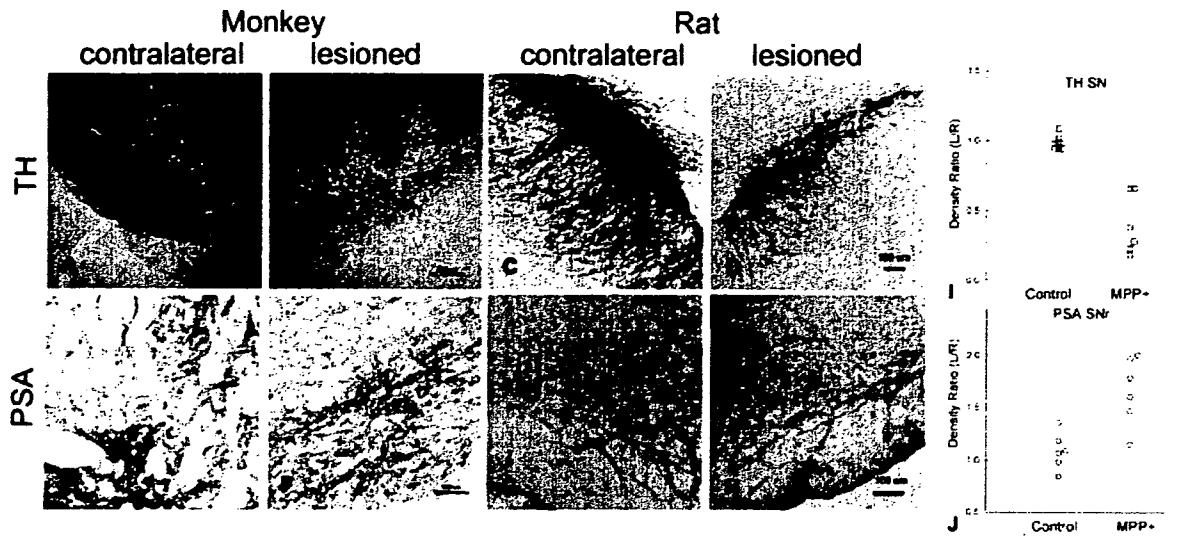


Fig 4. Changes in polysialic acid (PSA)–positive cells in hemilesioned animals. Substantia nigra of hemi-parkinsonian model monkey (A, B, E, F) and rat (C, D, G, H) showed reduced tyrosine hydroxylase (TH; A–D) and increased PSA (E–H) immunostaining. The substantia nigra of the monkey ipsilateral to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Sigma, St. Louis, MO)–injected caudate (B, F) and that of the rat ipsilateral to 1-methyl-4-phenylpyridinium salt (MPP^+)–injected medial forebrain bundle (D, H) are shown side by side to their respective contralateral sides (A, C, E, G). (I, J) Six intact and six hemi-MPP^+ lesioned rats were subjected to densitometric analysis, and the results are expressed as the ratio of staining density of MPP^+-injected side to the saline-injected side. Each dot *represents one animal, and the* horizontal bars *indicate the average of the group. Marked reduction in TH* ($p < 0.01$) *and a slight increase in PSA* ($p < 0.01$) *immunostaining were detected in the MPP^+-treated side (two-tailed* t *test), whereas the ratio was almost even in the intact rats. Scale bars = 500μm (A, B); 100μm (C–H).*

can label the proliferating cells in a small area.[19,22] However, unlike the migration from subventricular zone (SVZ) to the olfactory bulb, the number of GFP-labeled cells beside the aqueduct was small, especially in mice. Because injection of the same retroviral solution into the dorsal striatum close to the lateral ventricle succeeded in labeling a large number of neuronal stem cells,[22] the small number noted in our study is not likely to be because of leakage of the viral solution into the aqueduct. Our preliminary study showed nestin, Ki67 immunostaining, and BrdU-positive nuclei after the current method of BrdU-administration was not evident around the midbrain aqueduct, whereas marked staining of them was noted in SVZ of the lateral ventricle (data not shown). These results suggest that the aqueduct area is not likely the source of new neurons in the adult midbrain.

Does Polysialic Acid in Substantia Nigra Suggest Neurogenesis?

Many cells in the SNr in PD midbrain were PSA-positive. Similar results were noted in the monkey and six rats after dopaminergic deprivation in the left hemisphere. In the hippocampus, PSA expresses on neurons under plastic changes of synapses and newly generated neurons.[24] In the midbrain, it is unclear whether PSA can be a marker of newly formed neurons.[35] Despite the negative results of retroviral labeling, it is possible that neurogenesis is present but undetectable by BrdU or retroviral vector. Neurogenesis in SVZ is well documented[38] and can be separated into three steps: proliferation, migration, and neuronal differentiation. BrdU and retroviral vector label DNA during its duplication. These methods have been effective in detecting neurogenesis in SVZ and hippocampus, but they are not direct markers of neuronal differentiation. Neural progenitor cells may not necessarily differentiate to neurons sequentially after cell proliferation. Actually, the cells start the final step of neuronal differentiation a few days after they reach the olfactory bulb.

Immature cells that can differentiate into neurons in vivo on transplantation into the hilar region of the hippocampus have been isolated from the adult rat midbrain.[11] It is unclear whether immature cells can differentiate into neurons and express PSA without cell division. In this study, most GFP-labeled cells were microglia and NG2-positive glial precursors (see Table 2), which can recover the multipotency and differentiate into neurons in certain environments.[39,40] These precursor cells may start neuronal differentiation without further proliferation when more neurons are required in the brain.

Regeneration of nigral neurons is still an attractive therapeutic target in PD. It is not appropriate to give a negative conclusion to this therapeutic possibility simply because the proliferative cells failed to differentiate

Table 3. Characteristics of Patients and Disease Controls

	Subject Age (yr)	Sex	Diagnosis	Duration of Illness	Yahr Stage	Dopa Treatment (yr)	PSA-Positive Cells[a]	
							SNc	SNr
Control								
	81	M	Cerebral infarction				6.0	0.0
	63	M	Myotonic muscular dystrophy				13.5	1.5
	57	F	Myasthenia gravis				21.0	2.5
	77	M	Cerebral hemorrhage				10.5	8.5
	92	M	Cerebral hemorrhage				99.5	11.0
	67	F	Alzheimer's disease				4.0	11.5
Mean	72.8						25.8	5.8
SD	12.9						36.6	5.1
PD								
	85	F	PD	13	5	13	7.5	0.0
	77	M	PD	7	4	7	5.0	8.5
	74	F	PD	8	4	8	8.5	17.0
	69	F	PD	9	4	7	33.0	28.0
	62	M	PD	13	3	13	33.0	38.5
	70	M	PD	16	4	16	14.5	55.5
Mean	72.8						16.9	24.6
SD	7.8						12.8[b]	20.4[c]

[a]The average of duplicate counting of each sample by blinded observer is shown. Data are aligned from top to bottom in the order of PSA cell number in SNr.
[b]$p < 0.05$; and [c]$p < 0.01$, significant difference of distribution, F-test.

PSA = polysialic acid; SNc = substantia nigra pars compacta; SNr = substantia nigra pars reticulata; SD = standard deviation; PD = Parkinson's disease.

into dopaminergic neurons. Nigrostriatal dopaminergic projection shows considerable recovery after MPTP treatment of animals, and a small number of TH-positive neurons appear in the SN after such treatment.[41–43] We speculate that the PSA-TH double-positive cells identified in this study represent newly generated dopaminergic neurons in the adult SN. In this regard, a recent study showed that dopaminergic agonists stimulate neurogenesis in SVZ.[43] It has been suggested that certain therapeutic agents currently in use, such as selegiline, ropinirole, and pramipexole, can slow the progress of the disease.[44,45] Further studies are warranted to examine the effects of these compounds on neurogenesis in the midbrain.

This work was supported by grants from the High Technology Research Center; the Ministry of Education, Culture, Sports, Science, and Technology, Japan (grant-in-aid for Scientific Research, B16390255, H.M.; S.M., Y.S.), Fujimoto Pharmaceuticals Corporation, (K.Y.), Ministry of Health, Labor and Welfare (S.M.), and the Tokyo Metropolitan Institute of Gerontology (S.M.).

We are grateful to Dr. H. Mori for providing human brain samples, Drs Y. Imai and S. Kohsaka for providing anti-Iba-1 antibody; Dr H. Akiyama for providing anti-glial fibrillary acidic protein antibody; H. Hayakawa for technical assistance; and Dr H. Imai for providing hemi-parkinsonian monkey brain sample. We thank Dr S. Fukuda for technical advice in confocal microscopy and for fruitful discussion.

References

1. Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 2000;20:9104–9110.
2. Banasr M, Hery M, Brezun JM, Daszuta A. Serotonin mediates oestrogen stimulation of cell proliferation in the adult dentate gyrus. Eur J Neurosci 2001;14:1417–1424.
3. Santarelli L, Saxe M, Gross C, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 2003;301:805–809.
4. Armstrong RJ, Barker RA. Neurodegeneration: a failure of neuroregeneration? Lancet 2001;358:1174–1176.
5. Curtis MA, Penney EB, Pearson AG, et al. Increased cell proliferation and neurogenesis in the adult human Huntington's disease brain. Proc Natl Acad Sci U S A 2003;100:9023–9027.
6. Mikkonen M, Soininen H, Tapiola T, et al. Hippocampal plasticity in Alzheimer's disease: changes in highly polysialylated NCAM immunoreactivity in the hippocampal formation. Eur J Neurosci 1999;11:1754–1764.
7. Jin K, Peel AL, Mao XO, et al. Increased hippocampal neurogenesis in Alzheimer's disease. Proc Natl Acad Sci U S A 2004; 101:343–347.

