Of the 254 patients with PD identified in the second investigation, 34 (13.4%) were not diagnosed during the first investigation in Yonago. The H&Y scale score and mean duration of illness were significantly lower in the second investigation than in the first. We found many mild cases, and we could calculate the higher prevalence by performing the investigation twice. Two of the 34 patients were only diagnosed after sustaining a fracture, although their fractures might have been prevented, had the diagnosis been made earlier and adequate treatment and management been provided. Patients with apparent motor dysfunction should therefore receive detailed neurological examinations to detect PD, if present, in the early stages.

When we compared the results of the two areas over the same period, we found that the age- and sex-adjusted prevalence of PD in Daisen (192.6), in comparison with Yonago (166.8), was increased by 13.4% (25.8/192.6). In principle, variations in prevalence of PD may represent differences in environmental, geographic, and genetic factors, as well as diagnostic criteria, recognition of PD, and methods used in studies [3, 7, 20]. In our studies, the diagnostic criteria and recognition of PD were consistent, hence the observed difference in prevalence was more likely related to method. Service-based studies may not include patients who have not sought medical attention, and may thereby underestimate the prevalence of PD. As reported by the Europarkinson group, this underestimation may vary from 11 to 52% [21]. The difference in prevalence might also reflect a difference in population dynamics. Daisen had an aging and decreasing population. In contrast, Yonago similarly had an aging, but increasing population. Since Yonago is an urban area and Daisen is rural, environmental factors such as exposure to pesticides and herbicides might also have had an effect [13–15]. Although we described slight differences between these two areas, they are closely adjacent and show very similar environmental profiles. In Daisen, 9.5% of patients refused medical treatment, and the service-based study did not reveal this number, even in an area of present-day Japan with raised awareness of PD. This percentage may change as inhabitants gain broader access to medical education. Eighteen percent of inhabitants in Daisen did not respond to the questionnaire, which may have further obscured the true prevalence of PD.

Conclusion

We found both higher prevalence and incidence of PD in this study than in our previous studies. Our results suggest that these increases in prevalence and incidence of PD may primarily reflect the aging of the study population and increasing opportunities for diagnosis. Early detection of PD will lead to a better quality of life for patients with this disease through earlier intervention and education.

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Assessment of dementia in patients with multiple system atrophy

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Background and purpose: We investigated dementia in patients with multiple system atrophy (MSA) in order to characterize the prevalence and nature of impairments in these patients.

Methods: Fifty-eight MSA patients were recruited in our institution between April 1996 and December 2006 and investigated.

Results: Of 58 patients, 10 were diagnosed with dementia. There were no significant differences in age at onset, gender, duration of disease, or severity of cerebellar dysfunction between patients with and without dementia. The early and delayed heart to mediastinum (H/M) ratios obtained with ^{123}I-metaidobenylguanidine (MIBG) cardiac scintigraphy were significantly decreased in patients with dementia compared with those without dementia. Of the 10 patients with dementia, three were found to have cognitive decline that preceded onset of motor symptoms. White matter lesions were evident in these patients, whilst frontal atrophy was prominent in patients whose cognitive decline was preceded by onset of motor symptoms.

Conclusions: Dementia in patients with MSA may be more common than previously thought, furthermore, we speculate that clinical features of dementia in these patients might be heterogeneous.

Introduction

Multiple system atrophy (MSA) is a sporadic progressive neurodegenerative disease, characterized clinically by combinations of ataxia, pyramidal signs, parkinsonism, and autonomic dysfunction. MSA is separated into two major clinical subtypes: MSA-P (striatonigral degeneration) with predominant parkinsonian features and MSA-C (olivopontocerebellar atrophy) with predominant cerebellar ataxia. Inclusion of autonomic dysfunction, common to all forms of MSA and referred to previously as Shy-Drager syndrome, has been discouraged in the consensus criteria [1]. Neuropathology significantly affects subcortical areas; most specifically, the gray matter of the substantia nigra, striatum, inferior olivary nucleus, pontine nuclei, and cerebellum [2]. The histological hallmark is the presence of glial cytoplasmic inclusions (GCI) in oligodendroglia: Demonstration is required for a definite diagnosis [3]. Neuronal and astroglial cytoplasmic inclusions of similar composition are found in many brain areas. Recently, it was reported that the main components of these inclusions were α-synuclein, and MSA is now classified amongst the 'α-synucleinopathies' along with Parkinson's disease (PD) and dementia with Lewy bodies. Accumulation of α-synuclein in patients with MSA is also found in neuronal cell bodies and processes in several brain regions [4–7].

The frequency of cognitive impairment is 20–40% in patients with clinical MSA, although general intellectual dysfunctions such as dementia are excluded from criteria for diagnosis of MSA [1,8,9]. Reports suggest that cognitive impairment in patients with MSA involves the frontal lobe, presenting as frontal-executive dysfunction rather than impairment of memory [10–15]. However, cognitive dysfunction in these patients is not fully understood. In this research, we clinically evaluated hospitalized patients with MSA cared for by our department in order to clarify clinical features, especially those regarding dementia.

Methods

Participants

Participants were 58 patients with MSA who were admitted to the Department of Neurology, Tottori University Hospital, Japan between April 1996 and December 2006. Patients were diagnosed with possible

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or probable MSA according to the consensus criteria, excluding criteria of dementia because that has not been adopted into any formal criteria for MSA with dementia. Patients were examined by at least two neurologists board-certified by the Japanese Neurological Society. We used the International Cooperative Ataxia Rating Scale (ICARS) [16] and the Mini Mental State Examination (MMSE) for all patients. A complete neurological examination was also performed for all patients, including blood analysis, cerebrospinal fluid studies, and imaging of the head with magnetic resonance imaging (MRI). All MRI studies were performed on either 1.5-T or 3.0-T units. T1- and T2-weighted images and fluid attenuated inversion recovery sequences were obtained. Definitions and gradings of cerebral atrophy, especially frontal lobes were qualitatively estimated by two neurologists blind to the clinical findings. White matter hyperintensities severity ratings were attained from T2-weighted images according to the Fazekas scale [17]. Twenty-seven patients also underwent ^{123}I-meta-iodobenzylguanidine (MIBG) myocardial scintigraphy. Demographic features of patients are shown in Table 1.

Clinical diagnosis of MSA was based on consensus criteria excluding dementia [1]. Diagnosis of dementia was based on the Diagnostic and Statistical Manual of Mental Disorders, fourth edition-revised (DSM-IV) criteria, scored ≥1.0 on the Clinical Dementia Rating scale [18] and scored ≤24 on MMSE. All participants described in this study were approved by the Ethics Review Committee of School of Medicine, Tottori University and informed consent was obtained from each subject.

Statistical analysis

Data analysis was conducted with SPSS for Windows (version 15; SPSS Inc., Chicago, IL, USA). Results are presented as the mean ± standard deviation. Comparison of means was performed using the Mann–Whitney *U*-analysis for independent samples. Categorical variances were examined using the chi-squared test. A *P*-value <0.05 was accepted as significant. Differences in heart to mediastinum (H/M) ratios between groups were evaluated using analysis of covariance (ANCOVA) adjusted for patient age as the covariate.

Table 1 Comparison of clinical features between multiple system atrophy (MSA) patients with and without dementia

	MSA without dementia $n = 17$	MSA with dementia $n = 10$	*P*-value
Age at evaluation (year)	59.8 ± 8.1	64.3 ± 6.8	0.145
Age at onset (year)	56.2 ± 8.2	60.4 ± 6.5	0.258
Gender (M/F)	6/11	4/6	0.178[a]
Disease duration (year)	3.2 ± 2.1	3.9 ± 1.6	0.523
ICARS	41.5 ± 15.6	49.6 ± 22.6	0.419
MMSE	27.9 ± 2.3	21.3 ± 2.3	<0.001
Early H/M ratio	2.29 ± 0.26	1.78 ± 0.31	0.001
Delayed H/M ratio	2.30 ± 0.34	1.62 ± 0.46	0.003
Washout rate	27.3 ± 5.35	38.2 ± 9.70	0.018

P-value: Mann–Whitney *U*-test. [a]Chi-squared test; H/M, heart to mediastinum.

Results

Demographics of patients

Of all 58 patients, 49 (84%) were classified as MSA-C, nine patients (16%) were classified as MSA-P. Ten patients with MSA (17%) were diagnosed with dementia. All patients with dementia were clinically diagnosed as MSA-C type and have not experienced visual hallucinations.

Comparison of ^{123}I-MIBG cardiac scintigraphy between patients with and without dementia

We evaluated ^{123}I-MIBG cardiac scintigraphy in 27 patients, including 10 patients with dementia and 17 patients without dementia. Demographic features of these patients are shown in Table 1. Whilst age at onset, gender, disease duration, and severity of ataxia (ICARS) did not differ between groups, the early and delayed H/M ratio of ^{123}I-MIBG cardiac scintigraphy were significantly decreased in patients with dementia compared with those without dementia.

Clinical features of patients with dementia

The clinical features of MSA *patients with dementia* are shown in Table 2. Whilst seven patients were found to have cognitive decline preceded by ataxia, three patients had dementia develop within 1 year prior to onset of ataxia. The latter three patients were initially diagnosed as dementia with Alzheimer type (DAT).

The seven patients (cases 1–7) whose dementia occurred after onset of ataxia showed mild or moderate frontal lobe atrophy by MRI and decreased regional cerebral blood flow (rCBF) in the frontal lobe by SPECT, but none had cerebral white matter lesions by MRI. In contrast, the latter three patients (cases 8–10), whose dementia developed before onset of ataxia, had both moderate or severe cerebral atrophy and cerebral white matter lesions. Clinically, disorientation was more severe in these three patients than in the other seven patients. Clinically, memory

Table 2 Summary of multiple system atrophy patients with dementia

Case	Gender	Onset age	Disease duration	Initial symptom	ICARS	MMSE	Frontal lobe atrophy	WMH severity	H/M ratio (early/delayed)
1	F	71	2	Ataxia	32	23	+	–	1.88/1.72
2	F	52	2	Ataxia	45	24	+	–	1.73/1.43
3	F	54	3	Ataxia	33	22	+	–	2.04/2.29
4	M	53	3	Ataxia	49	21	+ +	–	1.61/1.38
5	M	62	5	Ataxia	53	20	+	–	1.82/2.09
6	F	58	6	Ataxia	90	23	+ + +	–	1.34/1.04
7	M	59	7	Ataxia	71	21	+ +	–	1.43/1.13
8	F	58	3	Dementia	23	18	+ + +	+	2.06/1.58
9	M	64	4	Dementia	94	9	+ +	+ +	1.47/1.21
10	F	69	6	Dementia	34	18	+ + +	+ + +	2.38/2.37

Gradings of frontal lobe atrophy were estimated qualitatively; +: slight, + +: moderate, + + +: severe, white matter hyperintensities (WMH) severity ratings were attained according to the Fazekas scale; –: grade 0, +: grade 1, + +: grade 2, + + +: grade 3; ICARS, International Cooperative Ataxia Rating Scale; MMSE, Mini Mental State Examination; H/M, heart to mediastinum.

impairment and disorientation in the same three patients were more severe than seen in the other seven patients.

