in NFE2L1-dependent slow-twitch muscle atrophy, because of interference in the NFE2L1 system by oxidative stress. Furthermore, fast-twitch plantaris muscles atrophied same extent by denervation in the presence and the absence of PARK2-mediated mitophagy, express lower levels of PARK2 and NFE2L1 than slow-twitch muscles during denervation atrophy. Therefore, we speculate that tissues regulated by the NFE2L1 system express more PARK2 to eliminate damaged mitochondria than do other tissues. Our findings highlight the linkage between mitochondria autophagy and the UPS, 2 major intracellular protein degradation systems, and their different roles in slow-twitch skeletal muscle atrophy.

Materials and Methods

Antibodies and reagents

Anti-ATG7 antibodies were described previously.[14] Anti-PARK2 (Parkin, 4211), anti-PDHA1 (pyruvate dehydrogenase, 3205), anti-PSMD4 (Rpn10/S5a, 3846), anti-GAPDH (2118), anti-TRP53 (p53, 2524), anti-NFE2L1 (TCF11/Nrf1, 8052), anti-BCL2 (Bcl-2, 2870) and anti-BCL2L1 (Bcl-xL, 2764) antibodies were obtained from Cell Signaling Technology. Anti-PSMA5 (Proteasome 20S α5 subunit, BML-PW8125), anti-PSMB7 (Proteasome 20S β2 subunit, BML-PW9300) and anti-PSMC6 (Proteasome 19S Rpt4 subunit, BML-PW8830) were obtained from Enzo Life Sciences. Anti-OPA1 (612606) and anti-DNM1L (Drp1, 611112) were obtained from BD transduction laboratories. Anti-SQSTM1 (GP62-C) was obtained from Progen. Anti-MYH7(myosin heavy chain I, Clone NOQ7.5.4D, M8421) was obtained from Sigma-Aldrich. Anti-multi ubiquitin (Clone FK2, D058-3) was obtained from MBL. Anti-PPARGC1A (PGC-1, AB3242) was obtained from Millipore. MitoProfile Total OXPHOS Rodent WB Antibody Cocktail (MS604) was obtained from MitoSciences. Anti-TOMM20 (Tom20, sc-11415), anti-CYCS (Cytochrome *c*, sc-13156), anti-NFE2L1 (Nrf1, H-285, sc-13031, for immunostaining of HeLa cells), anti-NFE2L2 (Nrf2, H-300, sc-13032) and anti-LMNB (Lamin B, sc-6216) were obtained from Santa Cruz Biotechnology. Anti-DMD (Dystrophin, ab15277) and anti-MUL1 (ab84067) were obtained from Abcam. Anti-MFN1 (H00055669-M04) was obtained from Abnova. Anti-FIS1 (10956-1-AP) was obtained from Proteintech. Anti-8-OHdG (MOG-020P) was obtained from the Japan Institute for the Control of Aging, NIKKEN SEIL Co, Ltd. The Protein Carbonyls Western Blot Detection Kit was obtained from SHIMA Laboratories. Alexa 488- and Alexa 594-conjugated secondary antibodies (A11034, A11029, A11037, A11032) were obtained from Molecular Probes. The M.O.M. Immunodetection kit and Texas Red Avidin DCS were obtained from VECTOR Laboratories. Tunicamycin (T7765), tBHQ (112941), CCCP (C-2759), rotenone (R-8875), antimycin (A-8674) and N-acetyl-cysteine (A9165) were obtained from Sigma-Aldrich. MG-132 (474790) was obtained from CALBIOCHEM. Succinyl-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin (Suc-LLVY-MCA, 3120-v) and epoxomicin (4381-v) were obtained from Peptide Institute, Inc.

Animals

HSA-Cre-ER^{T2} transgenic mice were a gift from Dr Pierre Chambon. To produce *Atg7*$^{Flox/Flox}$: HSA-ER^{T2}-Cre mice, *Atg7*$^{Flox/Flox}$ mice were bred with HSA-Cre-ER^{T2} transgenic mice. To delete the floxed *Atg7* gene from skeletal muscle, Cre-ER^{T2} recombinase activity was induced in 4-wk-old mice by i.p. injections of 1 mg tamoxifen for 5 consecutive days. GFP-LC3 transgenic and *Park2* knockout mice have been previously described. All mice were maintained in an environmentally controlled room (lights on from 8:00 to 20:00) and were fed a pelleted laboratory diet and tap water ad libitum, unless otherwise stated. Denervation was performed at 4 wk after tamoxifen injections. To standardize autophagic activity in the skeletal muscles, mice were fasted for 24 h before euthanasia. Experimental protocols were approved by the Ethics Review Committee for Animal Experimentation of Juntendo University.

Histological analysis and electron microscopy

Cryosections, 10 μm thick, from mouse hind limbs were stained with hematoxylin and eosin (H&E), stained for succinate dehydrogenase (SDH) or cytochrome *c* oxidase (COX) activities, or immunolabeled with anti-PARK2, anti-TOMM20, anti-myosin heavy chain I (MYH7), anti-DMD and anti-8-OHdG antibodies. To quantify the SDH or COX activities of soleus muscles, Image J software was used. For EM analysis, soleus muscles were directly fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer on ice. Embedding, sectioning and microphotography were performed by the Hanaichi Electron Microscopic Laboratory, Inc.