8. Hirsch EC, Hunot S, Faucheux B, et al. Dopaminergic neurons degenerate by apoptosis in Parkinson's disease. Mov Disord 1999;14:383–385.
9. Olanow CW, Goetz CG, Kordower JH, et al. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. Ann Neurol 2003;54:403–414.
10. Kay JN, Blum M. Differential response of ventral midbrain and striatal progenitor cells to lesions of the nigrostriatal dopaminergic projection. Dev Neurosci 2000;22:56–67.
11. Lie DC, Dziewczapolski G, Willhoite AR, et al. The adult substantia nigra contains progenitor cells with neurogenic potential. J Neurosci 2002;22:6639–6649.
12. Zhao M, Momma S, Delfani K, et al. Evidence for neurogenesis in the adult mammalian substantia nigra. Proc Natl Acad Sci U S A 2003;100:7925–7930.
13. Frielingsdorf H, Schwarz K, Brundin P, Mohapel P. No evidence for new dopaminergic neurons in the adult mammalian substantia nigra. Proc Natl Acad Sci U S A 2004;101: 10177–10182.
14. Nakatomi H, Kuriu T, Okabe S, et al. Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. Cell 2002;110: 429–441.
15. Zhu DY, Lau L, Liu SH, et al. Activation of cAMP-response-element-binding protein (CREB) after focal cerebral ischemia stimulates neurogenesis in the adult dentate gyrus. Proc Natl Acad Sci U S A 2004;101:9453–9457.
16. Kawai T, Takagi N, Miyake-Takagi K, et al. Characterization of BrdU-positive neurons induced by transient global ischemia in adult hippocampus. J Cereb Blood Flow Metab 2004;24: 548–555.
17. Rakic P. Adult neurogenesis in mammals: an identity crisis. J Neurosci 2002;22:614–618.
18. Gould E, Gross CG. Neurogenesis in adult mammals: some progress and problems. J Neurosci 2002;22:619–623.
19. Suzuki SO, Goldman JE. Multiple cell populations in the early postnatal subventricular zone take distinct migratory pathways: a dynamic study of glial and neuronal progenitor migration. J Neurosci 2003;23:4240–4250.
20. Kaneko S, Onodera M, Fujiki Y, et al. Simplified retroviral vector gcsap with murine stem cell virus long terminal repeat allows high and continued expression of enhanced green fluorescent protein by human hematopoietic progenitors engrafted in nonobese diabetic/severe combined immunodeficient mice. Hum Gene Ther 2001;12:35–44.
21. Suzuki A, Obi K, Urabe T, et al. Feasibility of ex vivo gene therapy for neurological disorders using the new retroviral vector GCDNsap packaged in the vesicular stomatitis virus G protein. J Neurochem 2002;82:953–960.
22. Yamada M, Onodera M, Mizuno Y, Mochizuki H. Neurogenesis in olfactory bulb identified by retroviral labeling in normal and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated adult mice. Neuroscience 2004;124:173–181.
23. Tanaka R, Yamashiro K, Mochizuki H, et al. Neurogenesis after transient global ischemia in the adult hippocampus visualized by improved retroviral vector. Stroke 2004;35:1454–1459.
24. Seki T, Arai Y. Highly polysialylated neural cell adhesion molecule (NCAM-H) is expressed by newly generated granule cells in the dentate gyrus of the adult rat. J Neurosci 1993;13: 2351–2358.
25. Fukuda S, Kato F, Tozuka Y, et al. Two distinct subpopulations of nestin-positive cells in adult mouse dentate gyrus. J Neurosci 2003;23:9357–9366.
26. Furuya T, Hayakawa H, Yamada M, et al. Caspase-11 mediates inflammatory dopaminergic cell death in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. J Neurosci 2004;24:1865–1872.
27. Przedborski S, Jackson-Lewis V, Naini AB, et al. The parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): a technical review of its utility and safety. J Neurochem 2001;76:1265–1274.
28. Altar CA, Heikkila RE, Manzino L, Marien MR. 1-Methyl-4-phenylpyridine (MPP+): regional dopamine neuron uptake, toxicity, and novel rotational behavior following dopamine receptor proliferation. Eur J Pharmacol 1986;131:199–209.
29. Imai H, Nakamura T, Endo K, Narabayashi H. Hemiparkinsonism in monkeys after unilateral caudate nucleus infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): behavior and histology. Brain Res 1988;474:327–332.
30. Mochizuki H, Imai H, Endo K, et al. Iron accumulation in the substantia nigra of 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP)-induced hemiparkinsonian monkeys. Neurosci Lett 1994;168:251–253.
31. Ito D, Imai Y, Ohsawa K, et al. Microglia-specific localisation of a novel calcium binding protein, Iba1. Brain Res Mol Brain Res 1998;57:1–9.
32. Dawson MR, Levine JM, Reynolds R. NG2-expressing cells in the central nervous system: are they oligodendroglial progenitors? J Neurosci Res 2000;61:471–479.
33. Cammer W, Zhang H. Localization of Pi class glutathione-S-transferase in the forebrains of neonatal and young rats: evidence for separation of astrocytic and oligodendrocytic lineages. J Comp Neurol 1992;321:40–45.
34. Yoshimi K, Iwata N, Takeda M, et al. Ischaemia-induced change in clathrin preceding delayed neuronal death. Neuroreport 1995;6:453–456.
35. Nomura T, Yabe T, Rosenthal ES, Krzan M, Schwartz JP. PSA-NCAM distinguishes reactive astrocytes in 6-OHDA-lesioned substantia nigra from those in the striatal terminal fields. J Neurosci Res 2000;6:588–596.
36. Kawano H, Ohyama K, Kawamura K, Nagatsu I. Migration of dopaminergic neurons in the embryonic mesencephalon of mice. Brain Res Dev Brain Res 1995;86:101–113.
37. Hokfelt-T, Martensson-R, Bjorklund-A, et al. Distributional maps of tyrosine-hydroxylase-immunoreactive neurons in the rat brain. Handbook of chemical neuroanatomy. Vol 2. New York: Elsevier, 1984:277.
38. Alvarez-Buylla A, Garcia-Verdugo JM. Neurogenesis in adult subventricular zone. J Neurosci 2002;22:629–634.
39. Belachew S, Chittajallu R, Aguirre AA, et al. Postnatal NG2 proteoglycan-expressing progenitor cells are intrinsically multipotent and generate functional neurons. J Cell Biol 2003;161: 169–186.
40. Yokoyama A, Yang L, Itoh S, et al. Microglia, a potential source of neurons, astrocytes, and oligodendrocytes. Glia 2004; 45:96–104.
41. Jackson-Lewis V, Jakowec M, Burke RE, Przedborski S. Time course and morphology of dopaminergic neuronal death caused by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Neurodegeneration 1995;4:257–269.
42. Ho A, Blum M. Induction of interleukin-1 associated with compensatory dopaminergic sprouting in the denervated striatum of young mice: model of aging and neurodegenerative disease. J Neurosci 1998;18:5614–5629.
43. Hoglinger GU, Rizk P, Muriel MP, et al. Dopamine depletion impairs precursor cell proliferation in Parkinson disease. Nat Neurosci 2004;7:726–735.
44. Marek K, Jennings D, Seibyl J. Do dopamine agonists or levodopa modify Parkinson's disease progression? Eur J Neurol 2002;9(suppl 3):15–22.
45. Stocchi F, Olanow CW. Neuroprotection in Parkinson's disease: clinical trials. Ann Neurol 2003;53(suppl 3):S87–S97.

Acta Neuropathol (2005) 109: 353–366
DOI 10.1007/s00401-004-0966-4

REGULAR PAPER

Kuniaki Tsuchiya · Shigeo Murayama · Kazuko Mitani
Tatsuro Oda · Kunimasa Arima · Masaru Mimura
Hiroshi Nagura · Chie Haga · Haruhiko Akiyama
Hiroshi Yamanouchi · Hidehiro Mizusawa

Constant and severe involvement of Betz cells in corticobasal degeneration is not consistent with pyramidal signs: a clinicopathological study of ten autopsy cases

Received: 13 September 2004 / Revised: 15 November 2004 / Accepted: 16 November 2004 / Published online: 25 February 2005

Abstract This report concerns a clinicopathological study of three additional patients with corticobasal degeneration (CBD), described here for the first time, and a clinicopathological correlation between pyramidal signs and upper motor neuron involvement, in ten autopsy cases of CBD, including seven cases reported by us previously. We investigated pyramidal signs, including hyperreflexia, Babinski sign, and spasticity, and involvement of the primary motor cortex and pyramidal tract, focusing on the astrocytosis of the fifth layer of the primary motor cortex. Pyramidal signs were observed in six (60%) of the ten cases. Hyperreflexia was evident in six patients (60%), with spasticity being observed in three patients (30%). Loss of Betz cells associated with prominent astrocytosis and presence of ballooned neurons in the fifth layer of the primary motor cortex was observed in all ten cases. In all cases, involvement of the pyramidal tract was obvious in the medulla oblongata, without involvement of the pyramidal tract in the midbrain. Constant and severe involvement of the fifth layer of the primary motor cortex, including the Betz cells, has not previously been reported in CBD. We suggest that the pyramidal signs in CBD have been disregarded.

Keywords Betz cells · Charcot-Rebeiz disease · Corticobasal degeneration · Pyramidal signs · Pyramidal tract degeneration

K. Tsuchiya (✉)
Department of Laboratory Medicine and Pathology,
Tokyo Metropolitan Matsuzawa Hospital, 2-1-1,
Kamikitazawa, Setagaya-ku, 156-0057 Tokyo, Japan
E-mail: ktsuchi@jcom.home.ne.jp
Tel.: +81-3-33037211
Fax: +81-3-33045109

K. Tsuchiya · C. Haga · H. Akiyama
Department of Neuropathology,
Tokyo Institute of Psychiatry, Tokyo, Japan

K. Tsuchiya · H. Mizusawa
Department of Neurology,
Tokyo Medical and Dental University, Tokyo, Japan

S. Murayama
Department of Neuropathology,
Tokyo Metropolitan Gerontology, Tokyo, Japan

K. Mitani · H. Nagura · H. Yamanouchi
Department of Neurology,
Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan

T. Oda
Department of Psychiatry, National Shimofusa Sanatorium,
Chiba, Japan

K. Arima
Department of Laboratory Medicine,
National Center Hospital for Mental,
Nervous and Muscular Disorders, Tokyo, Japan

M. Mimura
Department of Neuropsychiatry,
Showa University School of Medicine, Tokyo, Japan

Introduction

Corticobasal degeneration (CBD) [3, 9, 10, 11, 16, 17, 20, 21, 28, 29, 31, 38, 42, 43, 51, 56, 58, 59, 72, 73, 74] or cortical-basal ganglionic degeneration [4, 5, 37] was first described by Rebeiz et al. in 1967 and 1968 [52, 53] as corticodentatonigral degeneration with neuronal achromasia. It is a rare neurodegenerative disorder and can be classified as "Parkinson plus" together with multiple system atrophy and progressive supranuclear palsy [22, 23, 24, 25, 30]. CBD may occur more frequently as initially thought, as indicated by the growing number of reported cases since the report of three autopsy cases of CBD by Gibb et al. [18] in 1989. With

more autopsy cases and clinicopathological studies of CBD. Goetz [19] in 2000 noticed that the CBD prototype may be the "atypical Parkinson's disease" described by Jean-Martin Charcot. In 2000, Agid [1] proposed that the clinical diagnosis of CBD was evident when the following features were observed in a given patient: an akineto-rigid syndrome unresponsive to L-DOPA associated with dystonic postures, apraxia, and a marked asymmetry of symptoms. Furthermore, Agid noted that if Jean-Martin Charcot was really the first to point out this form of parkinsonism (atypical Parkinson's disease) at the end of the last century, that is, 75 years before Rebeiz et al. described the three cases that became the archetype of the syndrome, it might perhaps be more reasonable to name this affliction Charcot-Rebeiz disease, at least until its mechanism and causes are discovered. In 1999, Tsuchiya et al. [65] found that basal ganglia lesions of Group B Pick's disease, which have prominent degeneration of the pallidum and substantia nigra, and those classified by Constantinidis et al. [8] in 1974 and Constantinidis [7] in 1985, which macroscopically show frontal atrophy and histologically cortical degeneration characterized by ballooned neurons without Pick bodies, are fundamentally consistent with the basal ganglia lesions of CBD elucidated by Tsuchiya et al. in 1997 [63]. Recently, CBD has been regarded a member of the "Pick complex" [13, 60], a "unifying concept of overlapping clinical syndromes and neuropathological findings of neurodegenerative diseases causing focal cortical atrophy", as proposed by Kertesz and Munoz, emphasizing the similarities, rather than the differences, between them [32, 33, 44].