Representative cases

Case 7

The patient was a 66-year-old man with no family history of neurological disease and no history of major illness. He presented initially with gait disturbance and orthostatic hypotension at age 59 years and was diagnosed with MSA by a neurologist. The following year, he gradually developed dysarthria, ataxia and neuropathic bladder, and his family detected cognitive impairment. At age 62 years, he was admitted to our hospital, where he presented with ataxia, extrapyramidal signs, and dysautonomia. ICARS score was 36 points and MMSE score was 24 points. At age 65 years, his ICARS score was 71 points, MMSE score was 21 points, and intelligence quotient was 71 (VIQ 83, PIQ 60) with the Wechsler adult intelligence scale-revised (WAIS-R) and 10 points in frontal assessment battery. His lowest scored category in MMSE and WAIS-R was attention, calculation, and verbal frequency. MRI study disclosed typical findings of MSA such as severe cerebellar and pontine atrophy with the so-called 'hot cross bun sign' and moderate frontal lobe atrophy [Correction added after online publication 31 March 2009: in the preceding sentence, 'burn' was corrected to 'bun']. ^{99}mTc-ECD SPECT image revealed moderate decline of rCBF in the frontal lobe (Fig. 1). H/M ratio in ^{123}I-MIBG cardiac scintigraphy was 1.43/1.13 (early/delayed phase). Symptoms continued to progress gradually and he became bed-ridden at age 65 years.

Case 9

The patient was a 75-year-old man with no family history of neurological disease who had a several-year history of diabetes mellitus. At age 69, he had developed episodic memory impairment and disorientation; he was diagnosed with DAT by a neurologist. At this time, mild bilateral temporal atrophy was detected by MRI, whereas no cerebellar or pontine atrophy was evident. At age 70, gait disturbance gradually developed and he was admitted to our hospital. He was diagnosed with MSA based on the presence of severe ataxia, parkinsonism, and dysautonomia. He showed severe episodic memory impairment, disorientation, and constructional apraxia. MRI revealed typical MSA findings and moderate fronto-temporal lobe atrophy. Furthermore, there were moderate leukoaraiosis in deep white matter around anterior and posterior horn of lateral ventricles. ^{99}mTc-ECD SPECT image revealed severe decline of rCBF in the frontal and temporal lobes (Fig. 2). His ICARS score was 80 points. With cognitive assessment, he was found to have an MMSE score of 15 points and WAIS-R IQ of 62 (VIQ 76, PIQ 54). His lowest scored category in MMSE and WAIS-R was not only attention, calculation, and verbal fluency, but also severe disorientation. His symptoms progressed gradually and he became bedridden at age 72 years. At that time, ICARS score was 94 points and MMSE score was 9 points. H/M ratio in ^{123}I-MIBG cardiac scintigraphy was 1.46/1.12 (early/delayed phase). His symptoms continued to progress gradually and he died suddenly at age 73 years.

Discussion

In this study, we documented dementia in a significant proportion of patients with MSA in our hospital. Dementia is included in exclusion criteria in the consensus criteria for MSA. Of our patients, 10 (17%) were diagnosed with dementia. We classified two types of MSA with dementia. One group (cases 1–7) had

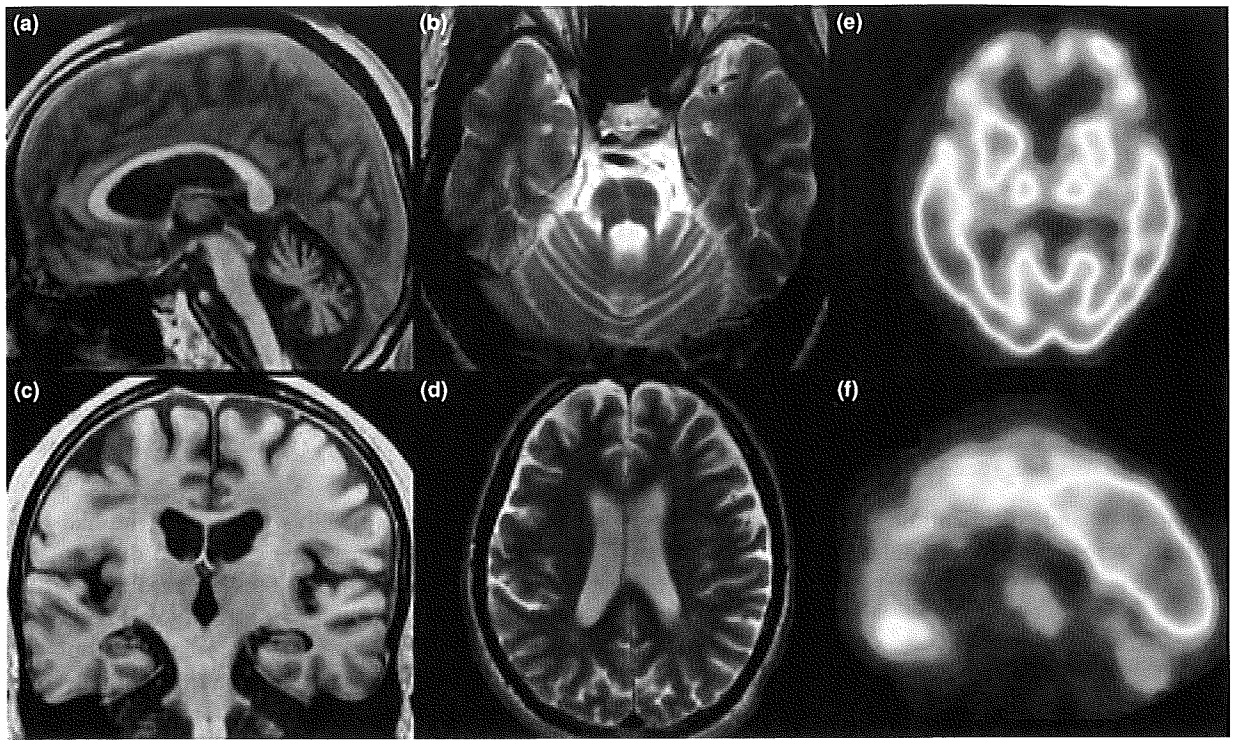

Figure 1 Neuroimaging studies at case 7. (a–d) Magnetic resonance imaging (MRI) revealed severe cerebellar and pontine atrophy with the so-called 'hot cross bun sign,' moderate fronto-temporal lobe atrophy [Correction added after online publication 31 March 2009: in the preceding sentence, 'burn' was corrected to 'bun']. White matter lesions were not noted. (e, f) 99mTc-ECD SPECT image showed moderate decline of regional cerebral blood flow in the frontal and temporal lobes. (a, c) T1 weighted MRI image. (b, d) T2 weighted MRI image.

dementia that was preceded by development of ataxia. The other three patients (cases 8–10) developed dementia before onset of ataxia. The common characteristics of cognitive impairment in each type of MSA with dementia included frontal executive dysfunction, findings similar to those of previous studies [10–15]. The latter type of dementia (cases 8–10) was diagnosed as DAT before criteria were reached for MSA. According to qualitative analyses of MRI, these patients showed more severe disorientation and more severe cerebral atrophy, with cerebral white matter lesions more prominent than in the other type of patient.

Moreover, we found that the patients with MSA and dementia had significantly reduced ^{123}I-MIBG cardiac uptake compared with the patients without dementia. Recent studies have reported that H/M ratio of ^{123}I-MIBG cardiac scintigraphy is a useful diagnostic tool for LBDs based on evidence of post-ganglionic cardiac sympathetic denervation in these patients [19–22]. Although MSA is also an α-synucleinopathy, H/M ratio of ^{123}I-MIBG cardiac scintigraphy in patients with MSA has been reported predominantly to be in the normal range. These published results support the hypothesis that post-ganglionic cardiac sympathetic denervation might be evident in MSA patients with dementia. However, it has not yet been clarified whether decreased cardiac uptake of MIBG is associated with neuropathological changes of the central nervous system and neuropsychological state. We have found that reduction of cardiac MIBG uptake might be associated with neuropsychological state in patients with PD [23]. Nagayama *et al.* [24] has reported a MSA case with reduction of MIBG uptake and Lewy body pathology. However, it has not been described whether the MSA case had dementia. In our study, whilst there was no neuropathological examination, we could not diagnose any type of dementia in these cases neuropathologically.

Recent studies reported that there are neuropathological changes in patients with MSA and dementia [25–27]. These reports described autopsy cases with remarkable frontal lobe atrophy, in which GCI were abundant in the deep layer of the cortex and were even more abundant in the white matter of the frontal and parietal lobes. Piao *et al.* [28] emphasized that α-synuclein and phosphorylated tau co-occurred in certain brain regions in two cases of combined MSA and AD. Moreover, only a few reports have described the

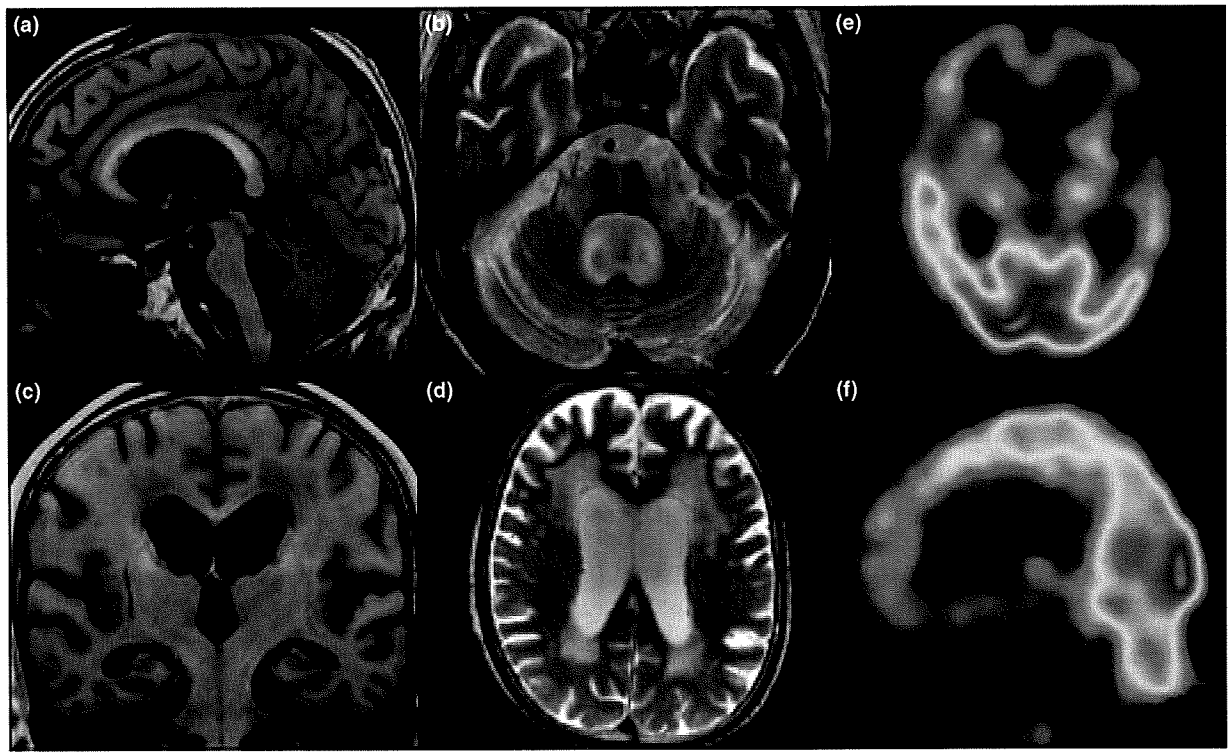

Figure 2 Neuroimaging studies at case 9. (a–d) Magnetic resonance imaging (MRI) revealed severe cerebellar and pontine atrophy with the so-called 'hot cross bun sign,' severe fronto-temporal lobe atrophy and moderate white matter lesions [Correction added after online publication 31 March 2009: in the preceding sentence, 'burn' was corrected to 'bun']. (e, f) 99mTc-ECD SPECT image showed severe decline of regional cerebral blood flow in the frontal and temporal lobes. (a, c) T1 weighted MRI image (b, d) T2 weighted MRI image.

co-existence of GCIs and Lewy bodies [29,30]. Neuropathological findings associated with dementia in patients with MSA are thought to be varied. As far as we know, patients with MSA whose dementia preceded motor dysfunction have not been described to date. As autopsy was not performed in any of the cases, we could not clarify the neuropathological correlates of dementia in our series. Therefore, neuropathological examination is necessary to clarify a new subtype of MSA. Thus, further study including postmortem neuropathological examination is needed.