Cell culture and siRNA transfection

C2C12 cells and HeLa cells were maintained in DMEM supplemented 10% fetal calf serum and antibiotics. For RNA interference experiments, ON-TARGETplus mouse *Nfe2l1* siRNA (Thermo Scientific Dharmacon, L-062252-01-0005) or nontargeting controls (Thermo Scientific Dharmacon, D-001810-01-05) were transfected into C2C12 cells using Lipofectamine RNAiMAX reagent according to the manufacturer's protocols (Invitrogen, 13778075).

Isolation of mitochondrial fractions and nuclear extracts

Mitochondrial fractions of soleus muscles were isolated using the Mitochondria Isolation Kit for Tissue (Pierce, 89801), and nuclear extracts of soleus muscles or C2C12 cells were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce, 78833) according to the manufacturer's protocols.

Western blotting

For tissue lysate preparation, mouse skeletal muscles were homogenized in 10 volumes of 50 mM TRIS-HCl (pH 7.4) containing 0.15 M NaCl, 1 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, a protease inhibitor cocktail (Roche Diagnostics, 11836170001), and a phosphatase inhibitor cocktail (Roche Diagnostics, 04906837001), using a motor-driven homogenizer (As One, S-203). For C2C12 cell lysate preparation, cells were lysed with the same buffer. The lysates were centrifuged at 12,000 × *g* for 10 min at 4 °C to remove debris. The supernatants, mitochondrial fractions, or nuclear extracts were analyzed by western blotting. Densitometric analysis was performed using ImageJ software.

Quantitative real-time PCR analysis

RNA was isolated using TRIzol reagent (Invitrogen, 15596026). cDNA was prepared using the Superscript III first strand synthesis kit (Invitrogen, 18080-044) according to the manufacturer's protocol. For mtDNA copy number quantification, genomic DNA was prepared. Quantitative real-time PCR was performed using the Fast SYBR Green Master Mix (Applied Biosystems, 4385612). The primers used for gene expression analysis are listed in **Table S1** and those used for mtDNA copy number analysis are listed in **Table S2.**

Measurement of proteasomal activity

Proteasome activities in soleus muscle extracts were measured using a fluorescent substrate, Suc-LLVY-MCA, as described previously.[46]

Statistics

All data are expressed as means ± s.d. Differences between groups were examined for statistical significance using one-way ANOVA, followed by Tukey-Kramer post hoc test or Student *t* test. A P value < 0.05 was considered statistically significant.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Materials

Supplemental materials may be found here:
www.landesbioscience.com/journals/autophagy/article/27785

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Brief communication

Clinicogenetic study of *GBA* mutations in patients with familial Parkinson's disease

Yuanzhe Li [a,b], Takeshi Sekine [b], Manabu Funayama [a,b], Lin Li [b], Hiroyo Yoshino [a], Kenya Nishioka [b], Hiroyuki Tomiyama [b,c], Nobutaka Hattori [a,b,c,*]

[a] *Research Institute for Diseases of Old Age, Juntendo University School of Medicine, Tokyo, Japan*
[b] *Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan*
[c] *Department of Neuroscience for Neurodegenerative Disorders, Juntendo University School of Medicine, Tokyo, Japan*

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ABSTRACT

The glucocerebrosidase gene (*GBA*) is a known risk factor of Parkinson's disease (PD). We sequenced entire coding exons and exon/intron boundaries of *GBA* in 147 Japanese familial PD (FPD) patients from 144 families and 100 unrelated control subjects. Twenty-seven of 144 (18.8%) of index patients were heterozygous for known Gaucher disease mutations, suggesting that *GBA* heterozygous mutations are strongly associated with FPD (odds ratio = 22.9, 95% confidence interval = 3.1–171.2). The frequency was significantly higher in autosomal dominant PD (ADPD) compared with autosomal recessive PD. According to clinical assessments, PD patients with *GBA* mutations exhibited typical manifestations of PD or dementia with Lewy bodies (DLB), such as L-dopa responsive parkinsonism with psychiatric problems and/or cognitive decline. Interestingly, they also presented with reduced myocardial ^{123}I-meta-iodobenzylguanidine uptake. Our findings suggest that heterozygous *GBA* mutations are strong risk factors in FPD, especially for autosomal dominant PD. Some patients with *GBA* heterozygous mutations develop clinical features of DLB. We speculate that *GBA* dysfunction may promote Lewy body formation, resulting in more severe PD or DLB phenotypes that are inherited in families.

1. Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative disorders. Patients develop disabled movement and complicating nonmotor symptoms, such as psychiatric disorders, cognitive dysfunction, olfactory nerve dysfunction, and sleep disorders (Weintraub and Burn, 2011). Cardinal features of PD are caused by marked loss of dopaminergic neurons in the substantia nigra, which is evidenced by the pathologic hallmark of Lewy bodies; however, PD is a more complicated and systemic disease. PD etiology was thought to be influenced mainly by the interactions between genetic and environmental factors. Nevertheless, recent developments in genetics have revealed that causative genes are involved in Mendelian-inherited parkinsonism (Hatano et al., 2009). Moreover, recent genome-wide association studies have also identified several common loci as genetic risk factors for PD (Hamza et al., 2010; Satake et al., 2009; Simon-Sanchez et al., 2009). During these studies and a subsequent meta-analysis, rare variants of the glucocerebrosidase gene (*GBA*; MIM#606463) have been found as risk factors of sporadic PD (SPD) (Aharon-Peretz et al., 2004; Sidransky et al., 2009). *GBA* is also known as the causative gene of Gaucher disease (GD), which is caused by a loss of function of hydrolytic enzyme activity and is inherited in an autosomal recessive pattern (Hruska et al., 2008; Tsuji et al., 1987).

Some genetic mutations in *GBA* were characterized as strong risk factors for SPD; however, there are few large studies of *GBA* mutations in familial PD (FPD) (Mitsui et al., 2009; Nishioka et al., 2010; Sidransky et al., 2009). Cosegregation was previously reported in a small number of families (Mitsui et al., 2009). One recent study emphasized an association between *GBA* mutations and cognitive impairment in PD (Alcalay et al., 2012).

In this study, we aimed to clarify the role of *GBA* mutations in PD, especially in FPD, by sequencing *GBA* in 147 FPD patients from 144 families and performing a comparative analysis of the clinical phenotype and severity to evaluate the association between *GBA* mutations and FPD.

2. Methods

2.1. Subjects

The study subjects comprised 147 FPD patients from 144 Japanese families and 100 Japanese controls (Table 1). The cohort of

* Corresponding author at: Department of Neurology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan. Tel.: +81 3 5802 1073; fax: +81 3 5800 0547.
E-mail address: nhattori@juntendo.ac.jp (N. Hattori).

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Table 1
Patient information

	n	Male/female	AAS (range)	AAO (range)
Patients	147	59/88	60.0 ± 12.8 (21–84)	50.8 ± 13.9 (13–81)
AD	85	35/50	57.7 ± 13.7 (21–84)	48.3 ± 14.2 (13–77)
AR	62	24/38	63.1 ± 10.8 (37–84)	54.3 ± 12.7 (25–81)
Controls	100	47/53	56.8 ± 16.3 (29–87)	

Key: AAO, age at onset; AAS, age at sampling; AD, autosomal dominant; AR, autosomal recessive.

Japanese patients consisted of 85 autosomal dominant PD (ADPD) and 59 autosomal recessive PD (ARPD). For the mode of inheritance to be considered autosomal dominant, there had to be affected family members in at least 2 consecutive generations; for autosomal recessive, we looked for affected siblings in the same generation. After detecting mutations, family members were analyzed to assess cosegregation. DNA samples were provided from various hospitals. Each patient submitted to a neurologic examination performed by expert neurologists and was given a diagnosis of PD on the basis of established criteria (Hughes et al., 1992). Diagnosis of dementia was given by the each clinician based on Mini Mental State Examination score (Folstein et al., 1975). All patients had good response to L-dopa. This study was approved by the ethics review committee of Juntendo University School of Medicine. All subjects provided informed and written consent prior to participation.

2.2. GBA *mutation analysis*

Genomic DNA was extracted from peripheral blood lymphocytes using standard protocols. Polymerase chain reaction was performed using previously reported primers to avoid amplifying the pseudogene (Mitsui et al., 2009). The purified polymerase chain reaction product obtained by ExoSAP IT (GE Healthcare, Salt Lake City, UT, USA) was subsequently used for dideoxy sequencing with BigDye Terminator Chemistry (Applied Biosystems, Foster City, CA, USA). The resulting products were loaded on ABI 310 or 3130 automated DNA sequence analyzers (Applied Biosystems) and analyzed with Sequencing Analysis Software v5.1 (Applied Biosystems). All exons and exon-intron boundaries were analyzed by direct sequencing.

To confirm Rec*Nci*I allele, we used TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA) for TA cloning and separating alleles. After separating each allele, direct sequencing was performed as described above.

2.3. *Clinical data analysis*

To clarify the clinical features of patients with *GBA* mutations, 19 items (Table 2) concerning prominent PD symptoms were statistically compared between *GBA* mutation–positive and *GBA* mutation–negative groups. Other affected members with *GBA* mutations were also included.

2.4. *Statistical analysis*

Statistical analysis included the *t* test, Fisher's exact test, odds ratio, and its confidence interval, using GraphPad Prism version 5.0d (GraphPad Prism Software). The Hardy-Weinberg equilibrium test was performed using SNPAlyze v5.1 software (Dynacom, Chiba, Japan). In all statistical analyses, p values ≤ 0.05 were considered statistically significant.