It is generally believed that pyramidal signs, including hyperreflexia, Babinski sign, and spasticity, are usually observed in cases of CBD [6, 36, 54, 55, 76]. Furthermore, it has been reported that the frequency of pyramidal signs in cases of CBD, ranged from extremely common [37] to about 27% [57]. In contrast, clinicopathological correlation studies of pyramidal signs with the lesions of the primary motor cortex and pyramidal tract in CBD have been rare [27, 53].

The purpose of this report is to describe the clinicopathological features of CBD in ten Japanese autopsy cases, including pyramidal signs and involvement of the primary motor cortex and pyramidal tract, focusing on the presence or absence of astrocytosis in the fifth layer of the primary motor cortex associated with presence of ballooned neurons and loss of Betz cells: i.e., small groupings of fat granule cells in the spaces in which Betz cells were present [66, 70]. We investigated the clinicopathological correlation between pyramidal signs and involvement of the pyramidal tract in ten autopsy cases. In addition, we address in the discussion the pathological heterogeneity in the primary motor cortex among multiple system atrophy (MSA), amyotrophic lateral sclerosis (ALS) with dementia, and CBD, paying attention to the clinicopathological dissociation of the pyramidal signs and lesions of the Betz cells and pyramidal tract in CBD, compared with those of MSA and ALS with dementia.

Materials and methods

The present investigation was carried out on ten autopsy cases from three Japanese institutions. The clinical records and tissue specimens in cases 1, 3, 6, 7, 8, and 9 were from the Department of Neuropathology, Tokyo Institute of Psychiatry. Those in cases 2, 5, and 10 were from the Department of Neuropathology, Tokyo Metropolitan Gerontology, and those in case 4 were from the Department of Laboratory Medicine, National Center Hospital for Mental, Nervous, and Muscular Disorders.

After fixation in formalin, the brains of the ten cases were sectioned in the coronal plane. The cerebral hemisphere and/or small blocks, including the frontal, temporal, parietal, and occipital lobes, and the striatum, pallidum, subthalamic nucleus, thalamus, amygdala, and hippocampus, were taken. Additional tissue blocks were taken from the midbrain, including the substantia nigra, brain stem, and cerebellum. The brains were embedded in paraffin and cut at a thickness of about 10 μm. The sections were stained with hematoxylin-eosin (HE), and also using the Klüver-Barrera, Holzer, Bodian, methenamine silver, and modified Gallyas-Braak methods. Immunocytochemistry was performed using antibodies against human-tau pool 2 (from Dr. H. Mori: Osaka City university), polyclonal neurofilament (200 kDa), and glial fibrillary acidic protein (GFAP).

The neuropathological diagnosis in the ten cases was made on the basis of the findings described below, which included many astrocytic plaques and ballooned neurons [14, 15, 34, 35] in the cerebral cortex and the widespread presence of argyrophilic threads in the central nervous system (Figs. 1, 2, 3). The neuropathological features of all ten cases were fundamentally consistent with the recently proposed neuropathological criteria for CBD by Dickson et al. [12].

The clinicopathological findings in cases 1, 3, 4, 6, 7, 8, and 9 have been reported previously by Tsuchiya et al. [61], Mimura et al. [39], Arima et al. [2], Oda et al. [49], Mitani et al. [40], Miyazaki et al. [41], and Oda et al. [50], respectively. The neuropathological hallmarks of case 7, including the abnormal cytoskeletal pathology peculiar to CBD, have been described by Uchihara et al. [71]. The distribution of cerebral cortical lesions identified by light microscopy, classified into three categories in cases 1, 6, 7, 8, and 9, has been reported by Tsuchiya et al. [62]. The distribution of basal ganglia lesions, classified into three categories in cases 1, 4, 6, 7, 8, and 9, has been investigated by Tsuchiya et al. [63]. Serial brain CT of cases 1 and 8 have also been described by Tsuchiya et al. [64].

Basal ganglia lesions, including the pallidum, caudate nucleus, putamen, and subthalamic nucleus, was

Table 1 Summary of clinical and pathological features (*M* male, *F* female, + presence, − absence,*severe* severe neuronal loss, *moderate*moderate neuronal loss, *slight* slight neuronal loss.*PD* Pick's disease, *CBD* corticobasal degeneration, *PSP* progressive supranuclear palsy)

Case	1	2	3	4	5	6	7	8	9	10
Clinical diagnosis	CBD	CBD	PD or frontal lobe dementia	PD	CBD	PSP	CBD	PD	PD	Atypical PSP
Heredity	−	−	−	−	−	−	−	−	−	
Sex	M	M	F	M	M	F	M	F	F	F
Age at onset (years)	65	71	62	64	64	45	63	58	62	67
Duration ofthe disease	2 years 1 month	4 years 8 months	2 years 9 months	9 years 2 months	5 years 8 months	8 years	3 years 10 months	3 years 7 months	7 years	11 years
Initial sign	Limbkinetic apraxia	Limbkinetic apraxia	Motor aphasia	Aphasia	Memory disturbance	Delusion of persecution	Limbkinetic apraxia	Abnormal behavior	Abnormal behavior	Memory disturbance
Muscular rigidity	+	+	−	+	+	+	+	−	+	+
Dementia	+	+	+	+	+	+	+	+	+	+
Brain weight (g)	1,370	1,345	1,200	1,120	1,100	1,050	1,040	987	940	810
Cerebral cortex										
Neuronal loss	+	+	+	+	+	+	+	+	+	+
Ballooned neurons	+	+	+	+	+	+	+	+	+	+
Astrocytic plaques	+	+	+	+	+	+	+	+	+	+
Pallidum	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe
Striatum (caudate nucleus and putamen)	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Subthalamic nucleus	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight
Neuronal loss in the Substantia nigra	+	+	+	+	+	+	+	+	+	+
References	[61, 62, 63, 64]		[39]	[2, 63]		[49, 62, 63]	[40, 62, 63, 71]	[41, 62, 63, 64]	[50, 62, 63]	

Table 2 Clinicopathological correlation between pyramidal signs and involvement of the primary motor cortex and pyramidal tract (+ present, – absent, *N.R.* not recorded)

Case	1	2	3	4	5	6	7	8	9	10
Pyramidal sign	+	–	N.R.	–	–	+	+	–	+	–
Hyperreflexia	+	–	N.R.	–	–	–	+	–	–	–
Babinski sign	+	–	N.R.	–	–	–		–	N.R.	–
Spasticity	+	+	N.R.	N.R.	–	N.R.	+	–	N.R.	N.R.
Loss of Betz cells	+	+	+	–	+	+	–	+	+	+
Astrocytosis of the primary motor cortex layer V	+	+	+	–	+	+	–	+	+	+
Degeneration of the pyramidal tract										
Midbrain	–	–	–	–	–	–	–	–	–	–
Medulla oblongata	+	–	+	+	+	–	+	+	+	+

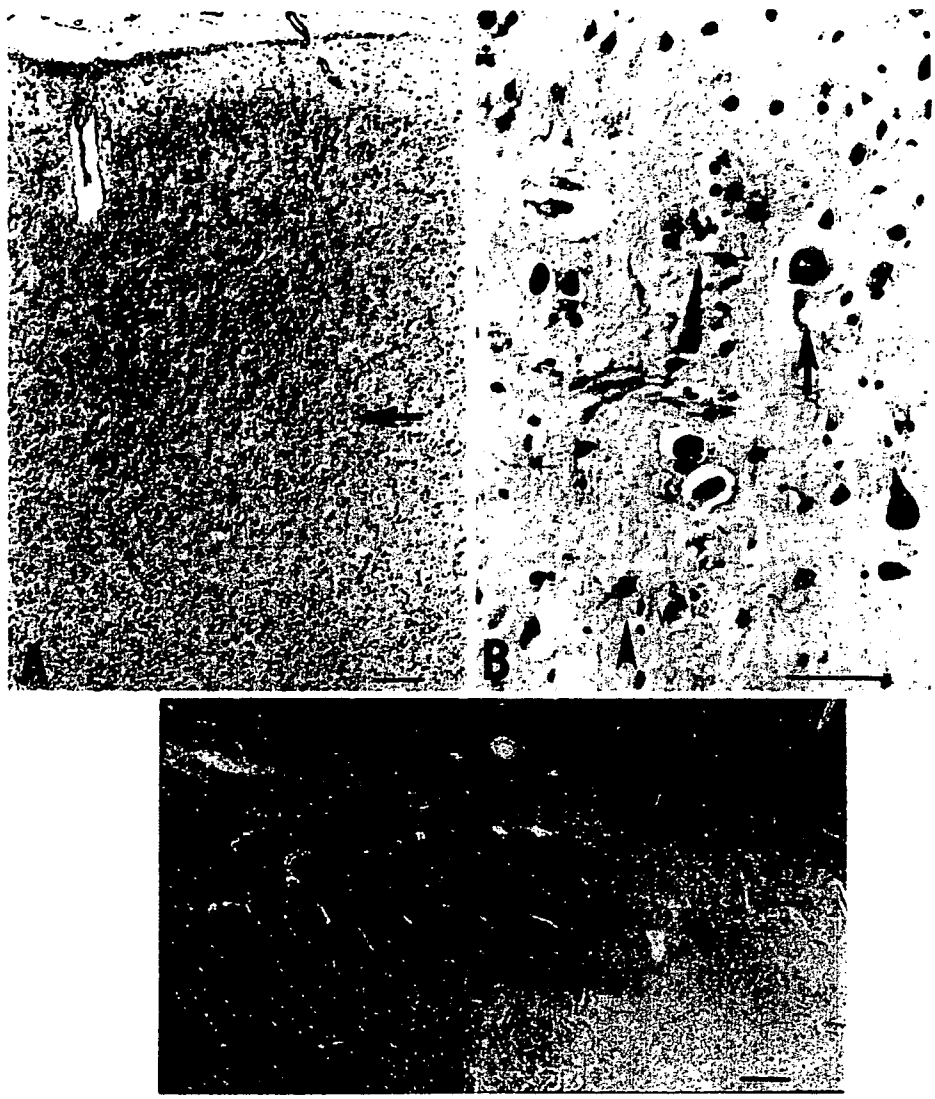


Fig. 1 A–C Case 2. A Superior frontal gyrus showing obvious neuronal loss. B. Enlargement of area indicated by *arrow* in A showing a ballooned neuron (*arrow*) and fibrillary glia (*arrowhead*). C Relative preservation of the subthalamic nucleus (*arrow*). A, B HE stain; C Klüver-Barrera stain; *bars* A 0.2 mm, B 0.05 mm, C 0.5 mm

classified as follows. Lesions identified by light microscopy were assigned to one of three categories: slight, showing relative preservation of the neurons with slight proliferation of the glia; moderate, showing obvious neuronal loss with evident astrocytosis and slight fibrillary gliosis; or severe, showing pronounced neuronal loss with neuropil rarefaction and/or prominent fibrillary gliosis. The classification of basal ganglia lesions described above is fundamentally consistent with the classification of basal ganglia lesions in CBD.