In conclusion, dementia in patients with MSA may be more common than previously thought. Etiology of cognitive decline in these patients may be varied, with heterogeneous underlying pathogenetic processes.

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Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease

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To identify susceptibility variants for Parkinson's disease (PD), we performed a genome-wide association study (GWAS) and two replication studies in a total of 2,011 cases and 18,381 controls from Japan. We identified a new susceptibility locus on 1q32 ($P = 1.52 \times 10^{-12}$) and designated this as *PARK16*, and we also identified *BST1* on 4p15 as a second new risk locus ($P = 3.94 \times 10^{-9}$). We also detected strong associations at *SNCA* on 4q22 ($P = 7.35 \times 10^{-17}$) and *LRRK2* on 12q12 ($P = 2.72 \times 10^{-8}$), both of which are implicated in autosomal dominant forms of parkinsonism. By comparing results of a GWAS performed on individuals of European ancestry, we identified *PARK16*, *SNCA* and *LRRK2* as shared risk loci for PD and *BST1* and *MAPT* as loci showing population differences. Our results identify two new PD susceptibility loci, show involvement of autosomal dominant parkinsonism loci in typical PD and suggest that population differences contribute to genetic heterogeneity in PD.

Parkinson's disease (MIM168600) is one of the most common neurodegenerative diseases worldwide, affecting 1–2% of individuals aged ≥65 years[1]. Clinical features of PD result primarily from loss of dopaminergic neurons in the substantia nigra. Various medical treatments improve PD symptoms but do little to deter disease progression. Identifying genetic risk factors for PD will be helpful in elucidating the pathogenesis of the disease. Linkage studies have been successful in mapping genes for mendelian forms of parkinsonism: *SNCA* (encoding α-synuclein)[2] and *LRRK2* (refs. 3,4) in autosomal dominant forms, and *PARK2* (encoding parkin), *PINK1*, *PARK7* (encoding DJ-1) and *ATP13A2* in autosomal recessive forms[5,6]. However, mendelian forms of parkinsonism are rare compared to the far more common typical PD, a complex disorder caused by multiple genetic and environmental factors[7]. Association studies have evaluated variants in many candidate genes for PD[7], but only a few, such as common variants of *SNCA*[8–10] and rare mutations of *GBA*[11], have been identified as PD-susceptibility genes with genome-wide significance. Recently, GWASs in PD have provided association evidence at several loci, but not at the genome-wide significant level[12–14].

We conducted a GWAS and two subsequent replication studies for PD to identify further common variants that contribute to disease. In the GWAS stage, we genotyped 561,288 SNPs on autosomal and sex chromosomes using the HumanHap550 array (Illumina). The GWAS stage included 1,078 PD cases and 2,628 controls in the Japanese population (**Supplementary Note**). After SNP and sample quality control analyses, we used genotype data from 435,470 SNPs in 988 cases and 2,521 controls in the GWAS analysis (see Online Methods).

We tested for association between each SNP and PD using the Cochran-Armitage trend test with 1 d.f. The quantile-quantile plot showed a close match to test statistics expected under the null distribution (genomic inflation factor $\lambda = 1.055$ for PD) (**Fig. 1a,b**). This indicates minimal overall inflation of genome-wide statistical results due to population stratification and also reveals a number of SNPs whose *P* values exceed those expected under the null hypothesis. Seventeen SNPs showed $P < 5 \times 10^{-7}$, the threshold for genome-wide significance suggested by the Wellcome Trust Case Control Consortium[15] (**Fig. 1c**). All these SNPs were located on 4q22, a region harboring *SNCA* that was previously identified by us and others as a definite susceptibility gene for PD[8–10].

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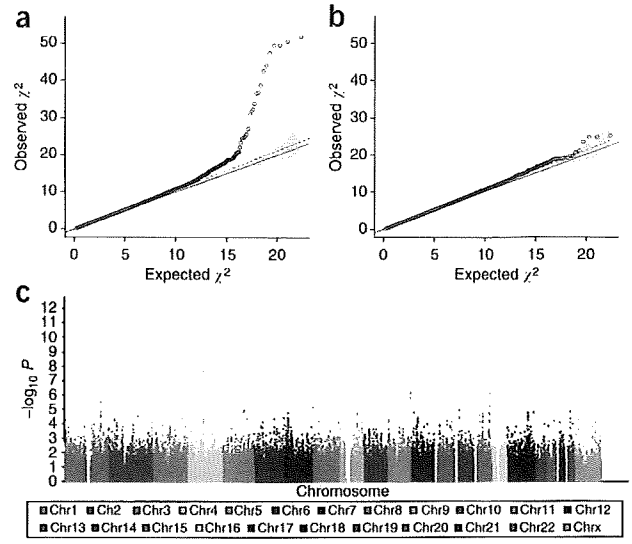

Figure 1 Genome-wide association results from the discovery phase. (a) Quantile-quantile plot for test statistics (Cochran-Armitage trend test) for 435,470 SNPs passing quality control. The solid line represents concordance of observed and expected values. Slope of the dashed line represents the genomic inflation factor ($\lambda = 1.055$). The shaded region is the 95% concentration band formed by calculating, for each order statistic, the 2.5th and 97.5th percentiles of the respective distribution under the null hypothesis. (b) Quantile-quantile plot for test statistics (Cochran-Armitage trend test) after the removal of the four loci with strong associations in this study (1q32, 4p15, 4q22 and 12q12). (c) Manhattan plot presenting the *P* values across the genome. The $-\log_{10} P$ (Cochran-Armitage trend test) from 435,470 SNPs in 988 Parkinson's disease cases and 2,521 controls is plotted according to its physical position on successive chromosomes.

For fast-track replication, we selected the 337 most associated SNPs ($P \leq 0.000533$) from analysis of GWAS data and genotyped them in a sample set of replication 1, which consisted of 612 cases and 14,139 controls from Japan (**Supplementary Note**). Thirty-two SNPs showed association of $P < 0.05$ in replication 1 (**Supplementary Fig. 1**). Combined analyses of the GWAS and replication 1 showed that 12 SNPs in 3 loci (1q32, 4p15 and 4q22) surpassed $P < 5 \times 10^{-7}$. Furthermore, we found association signals ($P = 3.06 \times 10^{-6}$, OR = 1.36) on 12q12, harboring *LRRK2*, which is a causative gene for autosomal dominant parkinsonism (**Table 1**).

In replication 2, we tested 24 SNPs at these four loci for association with PD. An independent sample set (321 cases and 1,614 controls) recruited from Japan was used in replication 2 (**Supplementary Note**). Association evidence was again found at these four loci: 1q32, $P = 2.80 \times 10^{-4}$, OR = 1.37; 4p15, $P = 7.70 \times 10^{-3}$, OR = 1.26; 4q22, $P = 0.02$, OR = 1.22; and 12q12, $P = 6.43 \times 10^{-4}$, OR = 1.57 (**Table 1**). The disease associations on 1q32 and 12q12 exceeded the conservative Bonferroni-corrected threshold for significance ($P = 0.0021$; calculated as 0.05/24). All the SNPs showed allele frequency differences in the same direction in the GWAS, replication 1 and replication 2. Furthermore, combined analysis of the GWAS and two replication stages provided strong evidence of association in the four regions with a significance level of $P = 2.72 \times 10^{-8}$ or less (**Table 1**).

We identified two new susceptibility loci with genome-wide significance on 1q32 and 4p15, which have not been reported to be associated with PD in previous studies[12–14]. On 1q32, seven SNPs (rs16856139, rs823128, rs823122, rs947211, rs823156, rs708730 and rs11240572) reached $P < 5 \times 10^{-7}$ in the overall analysis (**Fig. 2a**). rs947211 showed the strongest association to PD ($P = 1.52 \times 10^{-12}$, OR = 1.30) and is located 8.5 kb upstream of *RAB7L1* and 5.6 kb downstream of *SLC41A1*. Linkage disequilibrium (LD) analysis revealed that SNPs with significant associations to PD lie within several LD blocks containing the following five genes: *SLC45A3*, *NUCKS1*, *RAB7L1*, *SLC41A1* and *PM20D1* (also called *FLJ32569*) (**Table 1** and **Fig. 2a**). Three genes (*NUCKS1*, *RAB7L1* and *SLC41A1*) were contained in the same LD block as rs947211. rs947211 was weakly correlated with the other six SNPs ($r^2 = 0.07$–0.25), and we observed residual association signals when rs947211 and each of the other six SNPs were paired in conditional analyses of our overall data. This result suggests that this locus has multiple independent association signals (**Supplementary Table 1**). These data provide the first evidence, to our knowledge, of an association between 1q32 and PD susceptibility, and we designated this region as *PARK16*.

On 4p15, four SNPs (rs11931532, rs12645693, rs4698412 and rs4538475) reached $P < 5 \times 10^{-7}$ in the combined analysis (**Fig. 2b**). These four SNPs showed strong disease association with almost the same significance levels (ranging from $P = 3.94 \times 10^{-9}$ to $P = 1.78 \times 10^{-8}$, all OR = 1.24); among them, rs4538475 was the most strongly associated. The four SNPs were located from intron 8 to 4.1 kb downstream of *BST1* (bone marrow stromal cell antigen). LD analysis revealed that the four SNPs were correlated with $r^2 > 0.78$ and lie within a 15 kb LD block containing a single gene, *BST1*.

