of ataxic disease [15,16]. Hyperglutamatemia in our patients suggests some form of abnormality of glutamate metabolism, but it is difficult to speculate its role in the pathogenesis of their disease. Future investigation on genetic and biochemical aspects of this disease is important, because it might lead to therapeutic interventions by blocking NMDA- and non-NMDA-receptor mediated neurotoxicity.

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J Neuropathol Exp Neurol

Vol. 66, No. 5
May 2007
pp. 354–362

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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This work was supported by grants from Aid for Scientific Research on Priority Areas–Advanced Brain Science Project from the Ministry of Education, Culture, Sports, Science and Technology of Japan (SM, YS), Aid for Degenerative Disease from the Ministry of Health, Labor and Welfare (SM), Aid for Long-Term Comprehensive Research on Age-Associated Dementia from the Tokyo Metropolitan Institute of Gerontology (SM), and the Tokyo Metropolitan Geriatric Hospital (YS).

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)

		LB						
Stage	Substantia Nigra and Locus Ceruleus: Loss of Pigmentation	Nigrostriatal	Limbic-Neocortical	Spinal Cord	LB Score	Dementia	Parkinsonism	Diagnosis
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.

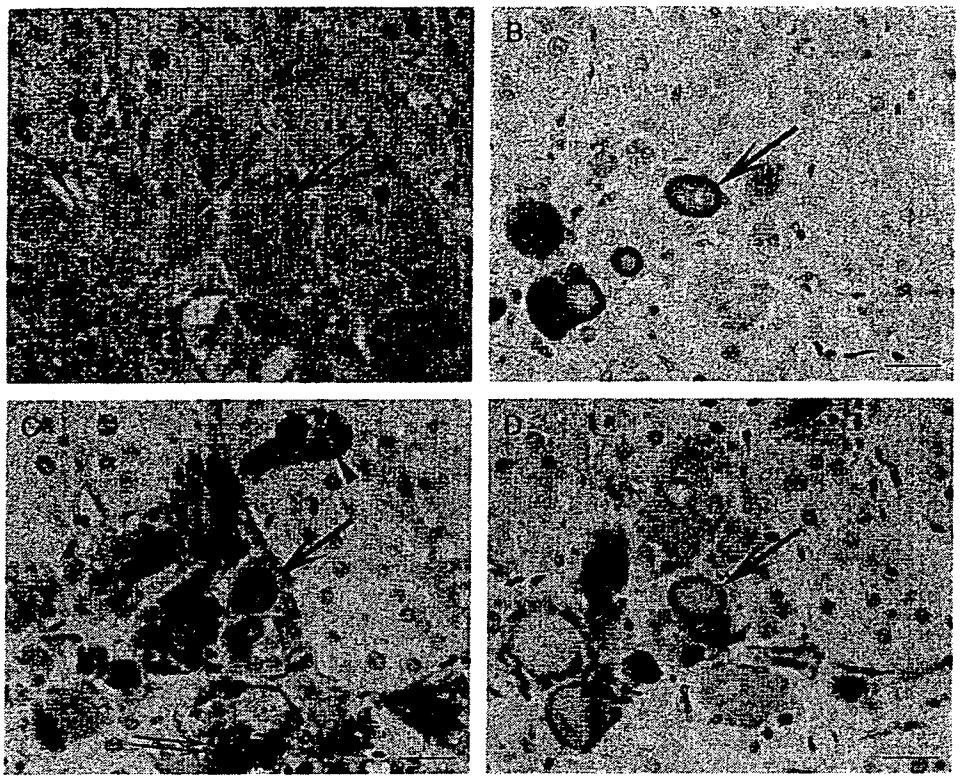

FIGURE 1. Lewy body-related α-synucleinopathy in the adrenal medulla. **(A)** Lewy bodies (arrow) are identified in a hematoxylin and eosin-stained section. **(B)** Anti-phosphorylated α-synuclein (Pser129) antibody clearly visualizes Lewy body-related inclusions, some of which show a central halo and 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.