Fig. 2 A–C Case 5. A Astrocytic plaque (*arrow*) in the cerebral cortex. B Substantia nigra showing prominent neuronal loss with free melanin (*arrow*). C Substantia nigra showing neurofibrillary tangles (*arrow*). A modified Gallyas-Braak stain. B Klüver-Barrera stain. C modified Gallyas-Braak stain; *bars* A–C 0.05 mm

Pick's disease with Pick bodies (PDPB) [65], a generalized variant of Pick's disease (gvPD) [67], diffuse neurofibrillary tangles (NFT) with calcification, reported by Tsuchiya et al. [63, 65, 67, 69], respectively. The clinical and pathological features of all cases are summarized in Table 1.

Pyramidal signs were judged to be present in patients who showed one or more signs of hyperreflexia in the extremities, Babinski sign, and spasticity in the extremities. Loss of Betz cells was judged to be present in cases that showed small groupings of lipofuscin-laden macrophages in the holes, from which Betz cells had presumably disappeared, in the primary motor cortex with the presence of normal and degenerated Betz cells in the absence of an internal granular layer [26, 45, 46, 47, 48, 75] (Fig. 4). Astrocytosis of primary motor cortex layer V was considered present in cases showing definite astrocytosis determined using HE and Holzer staining or immunohistochemistry using an antibody against GFAP (Fig. 4). Pyramidal tract degeneration was also judged as present in cases showing definite loss of myelinated fibers shown by Klüver-Barrera stain, accompanied by gliosis revealed using Holzer stain and immunohistochemistry using an antibody against GFAP (Fig. 5). The determination of loss of Betz cells, astrocytosis of primary motor cortex layer V, and the pyramidal tract degeneration described above was fundamentally consistent with that in MSA and ALS with dementia, as described by Tsuchiya et al. [66, 70]. The pertinent data are summarized in Table 2.

Case reports

Clinical course and neuropathological findings in case 2

The patient was a Japanese man aged 76 years at the time of death. He was in good health until the age of 71, when he became aware of clumsiness of the right hand and action tremor of the right upper extremity, followed by bradykinesia 3 months later and action tremor of the left upper extremity 7 months after the onset of the disease. A neurological examination at the age of 72 years (8 months after the disease onset), revealed right limbkinetic apraxia, mild agraphia, bilateral action myoclonus on the upper extremities, prominent on the right, muscular rigidity, bradykinesia, and hyperreflexia in the upper and lower extremities without Babinski sign. At this stage, obvious dementia was not observed. Neurological examination 1 year after disease onset disclosed evident dementia. At 2 years after the disease onset, he could no longer walk without assistance. Vertical ocular movement involvement and dysphagia were noticed 3 years after disease onset. During this period, he was bed-ridden. At 3 years 10 months after disease onset, severe dementia and spasticity were obvious. He died of pneumonia at age 76, 4 years 8 months after the onset of the disease. He was clinically diagnosed as having CBD.

The brain weighed 1,345 g. Macroscopic examination revealed atrophy of the posterior portion in the superior frontal gyrus abutting the precentral gyrus, atrophy of the pallidum, and depigmentation of the substantia nigra. A histological examination showed neuronal loss with astrocytosis, status spongiosus, many ballooned neurons, and astrocytic plaques in the cerebral cortex of the frontal and parietal lobes (Fig. 1A, B). In the primary mortor cortex layer V, there was loss of Betz cells associated with prominent astrocytosis and ballooned

Fig. 3 A–C Case 10. A Obvious fibrillary gliosis in the pallidum (*arrow*), in contrast to slight fibrillary gliosis in the putamen. B Obvious fibrillary gliosis in the pallidum. C Slight fibrillary gliosis in the putamen. A, B, C Holzer stain: *bars* A 1 mm; B, C 0.05 mm

neurons were present. Fibrillary gliosis was observed in the cerebral white matter. Neuronal loss was not observed in the hippocampus, parahippocampal gyrus, amygdala, nucleus basalis of Meynert, oculomotor nucleus, pontine nucleus, Purkinje cells, hypoglossal nucleus, dorsal motor nucleus of the vagus, or inferior olive. Severe neuronal loss was observed in the pallidum, prominently in the dorsal part. The caudate nucleus and putamen showed moderate neuronal loss, but the subthalamic nucleus disclosed relative preservation of the neurons with slight proliferation of the glia (Fig. 1C). In the substantia nigra, there was prominent neuronal loss with melanin pigment incontinence. In the dentate nucleus, there was mild neuronal loss and "grumose degeneration". Senile plaques were not observed using methenamine silver staining. Using modified Gallyas-Braak methods, a few NFT in the hippocampus CA1 and a small quantity of NFT in the parahippocampal gyrus were seen, compatible with stage II of Braak's classification, and many argyrophilic threads were encounterd in the central nervous system.

Clinical course and neuropathological findings in case 5

This autopsy case was a Japanese man who was 70 years old at the time of death. He was well until the age of 64, when he developed memory disturbance, followed by topographical disorientation 10 months later and aspontaneity 1 year 6 months after the onset of the disease. A neurological examination when the patient was 69 years old (4 years 4 months after the disease onset) revealed severe dementia (revised Hasegawa dementia rating scale 0), vertical ocular movement involvement, rigidity of the right upper and lower extremities, absence of Babinski sign and hyperreflexia in the upper and lower extremities. At 4 years 7 months after disease onset, he

could walk and eat a meal with assistance, but he was doubly incontinent. Dysphagia became evident 5 years 2 months after the onset of the disease. Severe dysphagia very often caused misswallowing, which necessitated a gastrostomy, performed 5 years 5 months after the disease onset. He died of repeated pneumonia, probably due to severe dysphagia, 5 years 8 months after the onset of the disease. He was clinically diagnosed as having CBD, mainly because of prominent dementia and obvious rigidity in the clinical stage without difficulty in walking, by one of the authors (K. Tsuchiya).

Fig. 4 Involvement of the primary motor cortex. A, B Case 1; C, D case 3; E–G case 7; H, I case 10. A Ballooned neuron (*large arrow*) and hypertrophic glia (*arrowhead*) in the primary motor cortex, including Betz cell (*small arrow*). B Loss of Betz cell (*large arrow*) in the primary motor cortex, including Betz cell (*small arrow*). C Ballooned neuron (*arrow*) in the primary motor cortex. D Loss of Betz cell (*large arrow*) in the primary motor cortex, including Betz cell (*small arrow*). E Obvious involvement of the primary motor cortex, including degenerated Betz cell (*arrow*), showing prominent spongy state in the upper cortical layers. F Enlargement of the area indicated by *arrow* in E, showing degenerated Betz cell (*arrow*) and hypertrophic glia (*arrowhead*). G Hypertrophic glia (*arrowhead*) in the primary motor cortex, including degenerated Betz cell (*arrow*). H Deep layer of the primary motor cortex, including Betz cell (*arrow*). I Enlargement of the area indicated by *arrow* in H, showing loss of Betz cell (*arrow*) and hypertrophic glia (*arrowhead*). A, B, E, F, H, I H.E. stain; C, D K-B stain; G Holzer stain; *bars* A–D, F, G, I 0.04 mm; E, H 0.2 mm

The brain weighed 1,100 g before fixation. Macroscopic examination revealed atrophy of the frontal and parietal lobes, with depigmentation of the substantia nigra. A histological examination showed neuronal loss with astrocytosis, status spongiosus, many ballooned neurons, and astrocytic plaques in the cerebral cortex of the frontal and parietal lobes (Fig. 2A). In the primary morter cortex layer V, there was loss of Betz cells associated with obvious astrocytosis and presence of ballooned neurons. In the cerebral white matter, hyalinosis of the small vessels was obvious. Neuronal loss was not observed in the hippocampus, parahippocampal gyrus, amygdala, pontine nucleus, Purkinje cells, hypoglossal nucleus, dorsal motor nucleus of the vagus, or inferior olive. Severe neuronal loss was encountered in the pallidum, prominently in the dorsal part. The caudate nucleus and putamen showed moderate neuronal loss, but the subthalamic nucleus disclosed relative preservation of the neurons with slight proliferation of the glia. In the substantia nigra, there was prominent neuronal loss with leakage of melanin pigment and the presence of NFT (Fig. 2B, C). In the dentate nucleus, there was mild neuronal loss and grumose degeneration. Senile plaques were not observed using methenamine silver staining. Using modified Gallyas-Braak staining, a few NFT in the hippocampus CA1 and a small quantity of NFT in the parahippocampal gyrus were seen, con-