3. Results

3.1. GBA *mutations observed in FPD*

In this study, we only observed heterozygous mutations; no individual had mutations in both alleles. We detected 6 nonsynonymous mutations: p.I(-20)V, p.G64V, p.R120W, p.D409H, p.L444P, and p.I489V; 1 nonsense mutation: p.W393X; 1 synonymous mutation: p.K466K; 1 frame-shift mutation: c.1447-1466delTGins; and 1 recombinant allele (Rec*Nci*I) in the Japanese population (Table 3). Among them, 5 mutations, p.R120W, p.D409H, p.L444P, c.1447-1466delTGins, and Rec*Nci*I, have been reported as causative mutations in GD patients. Patients with *GBA* mutation did not have causative *PARK2*, *PINK1*, and common *LRRK2* mutations.

p.I(-20)V is an amino acid change in the signal peptide region and was considered to be a single nucleotide polymorphism. The frequency of p.I(-20)V in patients with FPD (13 of 144, 9.0%) was not significantly different from the control subjects (10 of 100 = 10.0%, $p = 0.83$), and its genotype distribution was in Hardy-Weinberg equilibrium in both populations. Although p.K466K mutation was not detected in any control subjects, this synonymous mutation was also excluded for later clinical analyses because it is in a less important region of the final protein.

Two mutations, p.G64V and p.W393X (Fig. 1), were novel, and p.I489V was previously reported in SPD patients (Mitsui et al., 2009). The p.G64V mutation was caused by the substitution of the first amino acid of exon 4, and it seems to be segregated with PD (Figs. 1 and 2). In the family with p.W393X, no affected family member was confirmed for the mutation because of absent genomic DNA. The sequences around the region of mutation were interspecifically conserved in both mutations (Fig. 1).

The recombinant allele Rec*Nci*I was found in 1 patient and 1 control. p.L444P is more common, and p.R120W is less common in Japanese patients with GD, although p.R120W was frequently seen in Japanese FPD patients in the present study (9 of 144 = 6.3%, Table 3). However, p.L444P was the most frequent mutation seen in FPD patients (12 of 144 = 8.3%, Table 3).

In total, we found 31 FPD patients with heterozygous mutations that were reported in GD (p.R120W, p.D409H, p.L444P, c.1447-1466delTGins, and RecNciI) or unreported in GD (p.G64V,

Table 2
Comparison of clinical symptoms between *GBA*-positive and *GBA*-negative patients

Symptom	*GBA* mut (+)	*GBA* mut (−)	*p* value[a]
N	34	113	
Age at onset (mean ± SD)	49.1 ± 11.7	51.3 ± 14.5	
Resting tremor (%)	18 (52.9)	77 (68.1)	0.151
Bradykinesia (%)	27 (79.4)	99 (87.6)	0.265
Rigidity (%)	29 (85.3)	99 (87.6)	0.772
Gait disturbance (%)	28 (82.4)	95 (84.1)	0.795
Postural instability (%)	20 (58.8)	72 (63.7)	0.687
Wearing off (%)	20 (58.8)	49 (43.4)	0.112
Asymmetry at onset (%)	25 (73.5)	75 (66.4)	0.531
Orthostatic hypotension (%)	5 (14.7)	21 (18.6)	0.798
Incontinence (%)	7 (20.6)	11 (9.7)	0.131
Urinary urgency (%)	10 (29.4)	20 (17.7)	0.150
L-dopa-induced dyskinesia (%)	10 (29.4)	37 (32.7)	0.834
Sleep benefit (%)	3 (8.8)	21 (18.6)	0.288
Dystonia at onset (%)	4 (11.8)	11 (9.7)	0.750
Hyperreflexia (%)	5 (14.7)	17 (15.0)	1.000
Hallucination (%)	14 (41.2)	20 (17.7)	0.009[c]
Delusion (%)	8 (23.5)	6 (5.3)	0.004[c]
Other psychosis (%)	12 (35.3)	11 (9.7)	0.0009[d]
Dementia (%)	12 (35.3)	18 (15.9)	0.027[b]
Gaze palsy (%)	3 (8.8)	2 (1.8)	0.081

[a] Fisher's exact test.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$.

Table 3
Frequency of each *GBA* mutation found in this study

Mutations	FPD (n = 144)	AD (n = 85)	AR (n = 59)	Control (n = 100)	*p* Value[a] (FPD vs. control)	OR (95% CI) (FPD vs. Control)
Reported in GD						
p.R120W (%)	9 (6.3)	9 (10.6)	0 (0)	0 (0)	0.01	NA
p.D409H (%)	4 (2.8)	4 (4.7)	0 (0)	0 (0)	0.14	NA
p.L444P (%)	12 (8.3)	7 (8.2)	5 (8.5)	0 (0)	0.002	NA
indel (%)	1 (0.7)	1 (1.2)	0 (0)	0 (0)	NA	NA
RecNciI (%)	1 (0.7)	1 (1.2)	0 (0)	1 (1)	NA	NA
Unreported in GD						
p.G64V (%)	1 (0.7)	0 (0)	1 (1.7)	0 (0)	NA	NA
p.W393X (%)	1 (0.7)	0 (0)	1 (1.7)	0 (0)	NA	NA
p.I489V (%)	2 (1.4)	1 (1.2)	1 (1.7)	0 (0)	0.51	NA
Total (%)	31 (21.5)	23 (27.1)	8 (13.6)	1 (1)	<0.0001	27.2 (3.6–202.7)

Key: AD, autosomal dominant; AR, autosomal recessive; CI, confidence interval; FPD, familial PD; GD, Gaucher disease; indel, c.1447-1466delTGins; NA, not applicable; OR, odds ratio.
[a] Fisher's exact test. Italics denote novel mutations found in this study.

p.W393X, and p.I489V; Table 3). Interestingly, the frequency of *GBA* mutations among FPD patients was significantly higher than in the controls (31 of 144 = 21.5% vs. 1 of 100 = 1.0%, $p < 0.0001$, odds ratio = 27.2, 95% confidence interval = 3.6–202.7; Table 3). This cohort included 23 ADPD and 8 ARPD of a total of 31 FPD patients. When individual mutations were analyzed, the frequency of the p.R120W and p.L444P carriers was significantly higher in index PD patients than in the control subjects ($p = 0.01$ and $p = 0.002$, respectively).