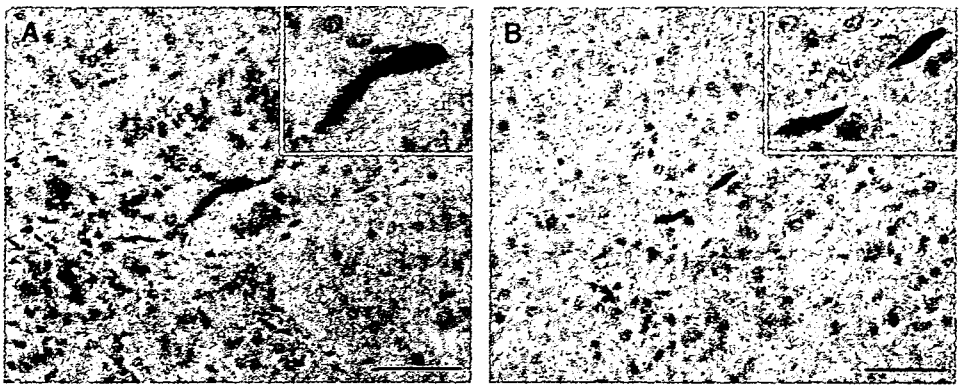

FIGURE 2. Lewy body-related α-synucleinopathy in the adrenal cortex. **(A, B)** Anti-phosphorylated α-synuclein antibody **(A)**, psyn#64, monoclonal; Pser129, polyclonal **(B)**. Both antibodies are raised against the same synthetic peptide and show the same specificity in immunoblots (15, 19). The 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

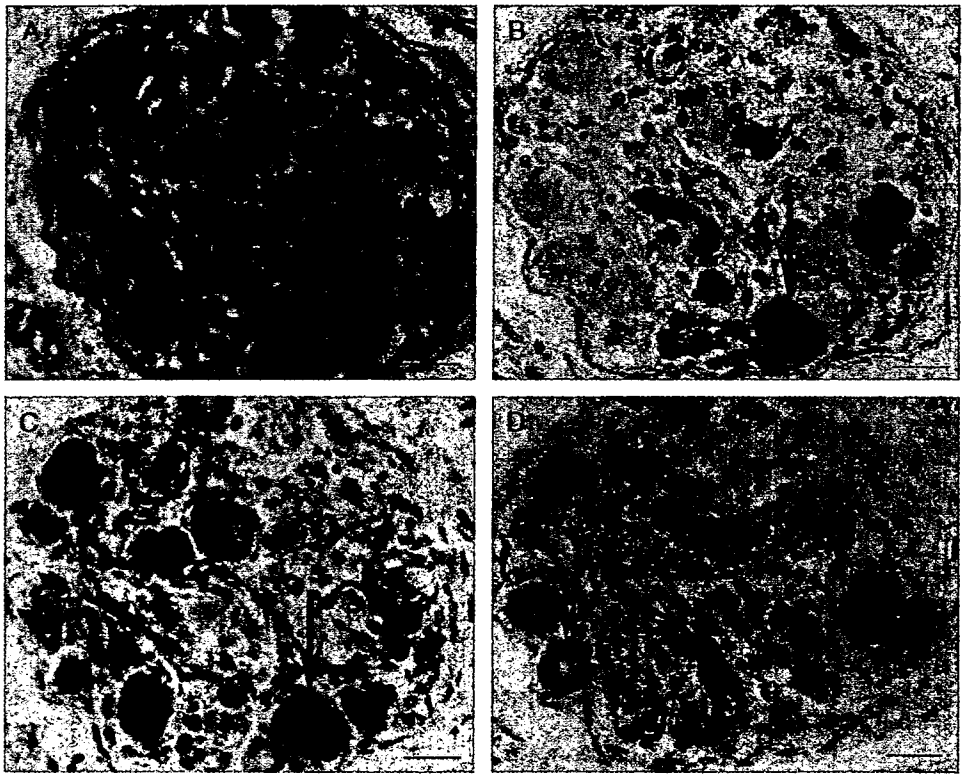

FIGURE 3. Lewy body-related α-synucleinopathy in a sympathetic ganglion from the fatty tissue surrounding the adrenal capsule. **(A)** Lewy bodies (arrow) are visible in a hematoxylin and eosin-stained section. **(B)** Anti-phosphorylated α-synuclein (Pser129) immunostaining visualizes the abundant Lewy 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.)