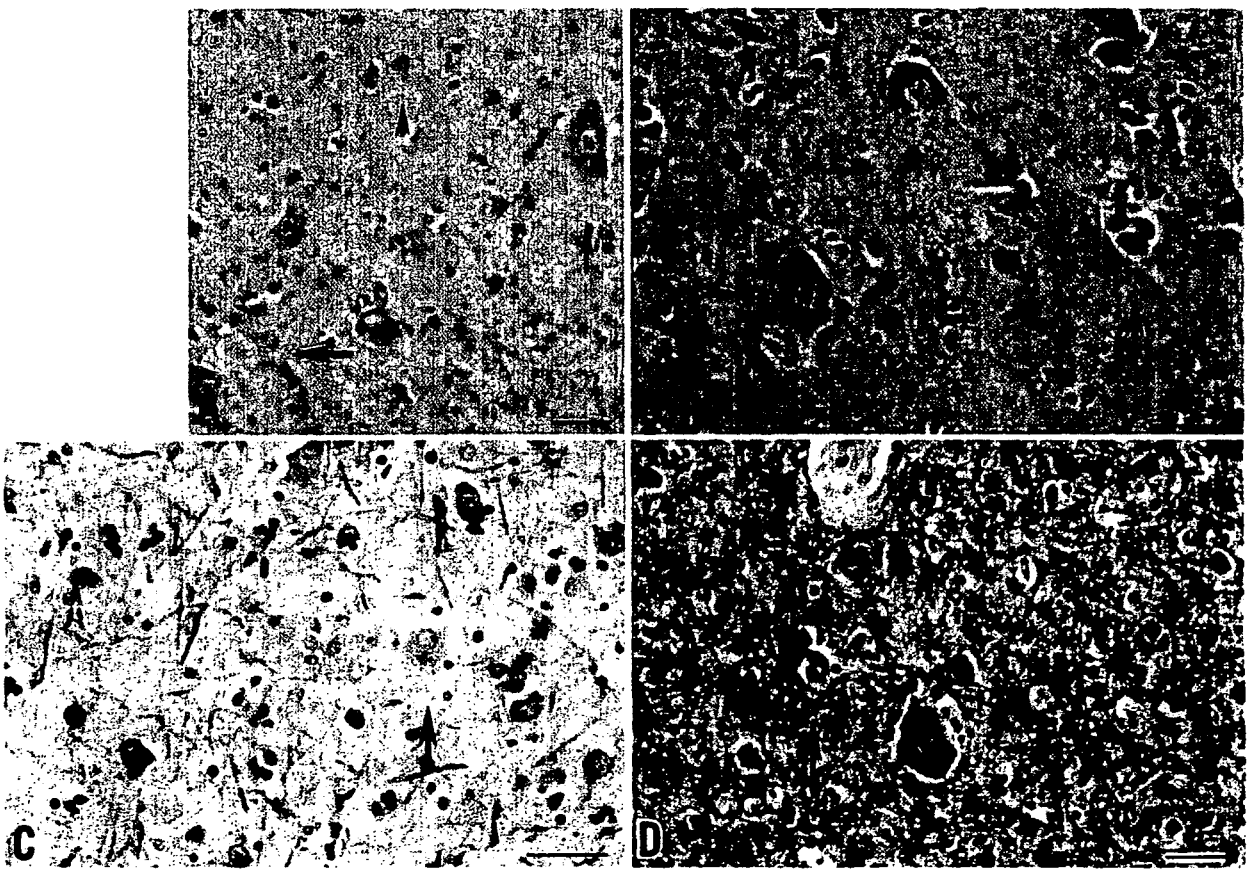
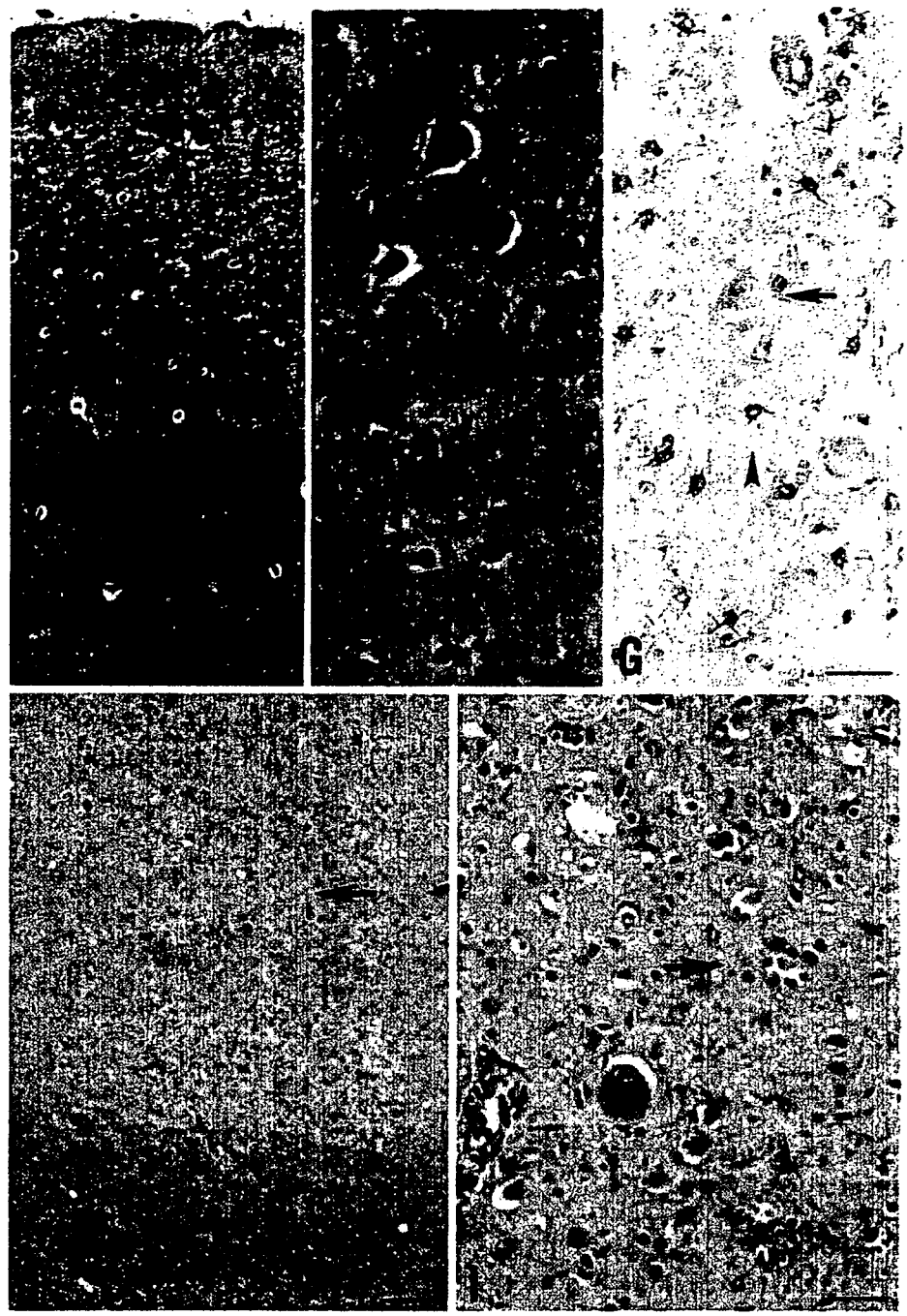


Fig. 4 (Contd.)



sistent with stage II of Braak's classification, as well as many argyrophic threads.

Clinical course and neuropathological findings in case 10

This patient was a Japanese woman aged 78 years at the time of death. She was in good health until the age of 67, when she developed memory disturbance, followed about 2 year later by action tremor, and at 3 years after the onset of the disease by difficulty in walking. At 5 years after the disease onset, memory disturbance worsened, followed by dyskinesia in the upper extremities. A neurological examination at the age of 75, 8 years after the disease onset, disclosed severe dementia, dyskinesia in the upper and lower extremities, hyperreflexia in the four extremities without Babinski sign. She died of

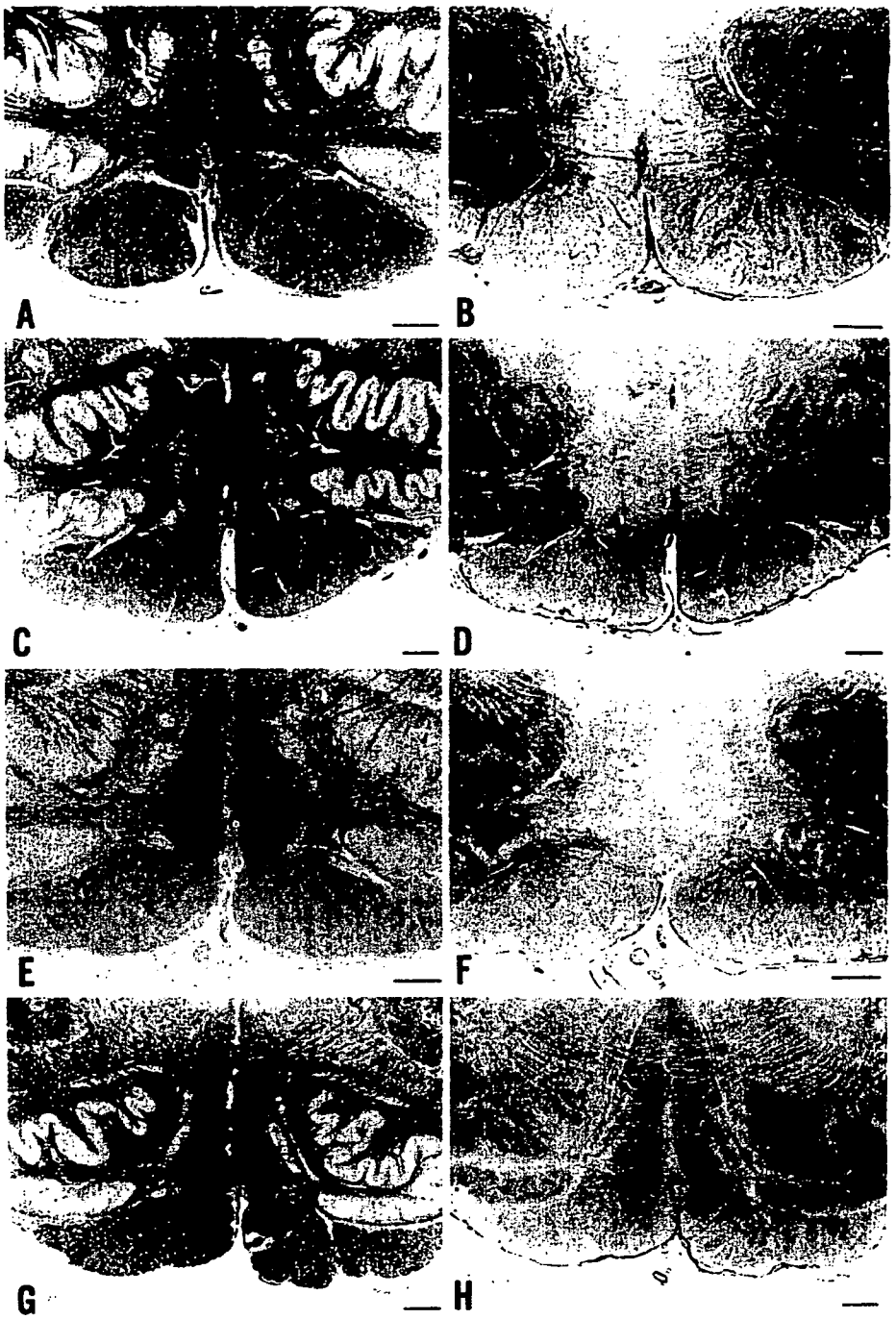


Fig. 5 A–H Involvement of the pyramidal tract in the medulla oblongata. A, B Case 1; C, D case 6; E, F case 7; G, H case 8. A, C, E, G Klüver-Barrera stain; B, D, F, H Holzer stain; *bars* A–H 1 mm

pneumonia at age 78, 11 years after the onset of the disease. She was clinically diagnosed as having atypical progressive supranuclear palsy.

The brain weighed 810 g. Macroscopic examination revealed atrophy of the frontal and parietal lobes, with depigmentation of the substantia nigra. A histological examination showed neuronal loss with astrocytosis, spongy state, many ballooned neurons, and astrocytic plaques in the cerebral cortex of the frontal and parietal lobes. In the primary motor cortex layer V, there was loss of Betz cells associated with prominent astrocytosis, and ballooned neurons were present. Fibrillary gliosis was observed in the cerebral white matter. Neuronal loss was not observed in the hippocampus, parahippocampal gyrus, amygdala, nucleus basalis of Meynert, oculomotor nucleus, trochlear nucleus, pontine nucleus, Purkinje

cells, hypoglossal nucleus, dorsal motor nucleus of the vagus, or inferior olive. Severe neuronal loss was observed in the pallidum, prominently in the dorsal part (Fig. 3A, B). The caudate nucleus and putamen (Fig. 3C) showed moderate neuronal loss, but the subthalamic nucleus disclosed relative preservation of the neurons with slight proliferation of the glia. In the substantia nigra, there was prominent neuronal loss with leakage of melanin pigment. In the dentate nucleus, there was mild neuronal loss and grumose degeneration. Senile plaques were not observed with methenamine silver staining. A few NFT in the hippocampus CA1 and a small quantity of NFT in the parahippocampal gyrus, compatible with stage II of Braak's classification, and many argyrophilic threads were encountered using modified Gallyas-Braak methods.