3.2. Mode of inheritance

We compared the differences between groups with AD and AR modes of inheritance and found that the frequency of mutations reported in GD was significantly higher in the AD group than in the AR group (22 of 85 = 25.9% vs. 5 of 59 = 8.5%, $p = 0.009$). Four known mutations reported in GD (p.R120W, p.D409H, c.1447-1466delTGins, and Rec*Nci*I) were all found in the AD group. In 1 AR family (p.L444P) with 2 brothers affected with PD/dementia with Lewy bodies (DLB), the parents had been recorded as asymptomatic, but they died at relatively young ages during the war. Thus, the possibility that this family also should be included in AD family with cosegregation remains (family 28; Fig. 2).

In 2 families with p.L444P and 1 family with p.G64V, both affected siblings had the same mutations, suggesting cosegregation (families 25, 28, and 31; Fig. 2). The present study indicates the incomplete penetrance of p.R120W (families 7 and 9; Fig. 2), which is consistent with previous reports suggesting cosegregation (Mitsui et al., 2009). p.I489V was not considered to show cosegregation (family 35; Fig. 2).

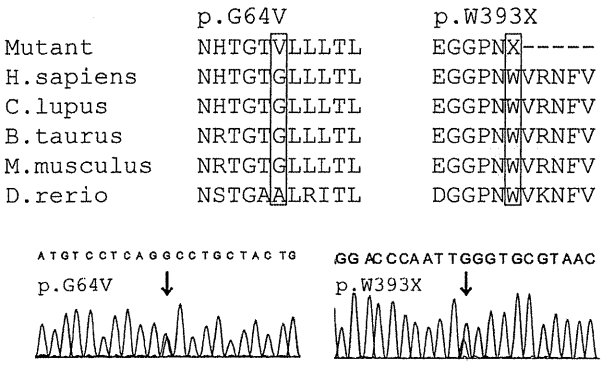

Fig. 1. Novel *GBA* mutations. Genomic sequence chromatogram and comparison of interspecific amino acids around the mutated site.

3.3. Clinical symptoms

In this study, we performed a comparative analysis between 2 groups: *GBA* mutation–positive (n = 34) and *GBA* mutation–negative patients (n = 113). Clinical and demographic data are listed in Table 2 and Supplementary Table 1. The mean age at onset (AAO) was not significantly different ($p = 0.437$). Statistical analysis of 19 symptoms in PD revealed significant differences in four indexes of hallucination, delirium, dementia, and other psychosis (Table 2). Notably, psychiatric symptoms and/or cognitive decline were more common in *GBA* mutation–positive patients (see Supplementary Table 2). Nine patients with *GBA* mutations underwent cardiac ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy (see Supplementary Table 3), and all patients showed marked reduction of myocardial MIBG uptake.

4. Discussion

Recently, the largest multicenter analysis of *GBA* mutations has proved an association between *GBA* mutations and PD (Sidransky et al., 2009). To disclose the role of *GBA* in FPD, we performed a *GBA* mutational analysis for in 147 FPD patients from 144 families. The frequency of *GBA* mutations was 21.5% and 25.9% in FPD and ADPD, respectively, which are higher values than reported previously, even compared with SPD patients (Lesage et al., 2011; Mitsui et al., 2009; Sidransky et al., 2009). Therefore, our data suggest that *GBA* mutations play an important role in not only SPD but also FPD, and especially in ADPD. This finding implies that *GBA* could play a major role in FPD and ADPD.

Rare *GBA* mutations have been reported to be a strong risk factor of PD, with a robust odds ratio of 28.0 (Mitsui et al., 2009). Thus, an analysis for *GBA* mutations in FPD was essential to clarify the role of each *GBA* mutation in PD patients. Indeed, p.L444P and p.G64V cosegregated with PD in some families. However, compared with a previous report (Mitsui et al., 2009). p.R120W did not cosegregate in this study. Regarding an association with PD, some mutations remained controversial because of the small number of patients with each mutation and restricted genetic testing in the families. Considering allele frequency, it might be reasonable that *GBA* mutations are incompletely inherited with PD. The carrier frequency of *GBA* mutations was extremely low among the normal Japanese population in this study and in a previous report (Mitsui et al., 2009), whereas many controls among the Jewish population have p.N370S, which is associated with type I GD (Aharon-Peretz et al., 2004), and many controls among the UK population have p.E326K, which is a risk factor for PD (Duran et al., 2013). These racial differences might be derived from the differences of effects of each mutation and other genetic risk factors, which have different