The remaining two intervals (4q22 and 12q12) harbored genes previously found to be causal for autosomal dominant forms of parkinsonism, specifically, *SNCA* and *LRRK2*, respectively. On 4q22, seven SNPs (rs3733449, rs11931074, rs3857059, rs2736990, rs3796661, rs6532194 and rs12233759) throughout the *SNCA* region showed genome-wide significant association in the combined analysis (**Fig. 2c**). The most significantly associated SNPs, rs11931074 ($P = 7.35 \times 10^{-17}$, OR = 1.37) and rs3857059 ($P = 5.68 \times 10^{-16}$, OR = 1.36), are approximately 35.7 kb apart, located 7.2 kb downstream from and in intron 4 of *SNCA*, respectively. The entire *SNCA* gene was divided into two LD blocks at intron 4. Both SNPs were positioned on the 3′ side of the LD block and showed a high degree of LD ($r^2 = 0.98$). Three SNPs (rs2736990, rs3796661 and rs6532194) were moderately correlated with rs11931074 ($r^2 = 0.81$, 0.76 and 0.63, respectively). The remaining two SNPs (rs3733449 and rs12233759) were weakly correlated with rs11931074 ($r^2 = 0.05$ and 0.24, respectively), and residual association signals were marginally observed when rs11931074 and each of these two SNPs were paired in conditional analyses of our overall data (**Supplementary Table 1**). These data confirm *SNCA* as a susceptibility gene for PD.

On 12q12, five SNPs (rs1994090, rs7304279, rs4768212, rs2708453 and rs2046932) surpassed $P < 5 \times 10^{-7}$ in the overall analysis (**Fig. 2d**). The five SNPs showed strong disease association with almost the same significance (ranging from $P = 2.72 \times 10^{-8}$ to $P = 1.09 \times 10^{-7}$, OR = 1.37–1.39); among them, rs1994090 was the most strongly associated to PD. These five SNPs were located from intron 2 of *SLC2A13* to 38.4 kb upstream of *LRRK2*. These SNPs were highly correlated with $r^2 > 0.83$ and were positioned within several LD blocks defined by the method of Gabriel *et al.*[16]. This is the first evidence that common variants proximal to *LRRK2* are associated with PD at genome-wide significance level.

Variants with the highest significance at the four loci detected in this study were common SNPs with risk allele frequencies of 0.50 (rs947211 on 1q32), 0.38 (rs4538475 on 4p15), 0.58 (rs11931074 on 4q22) and 0.08 (rs1994090 on 12q12) (**Table 1**). Population attributable risks for rs947211, rs4538475, rs11931074 and rs1994090 were estimated to be 13%, 8%, 18% and 3%, respectively.

Table 1 Summary of association results for representative SNPs that characterize the association of Parkinson's disease with 1q32 (*PARK16*), 4p15 (*BST1*), 4q22 (*SNCA*) and 12q12 (*LRRK2*)

				GWAS			Replication 1			GWAS + Replication 1			Replication 2			GWAS + Replication 1+2	
Locus	SNP	Pos (Mb)	Allele Minor/ major	MAF Case	Ctrl	P_{trend}	OR (95% CI)	MAF Case	Ctrl	P_{trend}	OR (95% CI)	P_{cmh}	OR (95% CI)	MAF Case	Ctrl	P_{trend}	OR (95% CI)	P_{cmh}	OR (95% CI)
New PD loci																			
1q32	rs16856139	203.91	T/C	0.10	0.14	2.55×10^{-6}	1.50 (1.26–1.77)	0.11	0.13	0.067	1.19 (0.99–1.44)	2.15×10^{-6}	1.35 (1.19–1.54)	0.10	0.13	0.015	1.42 (1.07–1.88)	1.02×10^{-7}	1.36 (1.22–1.53)
(*PARK16*)	rs823128	203.98	G/A	0.10	0.14	2.09×10^{-5}	1.43 (1.21–1.69)	0.11	0.13	0.0056	1.31 (1.08–1.59)	4.67×10^{-7}	1.38 (1.22–1.57)	0.09	0.14	0.0028	1.53 (1.16–2.03)	4.88×10^{-9}	1.41 (1.26–1.58)
	rs823122	203.99	C/T	0.10	0.14	7.98×10^{-5}	1.39 (1.18–1.64)	0.11	0.13	0.013	1.27 (1.05–1.54)	3.87×10^{-6}	1.34 (1.18–1.52)	0.09	0.14	0.0034	1.52 (1.15–2.01)	5.22×10^{-8}	1.37 (1.22–1.54)
	rs947211	204.02	A/G	0.43	0.48	1.15×10^{-4}	1.23 (1.11–1.37)	0.42	0.50	1.35×10^{-6}	1.35 (1.19–1.52)	1.12×10^{-9}	1.28 (1.18–1.38)	0.42	0.50	2.80×10^{-4}	1.37 (1.16–1.63)	1.52×10^{-12}	1.30 (1.21–1.39)
	rs823156	204.03	G/A	0.13	0.17	1.20×10^{-5}	1.40 (1.20–1.62)	0.14	0.17	0.012	1.25 (1.05–1.48)	6.45×10^{-7}	1.33 (1.19–1.49)	0.12	0.17	0.0013	1.52 (1.17–1.95)	3.60×10^{-9}	1.37 (1.23–1.52)
	rs708730	204.04	G/A	0.14	0.18	2.60×10^{-5}	1.37 (1.18–1.59)	0.15	0.17	0.022	1.22 (1.03–1.44)	2.89×10^{-6}	1.30 (1.17–1.46)	0.12	0.18	0.0019	1.48 (1.15–1.89)	2.43×10^{-8}	1.33 (1.21–1.48)
	rs11240572	204.07	A/C	0.13	0.16	1.66×10^{-4}	1.34 (1.15–1.56)	0.13	0.16	0.016	1.24 (1.04–1.48)	9.78×10^{-6}	1.30 (1.16–1.46)	0.12	0.17	0.0024	1.49 (1.15–1.92)	1.08×10^{-7}	1.33 (1.20–1.48)
4p15	rs11931532	15.33	T/C	0.45	0.40	2.75×10^{-4}	1.22 (1.09–1.35)	0.47	0.42	1.86×10^{-4}	1.26 (1.11–1.42)	2.02×10^{-7}	1.23 (1.14–1.34)	0.47	0.41	0.0077	1.26 (1.06–1.49)	5.13×10^{-9}	1.24 (1.15–1.33)
(*BST1*)	rs12645693	15.34	G/A	0.45	0.40	3.06×10^{-4}	1.21 (1.09–1.35)	0.47	0.42	3.00×10^{-4}	1.25 (1.11–1.41)	3.42×10^{-7}	1.23 (1.14–1.33)	0.47	0.41	0.0077	1.26 (1.06–1.49)	8.65×10^{-9}	1.24 (1.15–1.33)
	rs4698412	15.35	A/G	0.38	0.33	5.28×10^{-5}	1.25 (1.12–1.40)	0.40	0.35	4.91×10^{-4}	1.24 (1.10–1.40)	1.03×10^{-7}	1.25 (1.15–1.35)	0.38	0.34	0.055	1.19 (1.00–1.42)	1.78×10^{-8}	1.24 (1.15–1.33)
	rs4538475	15.35	A/G	0.41	0.36	4.05×10^{-5}	1.25 (1.12–1.40)	0.43	0.38	3.48×10^{-4}	1.25 (1.10–1.41)	5.98×10^{-8}	1.25 (1.15–1.35)	0.42	0.37	0.022	1.22 (1.03–1.46)	3.94×10^{-9}	1.24 (1.16–1.34)
Loci located in or near autosomal dominant parkinsonism genes																			
4q22	rs11931074	90.86	G/T	0.32	0.42	6.17×10^{-13}	1.50 (1.34–1.68)	0.36	0.42	2.12×10^{-5}	1.31 (1.16–1.48)	2.19×10^{-16}	1.41 (1.30–1.53)	0.38	0.43	0.034	1.21 (1.01–1.44)	7.35×10^{-17}	1.37 (1.27–1.48)
(*SNCA*)	rs3857059	90.89	A/G	0.32	0.41	1.17×10^{-12}	1.49 (1.34–1.67)	0.36	0.42	6.92×10^{-5}	1.29 (1.14–1.45)	1.54×10^{-15}	1.40 (1.29–1.52)	0.38	0.43	0.041	1.20 (1.01–1.43)	5.68×10^{-16}	1.36 (1.26–1.46)
	rs894278	90.95	G/T	0.43	0.38	7.68×10^{-5}	1.24 (1.11–1.37)	0.39	0.38	0.46	1.05 (0.93–1.18)	4.77×10^{-4}	1.15 (1.07–1.25)	0.42	0.37	0.020	1.22 (1.03–1.45)	3.28×10^{-5}	1.17 (1.09–1.25)
	rs6532194	91.00	C/T	0.31	0.40	6.93×10^{-11}	1.44 (1.29–1.61)	0.36	0.41	0.0014	1.22 (1.08–1.39)	1.77×10^{-12}	1.35 (1.24–1.46)	0.37	0.41	0.040	1.21 (1.01–1.44)	4.15×10^{-13}	1.32 (1.22–1.42)
12q12	rs1994090	38.71	G/T	0.11	0.08	4.45×10^{-5}	1.43 (1.20–1.70)	0.10	0.08	0.018	1.26 (1.04–1.54)	3.06×10^{-6}	1.36 (1.20–1.55)	0.12	0.08	0.0019	1.51 (1.16–1.97)	2.72×10^{-8}	1.39 (1.24–1.56)
(*LRRK2*)	rs7304279	38.75	T/C	0.11	0.08	5.17×10^{-5}	1.42 (1.20–1.69)	0.10	0.08	0.026	1.25 (1.03–1.52)	5.10×10^{-6}	1.35 (1.19–1.54)	0.12	0.09	0.0022	1.50 (1.15–1.95)	5.06×10^{-8}	1.38 (1.23–1.55)
	rs4768212	38.76	C/T	0.11	0.08	3.98×10^{-5}	1.43 (1.20–1.70)	0.10	0.08	0.057	1.21 (0.99–1.48)	1.10×10^{-5}	1.34 (1.18–1.52)	0.12	0.08	0.0020	1.51 (1.16–1.97)	1.09×10^{-7}	1.37 (1.22–1.54)
	rs2708453	38.76	T/G	0.11	0.08	7.46×10^{-5}	1.41 (1.19–1.68)	0.10	0.08	0.063	1.21 (0.99–1.48)	2.04×10^{-5}	1.33 (1.17–1.52)	0.13	0.08	6.43×10^{-4}	1.57 (1.21–2.04)	9.67×10^{-8}	1.38 (1.22–1.55)
	rs2046932	38.87	T/C	0.11	0.08	3.24×10^{-5}	1.44 (1.21–1.71)	0.10	0.08	0.039	1.23 (1.01–1.51)	5.47×10^{-6}	1.35 (1.19–1.54)	0.13	0.09	0.0017	1.52 (1.17–1.97)	4.34×10^{-8}	1.39 (1.23–1.56)

Nucleotide positions refer to NCBI build 36. *P* values obtained in the case-control analysis using the Cochran-Armitage trend test (1 d.f.) are listed (P_{trend}). Combined *P* values (P_{cmh}) and combined ORs of the Cochran-Mantel-Haenzel test statistics are shown. MAF, minor allele frequency.