Results

Clinical features

The main clinical information on the ten patients (five males, five females) is summarized in Table 1. The patients had no hereditary burden. The age at onset of symptoms was from the fifth to eighth decade of life (average of 62 years 1 month). The duration of the disease ranged from 2 years 1 month in case 1 to 11 years in case 10 (mean duration 5 years 9 months). Three patients presented with limbkinetic apraxia as the initial sign (cases 1, 2, and 7). Two patients initially developed aphasia and motor aphasia (cases 3 and 4). Memory disturbance was observed in two patients as the initial sign (cases 5 and 10). Delusion of persecution was noted as the initial sign in case 6. Abnormal behavior, reminiscent of Pick's disease, was noticed as the initial sign in cases 8 and 9. Muscular rigidity was noted in eight patients during the clinical course, but in cases 3 and 8, with relative shorter clinical courses, muscular rigidity was not noticed. All ten cases presented with dementia during the clinical course.

Pathological features

The neuropathological data are also summarized in Table 1. Brain weights at autopsy ranged from 1,370 to 810 g (average 1,096.2 g). In the cerebral cortex of all ten cases, neuronal loss and gliosis associated with the presence of ballooned neurons and astrocytic plaques were encounterd in the frontal and parietal lobes. In all cases, the pallidum revealed severe neuronal loss and prominent gliosis, while moderate lesions were evident in the caudate nucleus and putamen. In the subthalamic nucleus, slight lesions were found in each CBD case examined in this study. Neuronal loss of the substantia nigra was prominent in all cases.

Clinicopathological correlation between pyramidal signs and involvement of the primary motor cortex and pyramidal tract

Pyramidal signs and involvement of the primary motor cortex and pyramidal tract are summarized in Table 2. Pyramidal signs, including hyperreflexia and Babinski sign, were noted in six cases (cases 1, 2, 6, 7, 9 and 10). Spasticity was noticed in only three cases (cases 1, 2, and 7). Loss of Betz cells was observed in all ten cases (Fig. 4). Furthermore, astrocytosis of the primary motor cortex layer V, detected by HE, Holzer, and GFAP staining, was obvious in all ten cases (Fig. 4). Degeneration of the pyramidal tract was found in each case, and the distal portion (medulla oblongata) was more affected than the proximal portion (midbrain), suggestive of a dying back phenomenon (Fig. 5).

Discussion

Clinical features

Pyramidal signs, including hyperreflexia, Babinski sign, and spasticity, are said to be common in CBD cases, but the frequency of pyramidal signs in CBD patients remains unclear to date. In 1990, Riley et al. [54], who designated CBD as cortical-basal ganglionic degeneration, described 15 patients with CBD, including 2 autopsy-confirmed CBD cases (patients 1 and 2 in their report), noticed that in their 15 patients, hyperreflexia associated with Babinski sign was observed in 5 patients (33%), but that hyperreflexia without Babinski sign was found in 7 patients (47%). Rinne et al. [55], in 1994, conducted a clinical study of 36 CBD cases, including 6 pathologically confirmed CBD cases, noted that hyperreflexia was observed in 26 patients (72%), with Babinski sign in 17 patients (47%). In 1997, Schneider et al. [57], who investigated clinical and neuropathological heterogeneity in 11 cases of pathologically diagnosed CBD, observed that 3 patients manifested Babinski sign (27%). Kompoliti et al. [36], in 1998, who examined the clinical presentation and pharmacological therapy in 147 CBD patients, including 7 autopsy-proven CBD cases, noted that pyramidal signs were observed in 84 CBD patients (57%), but that they were encountered in 6 cases (86%) of the 7 autopsy-proven CBD cases. In 1998, Wenning et al. [76] analyzed the natural history and survival of 14 patients with CBD confirmed at postmortem examination, and noticed that hyperreflexia was observed in 5 cases (36%), with Babinski sign being found in 3 cases (21%), about 3 years after the disease onset, but that hyperreflexia was observed in 7 cases (58%), with Babinski sign being found in 5 cases (42%), respectively, about 6.1 years after the disease onset. Boeve et al. [6], in 1999, investigated the pathological heterogeneity in 13 clinically diagnosed CBD patients and found 7 autopsy CBD cases among

these patients. Furthermore, Boeve et al. noted that in their 7 autopsy CBD cases, pyramidal signs were obvious in 4 cases (57%).

Reviewing the literature regarding the frequency of the pyramidal signs in CBD, including hyperreflexia, Babinski sign, and spasticity, it becomes clear that there are many discrepancies between the frequencies of the pyramidal signs in CBD cases reported to date.

Pathological features

Neuropathological studies of CBD, focusing on the primary motor cortex and pyramidal tract, are very rare. Rebeitz et al. [53] noticed that in their three autopsy cases, in which case 1 had a very brisk left patellar reflex with cases 2 and 3 having prominent Babinski sign, there was evident pyramidal tract involvement in cases 2 and 3, but that in case 1 the Betz cells in the precentral cortex appeared normal with a good complement of Nissl granules. Gibb et al. [18] described three autopsy cases of CBD, in which three cases clinically presented with brisk tendon reflexes, but Babinski sign was only encountered in case 2. In their pathological findings, Gibb et al. noted that their three cases had mild to moderate corticospinal tract involvement, but they did not notice whether or not there was loss of Betz cells. In contrast, Horoupian and Chu [27] reported an autopsy case of CBD, in which bilateral Babinski signs, more prominent on the right, were clinically observed, and the pathological examination revealed prominent neuronal loss of the primary motor cortex, including Betz cells, associated with astrocytosis and presence of ballooned neurons.

From the literature concerning the involvement of the primary motor cortex and pyramidal tract in CBD, it becomes obvious that there have been few reports showing loss of Betz cells in the primary motor cortex and involvement of the pyramidal tract of CBD patients. Thus, our data, showing constant and severe involvement of Betz cells associated with constant involvement of the pyramidal tract in the medulla oblongata in ten cases of CBD, are important.

Clinicopathological correlation and pathological heterogeneity in the primary motor cortex among MSA, ALS with dementia, and CBD

Tsuchiya et al. [66] investigated the pyramidal signs, including spasticity, hyperreflexia, and Babinski sign, and the involvement of the primary motor cortex and pyramidal tract, in seven Japanese autopsy cases of MSA. In that study, pyramidal signs were observed in six (86%) of the seven MSA autopsy cases. Hyperreflexia and Babinski sign were each evident in five patients, but spasticity was observed in only one patient. Loss of Betz cells and presence of glial cytoplasmic inclusions (Papp-Lantos inclusions) in the primary motor cortex were noticed in all seven MSA autopsy cases. Astrocytosis in the fifth layer of the primary motor cortex was noted in five (71%) of the seven MSA autopsy cases. Involvement of the pyramidal tract in the medulla oblongata was observed in all seven MSA autopsy cases, but no involvement of the pyramidal tract in the midbrain was evident in any of the six autopsy cases in which this structure was examined.

Subsequently, Tsuchiya et al. [70] explored the pyramidal signs, including hyperreflexia, Babinski sign, and spasticity, as well as the involvement of the primary motor cortex and pyramidal tract, in eight Japanese autopsy cases of ALS with dementia. Pyramidal signs were observed in seven (88%) of the eight autopsy cases. Hyperreflexia and Babinski sign were evident in seven (88%) and three (38%) patients, respectively, but spasticity was not observed in any of the eight patients. Loss of Betz cells in the primary cortex was evident in all seven autopsy cases in which this structure was examined. In contrast, astrocytosis in the fifth layer of the primary motor cortex was noticed in only three cases (38%). Involvement of the pyramidal tract in the medulla oblongata was observed in all eight ALS with dementia autopsy cases, but no involvement of the pyramidal tract in the midbrain was found in any of the eight autopsy cases.

Given the high frequency of pyramidal signs in MSA (86%) [66] and ALS with dementia (88%) [70], the relatively low frequency of pyramidal signs (60%) in the ten CBD autopsy cases seen in the present study deserves a mention.

In this study, constant and severe involvement of the primary motor cortex, including loss of Betz cells and obvious astrocytosis of the fifth layer of the primary motor cortex, was observed in all ten CBD cases, suggesting that in CBD there is a clinicopathological dissociation between the involvement of the primary motor cortex and pyramidal tract, and pyramidal signs. In contrast, in seven MSA autopsy cases reported by Tsuchiya et al., astrocytosis in the fifth layer of the primary motor cortex was noticed in five cases (71%), consistent with the high frequency of pyramidal signs (86%), and in eight ALS with dementia cases reported by Tsuchiya et al., astrocytosis in the fifth layer of the primary motor cortex was noted in only three cases (38%), inconsistent with the high frequency of the pyramidal signs (88%).

On the basis of our data showing that pyramidal sign were observed in six (60%) of the ten CBD autopsy cases, and that astrocytosis in the fifth layer of the primary motor cortex and loss of Betz cells were obvious in all ten CBD autopsy cases, we believe that the pyramidal signs in CBD have been disregarded.

Acknowledgements We wish to express our gratitude to former Prof. H. Tsukagoshi (Department of Neurology, Tokyo Medical and Dental University) for his valuable advice. We also wish to thank to Mr. Y. Shoda and Ms E. Matsui for their photographic assistance.