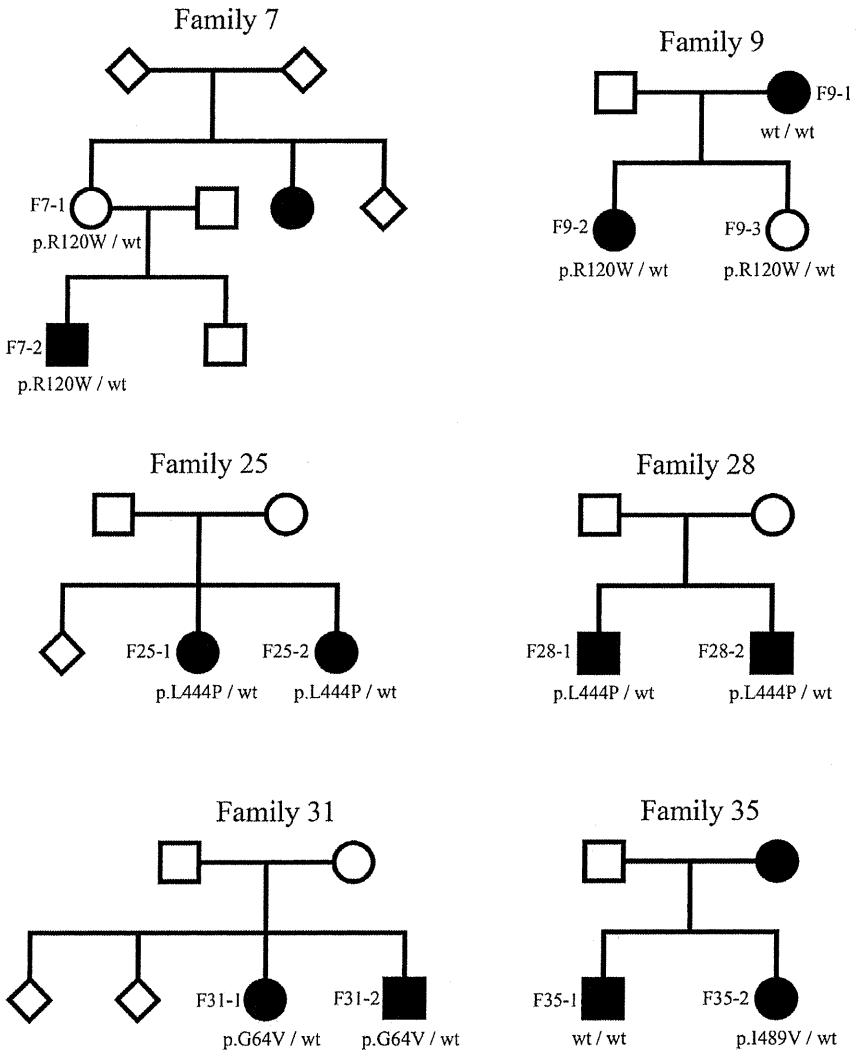

Fig. 2. Cosegregation study of *GBA*-positive families. Pedigrees of families with *GBA* mutations are shown. Affected Parkinson's disease patients are represented with a black symbol. The number of each family member represents his or her patient ID in Supplementary Table 1.

distributions due to founder effects (Lesage et al., 2011; Nishioka et al., 2010; Sidransky et al., 2009). Although racial or regional differences exist for each *GBA* mutation, our data emphasize that *GBA* mutations are more strongly associated with FPD rather than SPD.

A recent study reported a relatively high estimated penetrance ratio in *GBA* carriers, depending on age (7.6%, 13.7%, 21.4%, and 29.7% at 50, 60, 70, and 80 years, respectively). This result should lead to the consideration of *GBA* as a dominant causal gene with reduced penetrance (Anheim et al., 2012). Supporting this consideration, we detected heterozygous *GBA* mutations most frequently in ADPD patients. On the basis of our findings, we conclude that *GBA* is a strong and common risk factor but not a definite causal gene for ADPD and FPD.

In hereditary forms of PD, the frequency of heterozygous *GBA* mutations in ADPD (25.9%) was higher than that observed for ARPD (8.5%). Even allowing for classification bias by definition of ADPD and ARPD, the data imply that *GBA* mutations are strongly associated with ADPD, suggesting that heterozygous *GBA* mutations have a role in familial aggregation, especially in ADPD. In addition, heterozygous mutations have been identified among ARPD patients, suggesting the incomplete penetrance of forms even through the AR mode of inheritance.

Our PD patients with *GBA* mutations frequently developed psychiatric symptoms and/or cognitive decline. Our data support previous results in which PD patients with *GBA* mutations manifest exacerbated psychiatric symptoms and/or cognitive decline compared to those without *GBA* mutations (Alcalay et al., 2012; Sidransky et al., 2009; Winder-Rhodes et al., 2013). Some previous studies have reported the association of *GBA* mutations and DLB (Clark et al., 2009; Nalls et al., 2013; Tsuang et al., 2012). Our data further suggest that *GBA* heterozygous mutation carriers can develop clinical symptoms of PD and DLB. Accordingly, we found decreased cardiac MIBG uptake associated with *GBA* mutations. The heart-to-mediastinum ratio correlated with PD/DLB clinical severity; a decreasing ratio corresponded to an ascending Hoehn and Yahr stage (Nagayama et al., 2005). Thus, MIBG scintigraphy could be a useful biomarker for PD/Lewy body disease in patients with *GBA* mutations.