Next, we exchanged data with colleagues performing a GWAS of PD in individuals of European ancestry[17]. Their study found a strong association at the *MAPT* (microtubule-associated protein tau) region on 17q21. We genotyped our samples for six SNPs at the *MAPT* locus to evaluate these associations in the Asian population; however, the association with *MAPT* was not replicated in our study (**Supplementary Table 2** and **Supplementary Fig. 2**). Conversely, despite strong association signals in our scan of the samples from the Asian population, the association with *BST1* on 4p15 was not detected among individuals of European ancestry[17]. In contrast, the associations we found with *PARK16* and *LRRK2* were replicated among individuals of European ancestry[17]. These data provide evidence that *PARK16* and *LRRK2*, in addition to *SNCA*, are PD risk loci common to Asian- and European-descent populations and indicate that there is population genetic heterogeneity in the *MAPT* region and 4p15 (*BST1*) for PD susceptibility.

The *PARK16* region contains functionally interesting candidate genes for PD etiology. SLC41A1 is a magnesium (Mg^{2+}) transporter[18]. It is of interest that Mg^{2+} deficiency is thought to be an environmental risk factor for the amyotrophic lateral sclerosis

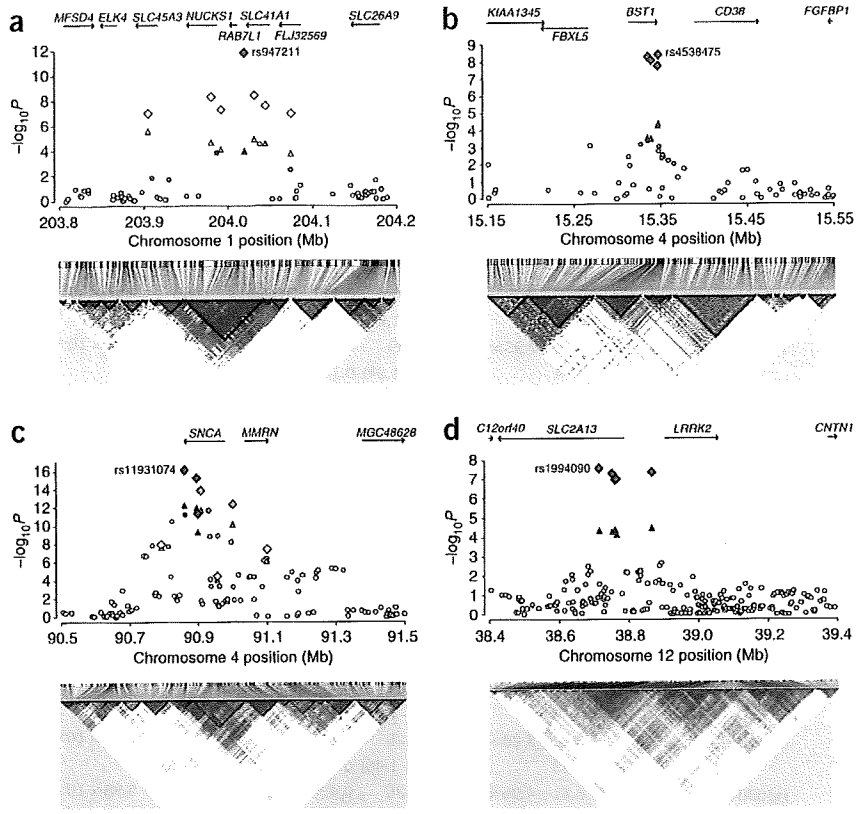

Figure 2 Regional association plots and linkage disequilibrium structure for the four PD risk loci. (a) 1q32 (*PARK16*). (b) 4p15 (*BST1*). (c) 4q22 (*SNCA*). (d) 12q12 (*LRRK2*). The $-\log_{10}$ *P* (Cochran-Armitage trend test) for association in the GWAS stage of SNPs across each region are shown as small triangles for SNPs that were selected for replication and as small circles for SNPs not selected. The $-\log_{10}$ combined *P* values (Cochran-Mantel-Haenszel test) for association in overall samples of SNPs selected for replication are shown as large diamonds. In each panel, the SNP with the most significant association in the combined analysis is listed. Proxies are indicated with colors determined from their pairwise r^2 from the JPT and CHB HapMap data (red, $r^2 > 0.8$; orange, $r^2 = 0.5–0.8$; yellow, $r^2 = 0.2–0.5$; white, $r^2 < 0.2$ or no information available). Positions are NCBI build 36 coordinates.

(ALS)-parkinsonism/dementia complex (MIM105500)[19]. Furthermore, RAB7L1 is a small GTP-binding protein that plays an important role in regulation of exo- and endocytotic pathways[20], and NUCKS1 is a nuclear protein containing several consensus phosphorylation sites for casein kinase II and cyclin-dependent kinases of unknown function[21]. We evaluated the relationships between the PD-associated SNPs and the transcript levels of genes in an available genome-wide gene expression database[22]. We found that rs947211 and ten tightly linked HapMap SNPs ($r^2 > 0.9$) were strongly associated with transcript levels of *NUCKS1* (rs947211, $P = 6.0 \times 10^{-15}$; rs823114, $P = 2.7 \times 10^{-34}$). These PD-susceptibility variants are the principal genetic determinants of variation in expression levels of *NUCKS1* (**Supplementary Fig. 3**). These data highlight *NUCKS1* as a promising candidate for association with PD that is worthy of additional follow-up.

The product of *BST1* on 4p15 catalyses formation of cyclic ADP-ribose (cADPR)[23]. cADPR mobilizes calcium (Ca^{2+}) from ryanodine-sensitive intracellular Ca^{2+} stores in the endoplasmic reticulum[24]. Disruption of Ca^{2+} homeostasis has recently been proposed as a possible cause of selective vulnerability of dopaminergic neurons in PD[25–27]. Associated SNPs in the *BST1* region may modify ADP-ribosylcyclase activity, thus leading to Ca^{2+} dyshomeostasis in dopaminergic neurons.

Two of the four susceptibility loci detected in our scan contained genes linked to autosomal dominant forms of parkinsonism. Gene overdosage is a potential mechanism for the influence of *SNCA* on PD because triplication and duplication of the *SNCA* locus has been seen in families with autosomal dominant parkinsonism[28]. SNPs with prominently low *P* values compared to other SNPs in the region were around the 3′ region of *SNCA*; these SNPs may function as enhancer or silencer elements, improve RNA stability or influence alternative splicing. The associated interval on 12q12 contains *SLC2A13* and the region upstream of *LRRK2*. Given prior evidence, *LRRK2* stands out as the most likely susceptibility gene for PD, although it remains possible that *SLC2A13*, which encodes a H^+-myoinositol cotransporter, may be the PD-related gene in this region[29]. Previous reports have investigated the association of SNPs in *LRRK2* with PD, but the results are a subject of dispute[30,31]. In the present study, it is noteworthy that the PD-associated intervals lie upstream of *LRRK2*. Increased kinase activity of mutant LRRK2 mediates neuronal toxicity[32,33]. PD-associated SNPs may play a role in transcriptional upregulation of *LRRK2*, leading to loss of dopaminergic neurons.

SNCA is a main component of Lewy bodies, a pathological hallmark of typical PD. The clinical features of individuals with *SNCA* duplication or *LRRK2* mutation similar to typical PD. 1.6% of sporadic PD cases among individuals of European ancestry have heterozygous *LRRK2* G2019S mutations[34]. These data support the close involvement of these genes with sporadic PD. Our data clearly show that the genes involved in autosomal dominant parkinsonism play a large part in the complex etiology of typical PD. Genes that cause autosomal dominant parkinsonism through their causative mutations also confer risk of typical PD through their common variants. Although further research is needed, this relationship between rare single-gene disorders and common multifactorial disorders may also be applicable for other disorders beyond PD.

Finally, *MAPT* mutations cause hereditary frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17), a type of autosomal dominant parkinsonism[35], and the *MAPT* H1 haplotype has been reported to be associated with several tauopathies[36–38]. Although the *MAPT* region is divided into two major haplotypes, H1 and H2, in Europeans, the H2 haplotype is absent in East Asians. Therefore, we believe that the differences observed between our study and the findings in populations of European descent reflect population differences in the genetic heterogeneity of PD etiology, although differences in allele frequencies and LD structure and a possible difference in the effect size between the European and East Asian populations may influence the detection power of the two scans.

Further increases in sample sizes for SNP-GWAS efforts and searches for copy number variation and rare variants will reveal additional genetic risk factors and further enhance our understanding of PD.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

T. Toda conceived the study. W.S., I.M. and T. Toda designed the study. W.S., Y.N., C.I., M.K. and T.Y. performed genotyping. W.S. and T. Toda wrote the manuscript. W.S., T.K. and T. Tsunoda performed data analysis. W.S., I.M., Y.H., M.W., A.T., H.T., K.N., K.H., F.O., H.K., S.S., M.Y., N.H., M.M. and T. Toda managed Parkinson clinical information and DNA samples. M.K. and Y.N. managed DNA samples belonging to BioBank Japan. T. Toda obtained funding for the study.

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ONLINE METHODS

Study participants. For the GWAS stage, 1,078 cases and 2,628 controls were recruited from Japan through multiple institutions. Two case-control sample sets, which were independent of each other, were used in the two subsequent replication stages (replication 1 and 2): the first sample set (replication 1) consisted of 612 cases and 14,139 controls and the second set (replication 2) consisted of 321 cases and 1,614 controls, all recruited in Japan. For replication 2, we used case samples from two facilities that had not provided any case samples for GWAS and replication 1, in order to eliminate false positive association due to a slight possibility of differences in PD diagnosis among facilities. Genomic DNA was extracted using established methods and quantified using PicoGreen (Invitrogen). Details for all study panels are given in the **Supplementary Note**. Informed consent was obtained from each participant, and approval for the study was obtained from the Ethical Committees of relevant institutions (Osaka University Graduate School of Medicine, National Center Hospital of Neurology and Psychiatry, Juntendo University School of Medicine, Kagawa Prefectural Central Hospital, University of Tsukuba, Tohoku University Graduate School of Medicine and Tottori University Faculty of Medicine).

Genotyping. Genome-wide genotyping was performed using the Illumina Infinium HumanHap550 array. Cases and controls were genotyped at the Division of Clinical Genetics of Osaka University Graduate School of Medicine and at RIKEN Center for Genomic Medicine, respectively. For two subsequent replication studies (replication 1 and 2), 337 SNPs were genotyped for replication 1 and 2 samples using Illumina GoldenGate technology for the VeraCode platform (335 SNPs; cases in replication 1 and cases and controls in replication 2), TaqMan (2 SNPs; cases in replication 1 and cases and controls in replication 2) and the Illumina Infinium HumanHap610 array (337 SNPs; controls in replication 1). For the replication study of the *MAPT* locus, we selected six SNPs (rs417968, rs17690703, rs242557, rs7225002, rs183211 and rs7224296) that showed significant association, including four SNPs showing association with genome-wide significance, in samples of European ancestry. Samples with a call rate >90% in replications 1 and 2 (877 cases and 15,616 contols) were genotyped. All genotyping was done according to the manufacturer's instructions. To assess consistency across genotyping platforms, we genotyped these SNPs in 95 samples included in the GWAS. After SNP and sample quality control analyses, the mean concordance rates were 99.8% and 99.5% for GoldenGate technology and TaqMan when compared with the HumanHap550 array.