References

1. Agid Y (2000) Conclusions. Adv Neurol 82:241–244
2. Arima K, Uesugi H, Fujita I, Sakurai Y, Oyanagi S, Andoh S, Izumiyama Y, Inose T (1994) Corticonigral degeneration with neuronal achromasia presenting with primary progressive aphasia: ultrastructural and immunocytochemical studies. J Neurol Sci 127:186–197
3. Armstrong RA, Cairns NJ, Lantos PL (2000) A quantitative study of the pathological lesions in the neocortex and hippocampus of twelve patients with corticobasal degeneration. Exp Neurol 163:348–356
4. Bergeron C, Pollanen MS, Weyer L, Black SE, Lang AE (1996) Unusual clinical presentations of cortical-basal ganglionic degeneration. Ann Neurol 40:893–900
5. Bergeron C, Davis A, Lang AE (1998) Corticobasal ganglionic degeneration and progressive supranuclear palsy presenting with cognitive decline. Brain Pathol 8: 355-365
6. Boeve BF, Maraganore DM, Parisi JE, Ahlskog JE, Graff-Radford N, Caselli RJ, Dickson DW, Kokmen E, Petersen RC (1999) Pathologic heterogeneity in clinically diagnosed corticobasal degeneration. Neurology 53:795–800
7. Constantinidis J (1985) Pick dementia: anatomoclinical correlations and pathophysiological considerations. In: Rose FC (ed) Interdisciplinary topics in gerontology, vol 19. Karger, Basel, pp 72–97
8. Constantinidis J, Richard J, Tissot R (1974) Pick's disease. Histological and clinical correlations. Eur Neurol 11:208–217
9. Dickson DW (1999) Neuropathologic differentiation of progressive supranuclear palsy and corticobasal degeneration. J Neurol 246 [Suppl 2]:II/6–II/15
10. Dickson DW, Litvan I (2003) Corticobasal degeneration. In: Dickson DW (ed) Neurodegeneration: the molecular pathology of dementia and movement disorder. ISN Neuropath Press, Basel, pp 115–123
11. Dickson DW, Liu W-K, Ksiezak-Reding H, Yen S-H (2000) Neuropathologic and molecular considerations. Adv Neurol 82:9–27
12. Dickson DW, Bergeron C, Chin SS, Duyckaerts C, Horoupian D, Ikeda K, Jellinger K, Lantos PL, Lippa CF, Mirra SS, Tabaton M, Vonsattel JP, Wakabayashi K, Litvan I (2002) Office of rare diseases neuropathologic criteria for corticobasal degeneration. J Neuropathol Exp Neurol 61:935–946
13. Duyckaerts C, Hauw J-J (2000) Diagnostic controversies: another view. Adv Neurology 82:233–240
14. Feany MB, Dickson DW (1995) Widespread cytoskeletal pathology characterizes corticobasal degeneration. Am J Pathol 146:1388–1396
15. Feany MB, Mattiace LA, Dickson DW (1996) Neuropathologic overlap of progressive supranuclear palsy, Pick's disease and corticobasal degeneration. J Neuropathol Exp Neurol 55:53–67
16. Ferrer I, Hernández I, Boada M, Llorente A, Rey MJ, Cardozo A, Ezquerra M, Puig B (2003) Primary progressive aphasia as the initial manifestation of corticobasal degeneration and unusual tauopathies. Acta Neuropathol 106:419–435
17. Forman MS, Zhukareva V, Bergeron C, Chin S S-M, Grossman M, Clark C, Lee V M-Y, Trojanowski JQ (2002) Signature tau neuropathology in gray and white matter of corticobasal degeneration. Am J Pathol 160:2045–2053
18. Gibb WRG, Luthert PJ, Marsden CD (1989) Corticobasal degeneration. Brain 112:1171–1192
19. Goetz CG (2000) Nineteenth century studies of atypical parkinsonism: Charcot and his Salpêtrière school. Adv Neurology 82:1–8
20. Grimes DA, Lang AE, Bergeron CB (1999) Dementia as the most common presentation of cortical-basal ganglionic degeneration. Neurology 53:1969–1974
21. Hattori M, Hashizume Y, Yoshida M, Iwasaki I, Hishikawa N, Ueda R, Ojika K (2003) Distribution of astrocytic plaques in the corticobasal degeneration brain and comparison with tuft-shaped astrocytes in the progressive supranuclear palsy brain. Acta Neuropathol 106:143–149
22. Hauw J-J, Agid Y (2003) Progressive supranuclear palsy (PSP) or Steele-Richardson-Olszewski disease. In: Dickson DW (ed) Neurodegeneration: The molecular pathology of dementia and movements disorder. ISN Neuropath Press, Basel, pp 103–114
23. Hauw J-J, Verny M, Delaère P, Cervera P, He Y, Duyckaerts C (1990) Constant neurofibrillary changes in progressive supranuclear palsy. Basic differences with Alzheimer's disease and aging. Neurosci Lett 119:182–186
24. Hauw J-J, Daniel SE, Dickson D, Horoupian DS, Jellinger K, Lantos PL, McKee A, Tabaton M, Litvan I (1994) Preliminary NINDS neuropathologic criteria for Steele-Richardson-Olszewski syndrome (progressive supranuclear palsy). Neurology 44:2015–2019
25. Hauw J-J, Verny M, Ruberg M, Duyckaerts C (1998) The neuropathology of progressive supranuclear palsy (PSP) or Steele-Richardson-Olszewski disease. In: Markesbery WR (ed) Neuropathology of dementing disorders. Arnold, London, pp 193–218
26. Holmes G (1909) The pathology of amyotrophic lateral sclerosis. Rev Neurol Psychiatry 8:693–725
27. Horoupian DS, Chu PL (1994) Unusual case of corticobasal degeneration with tau-Gallyas-positive neuronal and glial tangles. Acta Neuropathol 88:592–598
28. Ikeda K, Akiyama H, Iritani S, Kase K, Arai T, Niizato K, Kuroki N, Kosaka K (1996) Corticobasal degeneration with primary progressive aphasia and accentuated cortical lesion in superior temporal gyrus: case report and review. Acta Neuropathol 92:534–539
29. Ikeda K, Akiyama H, Arai T, Tsuchiya K (2002) Pick-body-like inclusions in corticobasal degeneration differ from Pick bodies in Pick's disease. Acta Neuropathol 103:115–118
30. Jellinger KA, Bancher C (1992) Neuropathology. In: Litvan I, Agid Y (eds) Progressive supranuclear palsy. Clinical and research approaches. Oxford University Press, New York, pp 44–88
31. Katsuse O, Iseki E, Arai T, Akiyama H, Togo T, Uchikado H, Kato M, Silva R de, Lees A, Kosaka K (2003) 4-report tauopathy sharing pathological and biochemical features of corticobasal degeneration and progressive supranuclear palsy. Acta Neuropathol 106:251–260
32. Kertesz A (1998) Pick's disease and Pick complex: introductory nosology. In: Kertesz A, Munoz DG (eds), Pick's disease and Pick complex. Wiley-Liss, New York, pp 1–11
33. Kertesz A, Munoz DG (1998) Clinical and pathological overlap in Pick complex. In: Kertesz A, Munoz DG (eds) Pick's disease and Pick complex. Wiley-Liss, New York, pp 281–286
34. Komori T (1999) Tau-positive glial inclusions in progressive supranuclear palsy, corticobasal degeneration and Pick's disease. Brain Pathol 9:663–679
35. Komori T, Arai N, Oda M, Nakayama H, Mori H, Yagishita S, Takahashi T, Amano N, Murayama S, Murakami S, Shibata N, Kobayashi M, Sasaki S, Iwata M (1998) Astrocytic plaques and tufts of abnormal fibers do not coexist in corticobasal degeneration and progressive supranuclear palsy. Acta Neuropathol 96:401–408
36. Kompoliti K, Goetz CG, Boeve BF, Maraganore DM, Ahlskog JE, Marsden CD, Bhatia KP, Greene PE, Przedborski S, Seal EC, Burns RS, Hauser RA, Gauger LL, Factor SA, Molho ES, Riley DE (1998) Clinical presentation and pharmacological therapy in corticobasal degeneration. Arch Neurol 55:957–961
37. Lang AE, Riley DE, Bergeron C (1994) Cortical-basal ganglionic degeneration. In: Calne DB (ed) Neurodegenerative diseases. Saunders, Philadelpia, pp 877–894
38. Mathuranath PS, Xuereb JH, Bak T, Hodge JR (2000) Corticobasal ganglionic degeneration and/or frontotemporal dementia? A report of two overlap cases and review of literature. J Neurol Neurosurg Psychiatry 68:304–312