Lewy body diseases are alpha-synucleinopathies characterized by abnormal accumulation of alpha-synuclein in neuronal cytoplasm. Recently, biochemical analysis using cell and animal models demonstrated that some *GBA* mutations lead to increased alpha-

synuclein concentration (Cullen et al., 2011). *GBA* mutation may promote alpha-synuclein accumulation and Lewy body development via the aberrant lysosomal function, resulting in severe parkinsonism and cognitive decline associated with DLB (Mazzulli et al., 2011; Tsuang et al., 2012; Yap et al., 2013).

Collectively, our findings have major implications for the genetic and pathogenic mechanisms of *GBA*. GD is an autosomal recessive disorder caused by mutations in both *GBA* alleles, which leads to a loss of function or reduced enzyme activity. In contrast to GD, our FPD and ADPD patients carried 1 mutant allele and 1 wild-type *GBA* allele. Therefore, the pathogenic mechanism may be due to haploinsufficiency, dominant-negative effect, or toxic gain of function rather than a loss of function.

In conclusion, heterozygous *GBA* mutations play a greater role in FPD, especially in ADPD, and are likely to facilitate the development of PD and Lewy body diseases via different genetic and pathogenic mechanisms. Our findings suggest that further functional analyses for *GBA* should elucidate the pathogenesis of PD and Lewy body diseases.

Disclosure statement

The authors have no actual or potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2013.09.019.

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III 変性疾患

錐体外路系疾患
パーキンソニズムを主とする疾患
その他

Perry症候群

Perry syndrome

坪井義夫

Key words：パーキンソン病，常染色体優性遺伝，Perry症候群，中枢性換気障害，体重減少，うつ/無気力

1. 概念・定義

パーキンソン病(Parkinson's disease: PD)は振戦，無動，固縮および姿勢反射障害を主な症状とする神経変性疾患である．PD患者の多くは孤発性に発症するが，一部のPD患者は家族性に発症することが知られている．1975年，カナダのPerryらにより常染色体優性遺伝を呈した，ユニークな家族性PDが報告された．臨床的な特徴は，進行の速いパーキンソニズムにうつ，体重減少，睡眠困難と中枢性換気障害を伴う家系であり[1,2]，2005年，フランスのLechevalierらが，Perry and Purdy's syndromeと命名し，トルコのElibolらも論文中にPerry syndromeと表記し，現在は慣例的にPerry症候群と呼ばれる[3-5]．類似の症候群を示す家系は，カナダ(2家系目)，米国，英国，更に我が国でも2002年に初めて同症候群が報告された[6-9]．

Perry症候群の発症者は，うつあるいは無気力などの精神症状で発症することが多く，遅れてパーキンソニズムを呈するとほぼ同時期に1年に約10kg以上の体重減少を示す．パーキンソニズムはL-DOPA反応性で，ウエアリングオフやジスキネジアなどの運動合併症も比較的早期に認める．この疾患に最も特徴的な症状は，中枢性換気障害で，そのためCO_2ナルコーシスのエピソードを繰り返し，人工呼吸器管理が必要となる．これまでの報告例でもPerry症候群は例外なく，重度の呼吸障害が出現し，それが原因で呼吸不全あるいは突然死で亡くなることが多い．一部はうつで自殺する場合もみられる．このように精神症状，進行性パーキンソニズムと進行期の呼吸不全はこの症候群の共通した特徴といえる．

2. 疫　　学

Perry症候群は現在確認されている中でも，**表1**に示す17家系が報告され，世界中に分布しているが，我が国は5家系と比較的多く，30人近い発症者が確認されている．うち4家系は九州にみられるが，それぞれの変異が異なるため創始者効果ではない．これは家族性PDの1%にも満たないまれな疾患である．

3. 病　　因

2002年に報告された我が国初の家系報告をきっかけに，Perry症候群の原因遺伝子の探求を目的とした国際共同研究が始まった．既に報告された8家系から7家系の主治医との接触に成功し，ハワイと我が国から未発表の家系も加え，臨床経過，剖検脳組織およびDNA解析が行われた．その結果，2009年にPerry症候群の発症者に，第2染色体短腕上にあるダイナクチン遺伝子(*DCTN1*)内に変異を発見した[10]．検討した8家系のすべてで*DCTN1*エクソン2上の非常に近接した部位に5つの突然変異(p.G71A/E/R, p.T72Pとp.Q74P)がみられ，これらの変異は，コントロールおよび他の家族性パーキンソン患

Yoshio Tsuboi: Department of Neurology, Fukuoka University School of Medicine　福岡大学医学部 神経内科学教室

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表1 これまでに報告されたPerry症候群

報告者	報告年	国	*DCTN1*遺伝子変異	文 献
Perry	1975, 1990	カナダ	G71R(c.211G>A)	1,2
Purdy	1979	カナダ	不明	6
Roy	1988	米国	T72P(c.214A>C)	7
Lechevalier	1992	フランス	G71E(c.212G>A)	3
Bhatia	1993	英国	G71A(c.212G>C)	8
Elibol	2002	トルコ	G71R(c.211G>A)	5
Tsuboi	2002	日本(福岡)	G71A(c.212G>C)	9
Farrer	2009	米国(ハワイ)	G71A(c.212G>C)	10
Ohshima	2010	日本(福岡)	Q74P(c.221A>C)	15
Newsway	2010	英国	G71R(c.211G>A)	16
Aji	2013	英国	G67D(c.200G>A)	17