Quality control. In the GWAS stage, case samples with a call rate <95% and control samples with a call rate <98% were excluded, according to each criterion of separate institutes which genotyped cases and controls. Remaining samples were reclustered using BeadStudio (Illumina), and genotypes of 1,012 cases and 2,573 controls were then obtained. We excluded samples with ambiguous sex (n = 18) by the check-sex function of PLINK 1.01 (ref. 39). We determined identity-by-state (IBS) similarity using PLINK 1.01, estimated the cryptic relatedness for each pair of samples, and excluded one individual from each pair of unexpected duplicates and first- or second-degree cryptic relatives (n = 55). To detect population outliers, we assessed 3,512 participants who remained after removal of samples with low call rates, ambiguous sex and familial relationships, together with 201 HapMap subjects without relationships (42 JPT, 45 CHB, 57 CEPH and 57 YRI)[40]. By computing IBS scores for 49,605 SNPs with $r^2 < 0.2$ and using multidimensional scaling, we identified three individuals who seemed to have non-Asian ancestry and excluded those from further analyses. Projection onto the two multidimensional scaling axes is shown in **Supplementary Figure 4**. We excluded SNPs with a call rate <95% in cases or controls (n = 7,927), a minor allele frequency <5% (n = 117,908) in all samples, or a P value of deviation from Hardy-Weinberg equilibrium (P_{HWE}) < 0.001 in the controls (n = 3,045). On visual inspection of the cluster plots of SNPs showing apparently strong association, we further removed 69 SNPs with poor clustering. The overall median genotype call rate for quality-controlled SNP was 99.9%. In replication 1, we excluded samples with a call rate <90%. We also removed SNPs with a call rate <130895% in cases or controls, a $P_{HWE} < 0.001$ in the controls, or poor clustering of SNP plot on visual inspection. Genotypes of 279 SNPs for 559 cases and 14,026 controls were then obtained for further analyses. The overall mean genotype call rate was 99.7% for quality-controlled SNPs. In replication 2, we excluded samples with a call rate <90% and then obtained genotypes of 318 cases and 1,590 controls. All 24 SNPs showed a call rate >90% and P_{HWE} >0.001. The overall mean genotype call rate was 99.7% for quality-controlled SNPs. Associated SNPs in each interval had high call rates in each sample set (**Supplementary Table 3**). In the replication study of the *MAPT* locus, all six SNPs showed a call rate >95% and $P_{HWE} > 0.001$.

Statistical analyses. To calculate the power of our GWAS stage, we used the CaTS program[41]. The GWAS stage had 80% power to detect common alleles that confer a genotype relative risk of 1.3 and 1.43 at a significance of $P < 0.00053$ and $P < 5 \times 10^{-7}$, respectively.

To test for association of each SNP with PD, we used the Cochran-Armitage trend test with 1 d.f. We estimated the odd ratios (OR) and their 95% confidence intervals using logistic regression. Association analysis of the combined samples was conducted using the Cochran-Mantel-Haenezel method. Heterogeneity among sample sets was examined using the Breslow-Day test. There was no heterogeneity among sample sets (rs947211, P = 0.38; rs4538475, P = 0.98; rs11931074, P = 0.09; rs3857059, P = 0.08; and rs1994090, P = 0.48). SNPs with combined $P < 5 \times 10^{-7}$ were considered to have genome-wide significant evidence for association. SNPs with genome-wide significant evidence for association in the combined analysis of the GWAS and replication 1 and $P < 0.05$ in replication 2 were considered to have confirmed association with PD. To assess whether single or multiple independent association signals existed within each locus, we investigated relationships among multiple SNPs that showed association with PD in the same region ($P < 5.0 \times 10^{-7}$), using logistic regression analysis. We assessed the impact of additional SNPs by a likelihood-ratio test with 1 d.f. A significant residual association signal was defined as $P < 0.05$ in the conditional analysis. We used R 2.8.1 or PLINK 1.01 for general statistical analysis.

The quantile-quantile plot was used to evaluate overall significance of the genome-wide association results and the potential impact of population stratification. Quantile-quantile plots were depicted using the qq.chisq function of the snpMatrix package with a concentration band[15]. The inflation factor λ was calculated by dividing the mean of the lower 90% of the test statistics by the mean of the lower 90% of the expected values from a χ^2 distribution with 1 d.f. Given that the impact of population stratification was found to be minimal, all statistical results are presented without correction for λ. Haploview 4.1 was used to infer the LD structure of the genome in regions containing loci associated with disease risk[42]. The LD patterns were created from the Asian (CHB and JPT) HapMap data (minor allele frequency > 5%, genotyping rate > 95%, and $P_{HWE} > 0.001$), using the methods of Gabriel *et al.*[16].

URLs. PLINK, http://pngu.mgh.harvard.edu/~purcell/plink; R, http://www.r-project.org; SNPmatrix, http://www-gene.cimr.cam.ac.uk/clayton; HapMap, http://www.hapmap.org; Haploview, http://www.broad.mit.edu/mpg/haploview; CaTS, http://www.sph.umich.edu/csg/abecasis/CaTS/; database of expression QTL analysis, http://www.sph.umich.edu/csg/liang/imputation/.

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特集 大脳生理学研究の最前線

神経変性疾患における REM睡眠行動障害(RBD)*

野村哲志**／井上雄一***／中島健二**

Key Words：REM sleep without atonia, synucleiopathies, International Classification of Sleep Disorders, sublaterodorsal nucleus, locus coerleus, Braak theory

はじめに

レム睡眠(rapid eye movement sleep：REM睡眠)は急速眼球運動，脳波の速波，骨格筋の脱力を特徴とする睡眠段階である[1]．このREM睡眠中に，REM睡眠行動障害(REM sleep behavior disorder：RBD)は夢内容に一致した異常行動を起こし，しばしば自分自身やベッドパートナーが怪我をしてしまう睡眠随伴症である[2]．病態の機序としては脳幹のREM睡眠制御の障害が考えられている．以前から神経疾患に合併する続発性のRBDの報告はあったが，最近Parkinson病(PD)，Lewy小体型認知症(demenita with Lewy bodies：DLB)，多系統萎縮症(multiple system atrophy：MSA)，純粋自律神経不全症(pure autonomic failure：PAF)などのαシヌクレイン陽性の細胞内封入体を有するシクレイノパチーと総称される神経変性疾患に進展する症例が報告され，これらの病前症状の可能性があるとして注目されている．

表1 RBDの典型的な臨床徴候[3]

- 男性に多い
- 平均発症年齢50～65歳
- 寝言，発汗，叫び声
- 簡易な四肢の動きから複雑な動きまであり，本人や添い寝している人が怪我するような行動まで多様な動作
- 動物や人に襲われる夢内容が多い
- 夢内容を行動する
- 睡眠後半に出現することが多い
- 数年から数十年認知症，Parkinson徴候に先行することが多い．

REM睡眠行動障害の診断

RBDは90％が50歳代以降の男性に認め，覚醒時の穏やかな言動と異なった暴力的な言動が特徴である．睡眠後半に出現することが多いが，悪夢，とくに動物や他人に襲われる夢をみて，夢の中での殴りあいや逃げ出すなどの行動を睡眠中に行ってしまう．表1にRBDの臨床上の特徴[3]を示す．終夜脳波(polysomnography：PSG)上REM睡眠は急速な眼球運動，骨格筋の脱力，α波，θ波の混在が特徴であるが(図1)，RBD患者では頤筋，下肢の骨格筋の脱力の消失したREM sleep without atonia(RWA)が出現する(図2)．

2005年に作成された睡眠障害国際診断分類

* REM sleep behavior disorder in neurodegenerative diseases.

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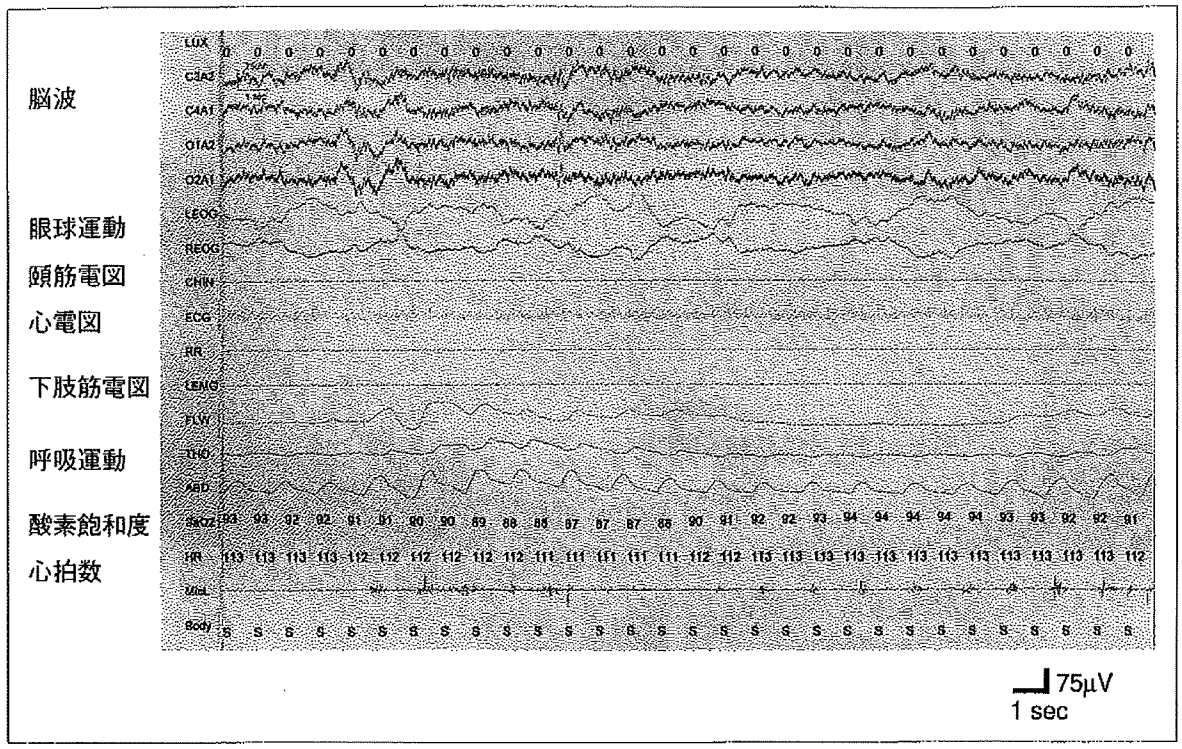