39. Mimura M, Oda T, Tsuchiya K, Kato M, Ikeda K, Hori K, Kashima H (2001) Corticobasal degeneration presenting with nonfluent primary progressive aphasia: a clinicopathological study. J Neurol Sci 183:19–26
40. Mitani K, Uchihara T, Tamaru F, Endo K, Tsukagoshi H (1993) Corticobasal degeneration: clinicopathological studies on two cases (in Japanese with English abstract). Clin Neurol (Tokyo) 33:155–161
41. Miyazaki H, Saito Y, Kijima Y, Akabane H, Tsuchiya K (1997) An autopsy case of corticobasal degeneration mimicking frontal Pick's disease (in Japanese with English abstract). No To Shinkei 49:277–282
42. Mizuno Y, Ozeki M, Iwata H, Takeuchi T, Ishihara R, Hashimoto N, Kobayashi H, Iwai K, Ogasawara S, Ukai K, Shibayama H (2002) A case of clinically and neuropathologically atypical corticobasal degeneration with widespread iron deposition. Acta Neuropathol 103:288–294
43. Mori H, Nishimura M, Namba Y, Oda M (1994) Coritcobasal degeneration: a disease with widespread appearance of abnormal tau and neurofibrillary tangles, and its relation to progressive supranuclear palsy. Acta Neuropathol 88:113–121
44. Munoz DG (1998) The pathology of Pick complex. In: Kertesz A, Munoz DG (eds), Pick's disease and Pick complex. Wiley-Liss, New York, pp 211–241
45. Murayama S, Inoue K, Kawakami H, Bouldin TW, Suzuki K (1991) A unique pattern of astrocytosis in the primary motor area in amyotrophic lateral sclerosis. Acta Neuropathol 82:456–461
46. Murayama S, Bouldin TW, Suzuki K (1992) Immunocytochemical and ultrastructural studies of upper motor neurons in amyotrophic lateral sclerosis. Acta Neuropathol 83:518–524
47. Nakano I, Donnenfeld H, Hirano A (1983) A neuropathological study of amyotrophic lateral sclerosis. With special reference to central chromatolysis and spheroid in the spinal anterior horn and some pathological changes of the motor cortex (in Japanese with English abstract). Neurol Med (Tokyo) 18:136–144
48. Nathan PW, Smith MC, Deacon P (1990) The corticospinal tract in man. Course and location of fibers at different segmental leves. Brain 113:303–324
49. Oda T, Kogure T, Tominaga I, Onaya M, Mimura M, Kato Y, Iwabuchi K, Haga C (1994) An autopsy case of progressive supranuclear palsy with echolalia showing psychiatric symptoms at the beginning (in Japanese). Seishinigaku 36:1159–1166
50. Oda T, Ikeda K, Akamatsu W, Iwabuchi K, Akiyama H, Kondo H, Seta K, Kato Y, Kogure T, Hori K, Tominaga I, Onaya M (1995) An autopsy case of corticobasal degeneration clinically misdiagnosed as Pick's disease (in Japanese with English abstract). Psychiatr Neurol Jpn 97:757–769
51. Oyanagi K, Tsuchiya K, Yamazaki M, Ikeda K (2001) Substantia nigra in progressive supranuclear palsy, corticobasal degeneration, and parkinsonism-dementia complex of Guam: specific pathological features. J Neuropathol Exp Neurol 60:393–402
52. Rebeiz JJ, Kolodny EH, Richardson EP Jr (1967) Corticodentatonigral degeneration with neuronal achromasia: a progressive disorder of late adult life. Trans Am Neurol Assoc 92:23–26
53. Rebeiz JJ, Kolodny EH, Richardson EP Jr (1968) Corticodentatonigral degeneration with neuronal achromasia. Arch Neurol 18: 20-33
54. Riley DE, Lang AE, Lewis A, Resch L, Ashby P, Hornykiewicz O, Black S (1990) Cortico-basal ganglionic degeneration. Neurology 40:1203-1212
55. Rinne JO, Lee MS, Thompson PD, Marsden CD (1994) Corticobasal degeneration. A clinical study of 36 cases. Brain 117:1183–1196
56. Rossor MN, Tyrrell PJ, Warrington EK, Thompson PD, Marsden CD, Lantos P (1999) Progressive frontal gait disturbance with atypical Alzheimer's disease and corticobasal degeneration. J Neurol Neurosurg Psychiatry 67:345–352
57. Schneider JA, Watts RL, Gearing M, Brewer RP, Mirra SS (1997) Corticobasal degeneration: Neuropathologic and clinical heterogeneity. Neurology 48:959–969
58. Su M, Yoshida Y, Hirata Y, Satoh Y, Nagata K (2000) Degeneration of the cerebellar dentate nucleus in corticobasal degeneration: neuropathological and morphometric investigations. Acta Neuropathol 99:365–370
59. Tolnay M, Probst A (2002) Frontotemporal lobar degeneration—tau as a pied piper? Neurogenetics 4:63–75
60. Tsuchiya K, Ikeda K (2002) Basal ganglia lesions in 'Pick complex': A topographic neuropathological study of 19 autopsy cases. Neuropathology 22:323–336
61. Tsuchiya K, Ikeda K, Watabiki S, Shiotsu H, Hashimoto K, Mitani K, Okiyama R, Sano M, Kondo H, Shimada H (1996) An unusual autopsy case of corticobasal degeneration—with special reference to clinicopathological differentiation from progressive supranuclear palsy and slowly progressive aphasia (in Japanese with English abstract). No To Shinkei 48:559–565
62. Tsuchiya K, Ikeda K, Uchihara T, Oda T, Shimada H (1997) Distribution of cerebral cortical lesions in corticobasal degeneration: a clinicopathological study of five autopsy cases in Japan. Acta Neuropathol 94:416–424
63. Tsuchiya K, Uchihara T, Oda T, Arima K, Ikeda K, Shimada H (1997) Basal ganglia lesions in corticobasal degeneration differ from those in Pick's disease and progressive supranuclear palsy: a topographic neuropathology of six autopsy cases. Neuropathology 17:208–216
64. Tsuchiya K, Miyazaki H, Ikeda K, Watabiki S, Kijima Y, Sano M, Shimada H (1997) Serial brain CT in corticobasal degeneration: radiological and pathological correlation of two autopsy cases. J Neurol Sci 152:23–29
65. Tsuchiya K, Arima K, Fukui T, Kuroiwa T, Haga C, Iritani S, Hirai S, Nakano I, Takemura T, Matsushita M, Ikeda K (1999) Distribution of basal ganglia lesions in Pick's disease with Pick bodies: a topographic neuropathological study of eight autopsy cases. Neuropathology 19:370–379
66. Tsuchiya K, Osawa E, Haga C, Watabiki S, Ikeda M, Sano M, Ooe K, Taki K, Ikeda K (2000) Constant involvement of the Betz cells and pyramidal tract in multiple system atrophy: a clinicopathological study of seven autopsy cases. Acta Neuropathol 99:628–636
67. Tsuchiya K, Ishizu H, Nakano I, Kita Y, Sawabe M, Haga C, Kuyama K, Nishinaka T, Oyanagi K, Ikeda K, Kuroda S (2001) Distribution of basal ganglia lesions in generalized variant of Pick's disease: a clinicopathological study of four autopsy cases. Acta Neuropathol 102:441–448
68. Tsuchiya K, Ikeda M, Hasegawa K, Fukui T, Kuroiwa T, Haga C, Oyanagi S, Nakano I, Matsushita M, Yagishita S, Ikeda K (2001) Distribution of cerebral cortical lesions in Pick's disease with Pick bodies: a clinicopathological study of six autopsy cases showing unusual clinical presentations. Acta Neuropathol 102:553–571
69. Tsuchiya K, Nakayama H, Iritani S, Arai T, Niizato K, Haga C, Matsushita M, Ikeda K (2002) Distribution of basal ganglia lesions in diffuse neurofibrillary tangles with calcification: a clinicopathological study of five autopsy cases. Acta Neuropathol 103:555–564
70. Tsuchiya K, Ikeda K, Mimura M, Takahashi M, Miyazaki H, Anno M, Shiotsu H, Akabane H, Niizato K, Uchihara T, Tominaga I, Nakano I (2002) Constant involvement of the Betz cells and pyramidal tract in amyotrophic lateral sclerosis with dementia: a clinicopathological study of eight autopsy cases. Acta Neuropathol 104:249–259
71. Uchihara T, Mitani K, Mori H, Kondo H, Yamada M, Ikeda K (1994) Abnormal cytoskeletal pathology peculiar to corticobasal degeneration is different from that of Alzheimer's disease or progressive supranuclear palsy. Acta Neuropathol 88:379–383
72. Uchihara T, Mizusawa H, Tsuchiya K, Kondo H, Oda T, Ikeda K (1998) Discrepancy between tau immunoreactivity and argyrophilia by the Bodian method in neocortical neurons of corticobasal degeneration. Acta Neuropathol 96:553–557

73. Wakabayashi K. Takahashi H (2004) Pathological heterogeneity in progressive supranuclear palsy and corticobasal degeneration. Neuropathology 24:79–86
74. Wakabayashi K. Oyanagi K, Makifuchi T. Ikuta F. Homma A. Homma Y. Horikawa Y. Tokiguchi S (1994) Corticobasal degeneration: etiopathological significance of the cytoskeletal alterations. Acta Neuropathol 87:545–553
75. Wakabayashi K. Mori F. Oyama Y. Kurihara A. Kamada M. Yoshimoto M. Takahashi H (2003) Lewy bodies in Betz cells of the motor cortex in a patient with Parkinson's disease. Acta Neuropathol 105:189–192
76. Wenning GK. Litvan I. Jankovic J. Granata R. Mangone CA. McKee A. Poewe W. Jellinger K. Ray Chaudhuri K. D'Olhaberriague L. Pearce RKB (1998) Natural history and survival of 14 patients with corticobasal degeneration confirmed at postmortem examination. J Neurol Neurosurg Psychiatry 64:184–189

Human Molecular Genetics, 2006, Vol. 15, No. 7 *1151–1158*
doi:10.1093/hmg/ddl030
Advance Access published on February 24, 2006

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

Ikuko Mizuta[1], Wataru Satake[1], Yuko Nakabayashi[1,2], Chiyomi Ito[1,2], Satoko Suzuki[1,2], Yoshio Momose[1,†], Yoshitaka Nagai[1], Akira Oka[3], Hidetoshi Inoko[3], Jiro Fukae[4], Yuko Saito[5,6], Motoji Sawabe[5], Shigeo Murayama[6], Mitsutoshi Yamamoto[7], Nobutaka Hattori[4], Miho Murata[8] and Tatsushi Toda[1,2,*]

[1]Division of Clinical Genetics, Department of Medical Genetics, Osaka University Graduate School of Medicine, 2-2-B9 Yamadaoka, Suita, Osaka 565-0871, Japan, [2]Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Saitama 332-0012, Japan, [3]Department of Molecular Life Science, Tokai University School of Medicine, Kanagawa 259-1193, Japan, [4]Department of Neurology, Juntendo University School of Medicine, Tokyo 113-8421, Japan, [5]Department of Pathology, Tokyo Metropolitan Geriatric Hospital, Tokyo 173-0015, Japan, [6]Department of Neuropathology, Tokyo Metropolitan Institute of Gerontology, Tokyo 173-0015, Japan, [7]Department of Neurology, Kagawa Prefectural Central Hospital, Takamatsu 760-8557, Japan and [8]Department of Neurology, Musashi Hospital, National Center of Neurology and Psychiatry, Tokyo 187-8551, Japan

Received December 22, 2005; Revised and Accepted February 15, 2006

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.

*To whom correspondence should be addressed. Tel: +81 668793380; Fax: +81 668793389; Email: toda@clgene.med.osaka-u.ac.jp
†Present address: Department of Clinical Bioinformatics, Graduate School of Medicine, University of Tokyo, Tokyo 113-8655, Japan.

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 ($P = 5.0 \times 10^{-10}$ 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 ($P = 2.0 \times 10^{-9}$–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 *SNCA* 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^{-3}	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.

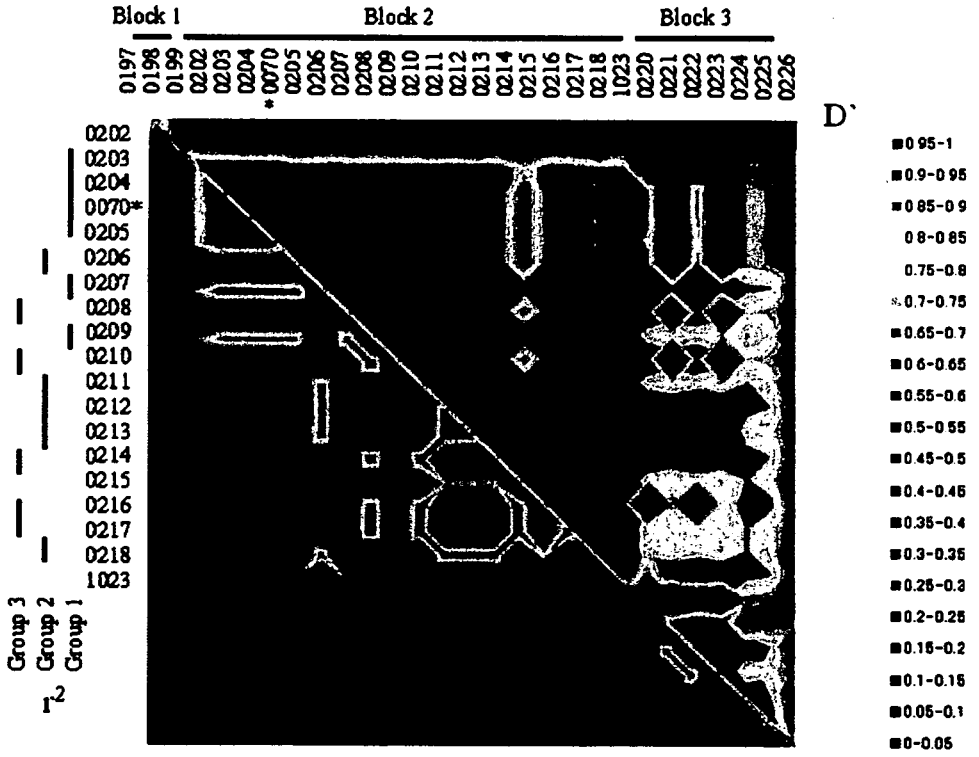


Figure 1. LD structure of the susceptibility region for sporadic PD. Pairwise LD between SNPs, as measured by D' in 134 controls, is graphically indicated. The region spanning 430 kb around the originally screened SNP0070(*) was divided into three LD blocks ($D' > 0.9$) (upper right). On the basis of 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