図1　正常なREM睡眠

脳波上α,θ波の混在があり，急速眼球運動が出現している．同時に，頤，下肢筋電図とも収縮が抑制されている．

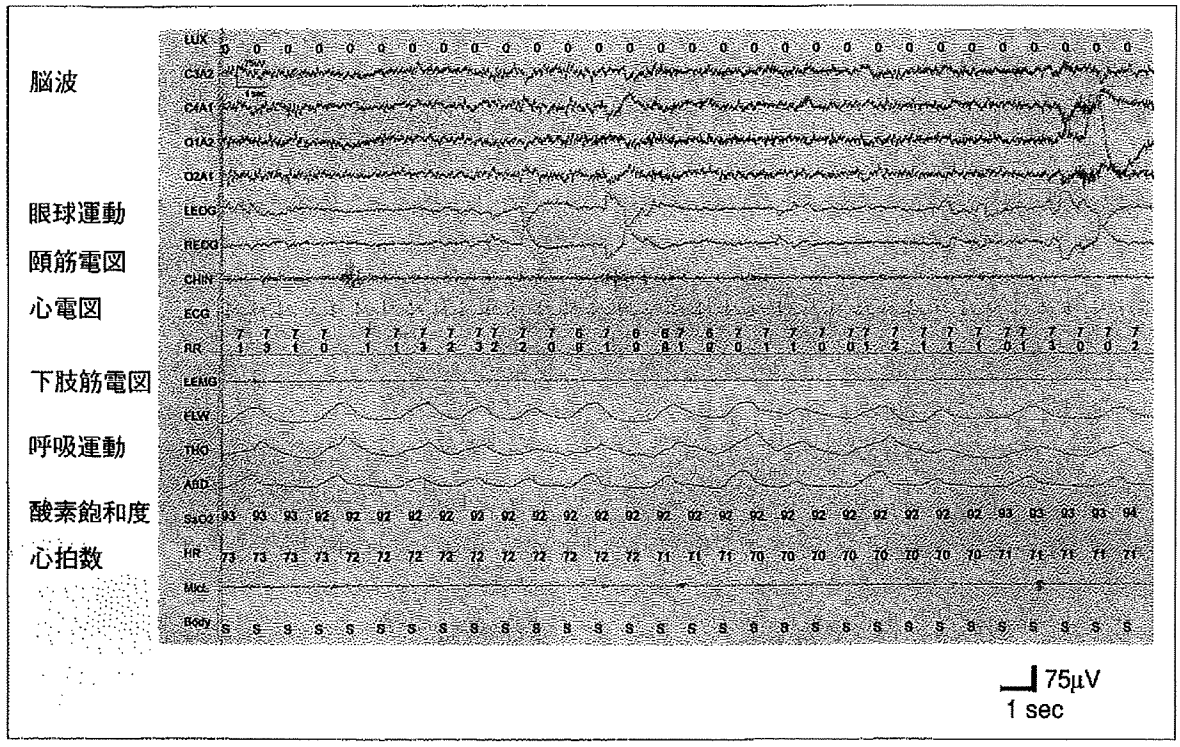

図2　REM sleep without atonia（RWA）

同様に，脳波上α,θ波の混在があり，急速眼球運動が出現している．しかし，正常なREM睡眠と異なり頤筋電図に筋放電の出現を認めている．

(International Classification of Sleep Disorders：ICSD)第二版の診断基準[2]において，夢内容の行動化により怪我をしたり，怪我をしてもおかしくないような睡眠中の行動化の病歴があるか，PSG実施中にREM睡眠期に異常な行動化があるかの少なくともどちらかの事象があるとともに，PSG上RWAの存在が確認されることがRBD診断の必須項目となっている．RBDと類似した夜間の異常行動はてんかん発作でもみられることがあり，他の睡眠疾患，内科・神経・精神科疾患，薬剤やアルコールの影響によってもRBD類似症状が生じることがあるため，これらを除外の上診断確定となる(表2)．

表2　RBDの診断基準(ICSD, 2005)[2]

1. 終夜脳波上のREM sleep without atnoiaの出現
2. 下記のどちらか一つの症状：
(1)病歴により怪我をしたり，怪我をしてもおかしくないような睡眠に関連した行動がある(夢内容の行動化など)
(2)終夜脳波中にREM睡眠時に異常な行動が出現する
3. REM睡眠に関連した発作を起こすような脳波上てんかん発作波を除外する
4. その他の睡眠疾患，内科疾患，神経疾患，精神疾患，薬剤服用，アルコール飲用などを除外する

REM睡眠行動障害の病態生理

REM睡眠中には鮮明な夢をみることが多いが，これは情動系が活発になっているためと考えられる．この情動系は脊髄の前角細胞に入力しているが，REM睡眠中には脳幹のREM睡眠制御系が前角細胞に対し抑制性に作用する．これによって情動系から出力される骨格筋の収縮は抑制されている．

Sastreらは，ネコを使った実験で脳幹の破壊実験を行い夢幻様運動を起こし，この病態の脳幹の関与を報告した[4](図3)．さらに最近のネコを使った実験からREM睡眠は情動系の抑制と骨格筋脱力の出現が重要であり，骨格筋脱力はコリン系の橋脚被蓋核(peduclopontine nucleus：PPN)と外背側被蓋核(laterodorsal tegmental nucleus：LDTN)，アドレナリン系の青斑核(locus coeruleus：LC)が延髄巨大細胞網様体(medullary magnocellular reticular formation：MCRF)を介して起こしていることがわかっている．また，ネズミの実験よりネコのLCに相当する下外側背側核(sublaral dorsal nucleus：SLD)がREM睡眠を促進する働きをもっており，反対に中脳水道周辺の腹外側灰白質(ventrolateral part of the periaqueductal grey matter：vlPAG)，外側橋被蓋(lateral pontine tegmentum：LPT)はREM睡眠を抑制していることが示されている[1]．ネコのLC，ネズミのSLDが直接障害された時にRWAは出現

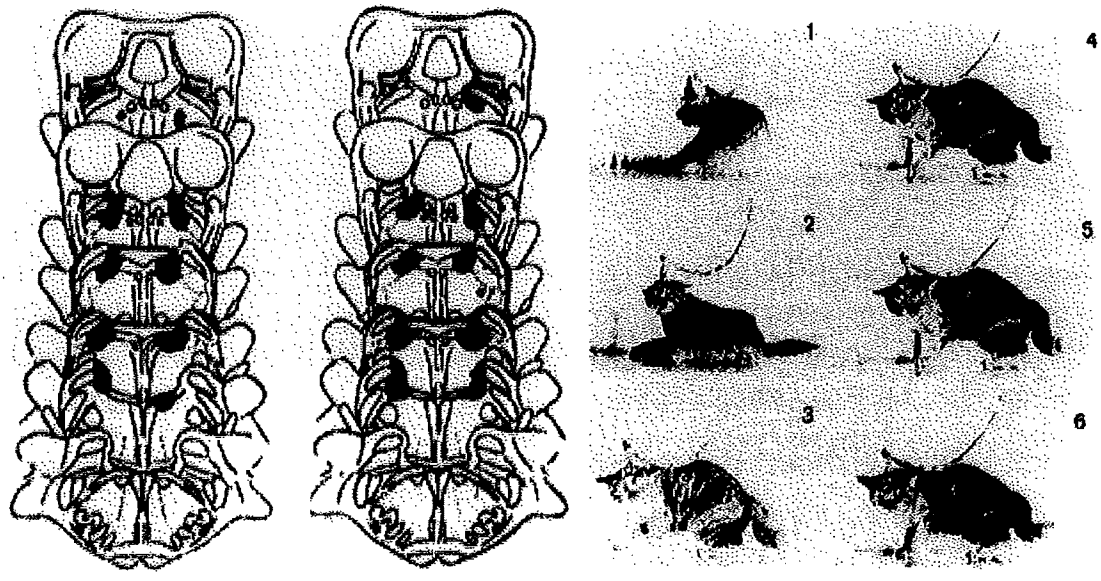

図3　脳幹破壊実験によるネコの夢幻様運動[4]
左図の脳幹部位を破壊したところ，右図のような夢幻様運動が出現している．

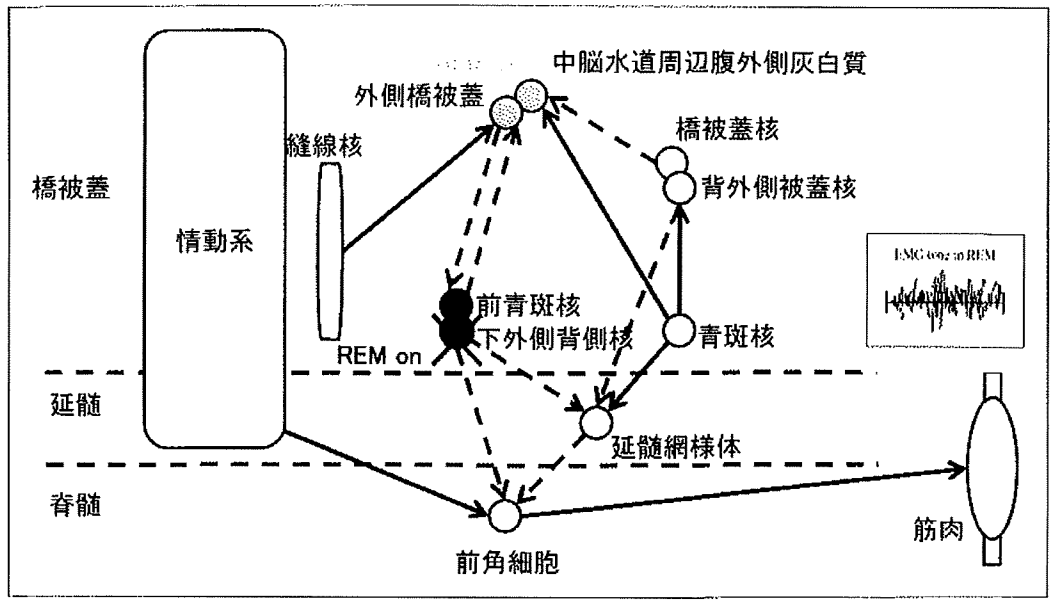

図4 REM睡眠行動障害の病態生理[5]

下外側背側核の病変＋情動系の入力＝REM睡眠行動障害．REM睡眠を促進するREM on（下外側背側核，前青斑核）とREM睡眠を抑制するREM off（中脳水道周辺腹外側灰白質，外側橋被蓋）が相互に干渉してREM睡眠の制御を行っている．REM睡眠時には，下外側背側核より直接・間接（延髄網様体を介して）的に脊髄前角細胞に抑制を行っているが，下外側背側核の障害により情動系からの出力への抑制が弱くなり，RWAの出現，夢内容の行動化が起こる．

し，間接路であるMCRFのみが障害されてもRWAは出現しない．このことからLC, SLDの障害がRBDの主病変と考えられている．

一方，ヒトにおいては頭部MRIの検討で中脳と橋被蓋部の病変が示唆されており，病理解剖より黒質（substantia nigra：SN），LC, PPN, LDTNの神経脱落を示唆する所見が示されている．ヒトにおけるネコのLC, ネズミのSLDに相当する部分が明らかでないが，Boeveらは，上記の結果からRBDの発症機序として図4のような仮説を提唱している[5]．

REM睡眠行動障害と神経疾患

RBDは脳幹のREM睡眠制御障害により出現するため，以前からシヌクレイノパチーのみでなく，脳血管障害，脳炎後遺症，ナルコレプシーとともに，タウパチーである進行性核上性麻痺（progressive supranuclear palsy：PSP），大脳皮質基底核変性症（corticobasal degeneration：CBD）での合併例が報告されていた．これらは続発性RBDと考えられていたが，特発性RBDよりパーキンソンニズムを呈する例が報告されて以来，特発性RBDへの注目が増している．

REM睡眠行動障害とシヌクレイノパチーの関連

RBDは，PD, DLB, MSA, PAFなどのシヌクレイノパチーとの合併が多く報告されている．Gangnonらは，PD患者33人のうち19人（58%）にPSG上REM睡眠中の20%以上RWAを認める症例を認め，そのうち11人がRBD症状を認めたと報告している[6]．Onofriらは，80人のPD患者を8年間フォローし，27人にRBD症状の出現を認めている[7]．またMSAにおいては，Tachibanaらが21人のMSA中18人にRBD関連症状があり，20人でRWAを認めたと報告している[8]．Plazziらは，39人のMSA中27人でRBDが認め，このうちRBD先行例が12人，MSA発症同時期にみられたものが7人，MSA発症後が8人であったと報告している[9]．さらにVetrungoらは，19人のMSAのうち3人でRBDがMSAに先行し，12人が病初期に出現し，全体で15人にRBDを認めたと報告している[10]．このような報告をまとめると，RBDはPDの33～60%，DLBの50～80%，MSAでの80～95%にみられたということになり，RBDとシヌクレイノパチーの合併が高頻度であることがわかる．

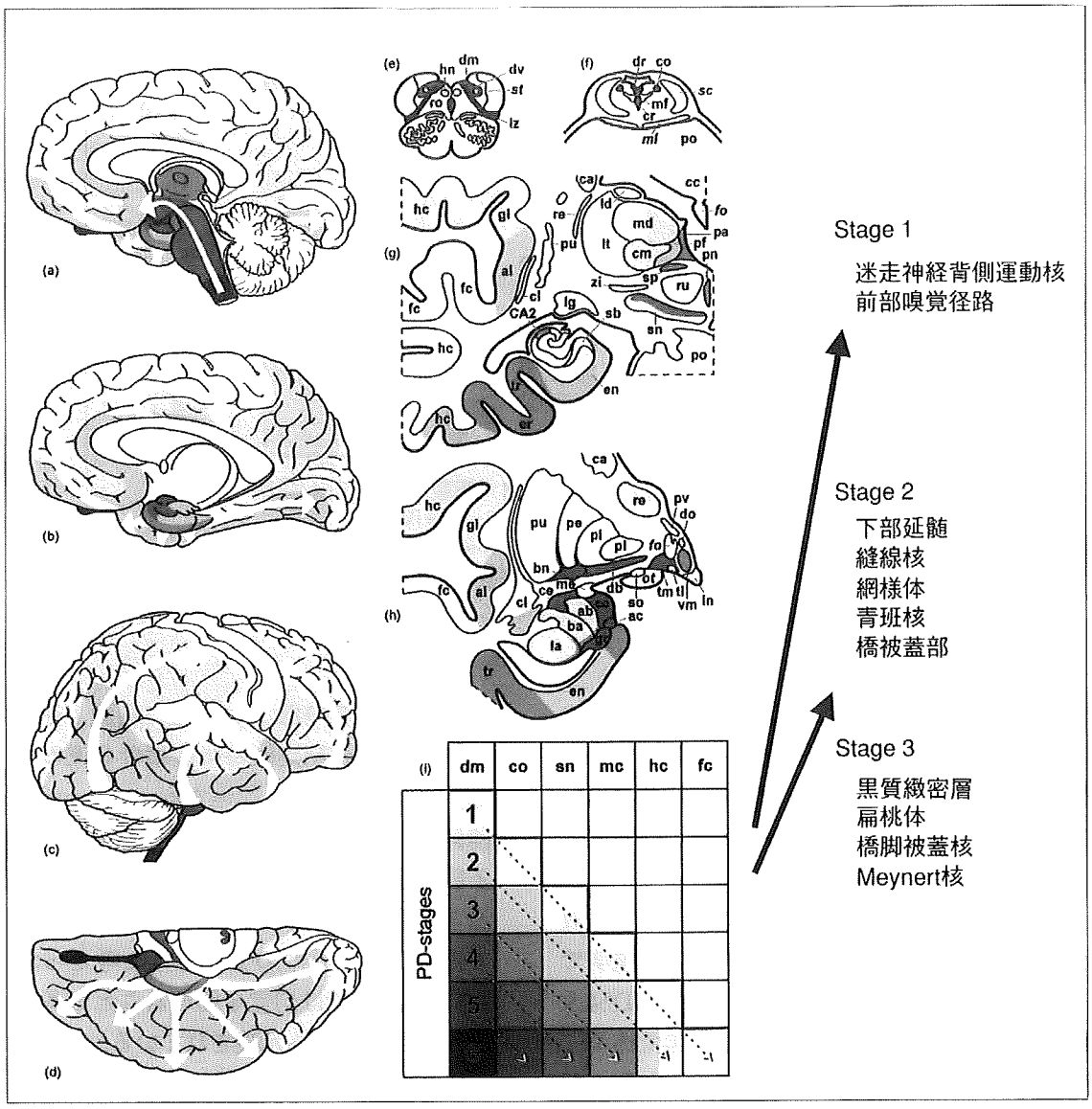

図5　Parkinson病の神経病理段階[16]

Braak仮説によれば，PD症状に出現する黒質緻密層に神経変性が出現するstage 3の前にRBDの責任病巣と考えられる青斑核などがstage 2で神経変性が出現することになる．このことはRBDのPDへの病前症状を支持している．

さらにSchenckらが，RBD患者29人のうち38%がRBD発症後平均12.7年でパーキンソニズムを発症したという報告[11]以来，RBDがPDの病前症状として関心を集めているが，彼らは追跡調査を行い65%の症例でパーキンソンニズムや認知症が出現していると報告している[12]．同様にOlsonらは，25人のRBD患者のうち3年間で13人(52%)がPD症状を呈したと報告している[13]．さらにIranzoらは，39人のRBD患者を平均11.5年フォローし20人(45%)が神経疾患を発症しており，そのうち9人がPD，6人がDLB，1人がMSA，4人が軽度認知機能障害(mild cognitive impairment)であったと報告し，RBDから神経変性疾患へのリスクとして視空間機能障害，自律神経障害，嗅覚機能障害の出現をあげている[14]．このように，高率にPDを始めとしたシヌクレイノパチーに進展する症例が報告されている．

またUchiyamaらは，特発性RBDの剖検例においてSN，LCにincidental Lewy小体が発見されたという症例報告をしているが[15]，これもRBDとPDの関連を示唆するものである．

最近，さらに両疾患の関連を示唆する仮説としてBraak仮説の存在が大きい[16](図5)．この説では，PDの運動症状はBraak stage 3でのSNの

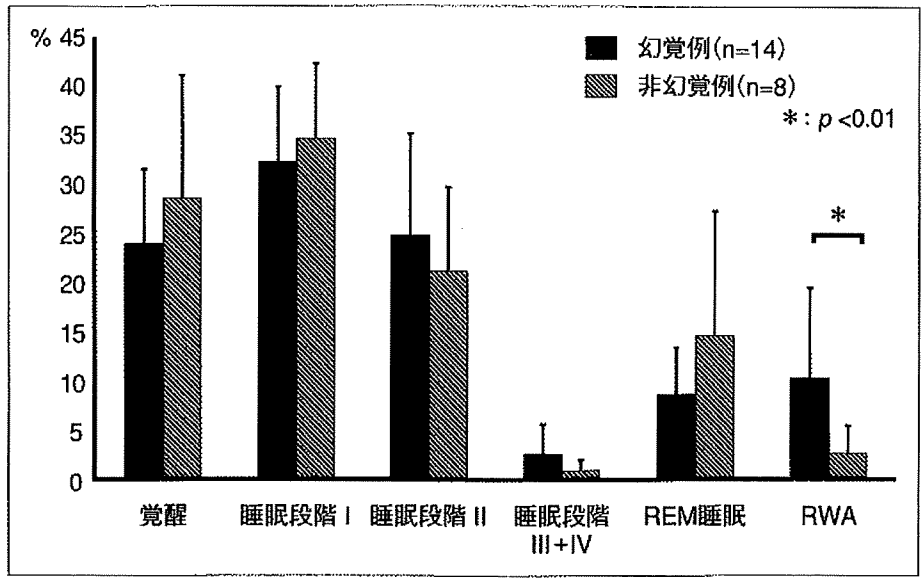

図6　PD患者の幻覚例，非幻覚例での終夜脳波所見の比較[24]
PD患者の終夜脳波の睡眠段階の割合である．幻覚例において有意に非幻覚例よりRWAの出現が多くなっている．

神経細胞脱落で出現する．RBDの病変が延髄のLC, MCRFなどであればBraak stage 2においてRBDが出現することは妥当であり，RBD出現はPDの発症前であることはBraak仮説においても支持されることになる．

さらに，RBDがPDの前駆症状であるという仮説は特発性RBDのPD患者との類似症状で説明できるかもしれない．Fantiniらは特発性RBD54人に嗅覚検査を施行し33人(61.1%)が異常であり，RBD患者ではPDと同様に嗅覚機能低下が認められたことを報告している[17]．さらにPostumaらは，特発性RBD患者においてUPDRSによる寡動症状の出現や歩行速度の低下，色識別の異常を示している[18]．Ferini-Strambiらは，RBDで視覚空間構成機能障害や視覚空間学習の低下を報告している[19]．またMazzaらは，RBD患者で橋，両側の被殻，右の海馬の血流増加，前頭葉，側頭－後頭葉の血流低下を報告し，早期のPD患者との共通性を指摘している[20]．Eisensehrらは，RBD患者では線条体のdopamine transporterが健常成人より消失しており，PD患者の病側と同程度であることを報告している[21]．日本においても，PDの鑑別診断として使用するMIBG心筋シンチでMiyamotoらは，特発性RBD患者がPDと同程度に心筋シンチの集積が低下していることを報告している[22]．Fantiniらは，RBD患者での覚醒時の後頭葉のβ波の低下と前頭，側頭，後頭葉のθ波の増加，REM睡眠時後頭葉のβ波の低下を示し，神経変性疾患と関連するsubclinicalな認知機能低下と関連する中枢神経機能障害の早期兆候と報告している[23]．これらの特発性RBDでのPD患者の症状との類似性は，RBDがPDの病前症状であることの一つの支持所見と考えられる．また，PD患者では幻覚を認めることが多いが，幻覚とRWAの関連も示唆されている．われわれは，幻覚を呈したPD症例に対してPSGを施行し，RWA出現時に夢内容の行動化を行い覚醒後も夢内容の言動を確認した．このことより14例の幻覚例と8例の非幻覚例にPSGで検討を行った結果，幻覚例にRWAを高率に認め，幻覚とRWAの関連を示唆する結果が得られた(図6)．さらに幻覚例にRBDの治療薬であるクロナゼパム内服を行ったところ8人中5人で幻覚が軽快し，RWAの量も減少した．また，日中の覚醒度を検査する反復睡眠潜時検査(multiple sleep latency test)を3例に施行し入眠早期にREM睡眠の出現するsleep onset REM periods(SOREMP)が高率に出現していた[24]．SOREMPはナルコレプシーで出現する特徴的な所見であり，これらのことより幻視とREM制御の障害の関連が示唆される．

RBDとDLBの関連としては，Boeveらは，RBD患者において視空間機能障害，構成失行，言語