Purdyらの報告以外ダイナクチン1(*DCTN1*)遺伝子変異が確定し，論文化されたもののみ掲載．論文化されていない我が国の3家系(宮崎，福岡，北海道：学会報告あり)，未発表のColombia，米国Georgia，New Zealand(personal communication)は未掲載．

者ではみられず，Perry症候群の原因遺伝子であることが確認された．

*DCTN1*は，ダイナクチンタンパク複合体の主要なサブユニットp150 gluedをコードし，p150 gluedは二量体として存在し，ダイナクチン複合体が直接微小管結合する部位を構成する．ダイナクチンを含む複合多重結合物質は，微小管とp150 gluedサブユニットを介してダイニンと作用し，ER，Golgiなど細胞小器官の間における物質輸送および，逆行性軸索輸送などの細胞内物質輸送に関与する．p150 gluedはそのN末端部位に細胞骨格関連タンパクCAP-Gly領域を有し[11]，Perry症候群にみられた5つの*DCTN1*変異はいずれもCAP-Gly領域内に存在する．実験的に，Perry症候群変異体(p.G71R，p.Q74P)は，p150 gluedの微小管との結合能が低下している[10]．更に，*DCTN1* p.G71R，p.Q74P，p.G59Sの遺伝子導入細胞は，野生株と比較して，ダイナクチンタンパクの細胞内分布が異なっていた[12]．これらから変異タンパクがダイナクチン凝集(toxic gain of function)を引き起こす機序と，ダイナクチン/ダイニン機能異常(loss of function)との両方の病態が示唆された．

4. 病　　態

FUK-1家系は，3世代にわたり8人の発症者あるいはその疑いが確認されている．壮年期まで運動，精神発達に問題がなく，パーキンソニズムの平均発症年齢は48歳(範囲：35-61)と孤発性PD病に比べて若年で，平均罹病期間は5年(範囲：2-10)と経過が早い．また，非運動症状がパーキンソン症候群に相前後して出現する特徴がある．

発端者は発症時41歳の会社員で，会社の産業医から無気力の指摘を受けた．その後意欲低下で，仕事に支障が生じるようになった．また同時期より歩行などの動作が緩慢になり，43歳時に当院神経内科を受診した．

そのとき自覚症状はなく，他覚的には明らかに自発性の欠如と，動作の緩慢がみられた．表情も乏しく，小声で，四肢に軽度の筋固縮を認めた．認知機能は保たれていた．歩行はやや前屈で腕の振りが消失．軽度の姿勢時振戦がみられた．腱反射は四肢で活発でBabinski徴候は陰性，姿勢反射障害は認めず，感覚障害，運動失調も認めなかった．パーキンソニズムに対してL-DOPAの内服を開始し，動作緩慢は改善した．その後誘因なく体重が極端に落ち始めて，2カ月で約10 kg，その後1年で計15 kgの体重減少がみられた．

44歳時には動作緩慢の悪化や姿勢反射障害が出現し，日常生活に一部介助が必要になった．

また不安，夜間の息切れが出現し精神科病院に入院．抗うつ薬の開始，L-DOPA内服を中止した後は混迷，姿勢時振戦，無動の悪化がみられた．この症状はL-DOPA再開でやや改善した．その後夜間の呼吸苦，頻呼吸などの症状が出現した．当院における再評価では体幹を含めた左右差のない筋固縮と無動，姿勢反射障害が顕著に認められ，パーキンソニズムの悪化が著明で，ウエアリング現象やジスキネジアもみられた．胸部X-Pや胸部CTで異常がなく，肺機能検査でも正常．睡眠ポリグラフではapnea indexが6.96と軽度の異常があり，中枢性の低換気が認められた．動脈血液ガス分析でPCO_2が47 mmHg(正常：35-45 mmHg)で高値を示し，PO_2は85.2 mmHg(正常：90-105 mmHg)であった．

その後転院先の病院にて，朝に昏睡状態で発見され，そのときの動脈血液ガス分析ではPCO_2が95 mmHgで，呼吸不全によるCO_2ナルコーシスの状態であり，当院に再入院した．気管内挿管，人工呼吸器管理にて意識は清明になり，その後人工呼吸器からの離脱も可能であったが呼吸不全を繰り返し，最終的には終日人工呼吸器管理が必要になった．46歳時に肺炎をきっかけに全身状態が悪化し，敗血症のため永眠した．

病理学的検討では黒質と青斑核の神経細胞脱落が強く，明らかな封入体を認めなかった．これまでの報告でも同様で，Lewy小体の記載はみられないか，あるいはごく少数との報告がほとんどであった[1-7]．著者らはこれまで行われなかったαシヌクレインの免疫組織染色を行ったが，黒質あるいはその他の部位でもLewy小体などの陽性封入体は確認できなかった[9]．一方，Widerらは著者らの症例も含め8例のPerry症候群の神経病理を検討し，共通して黒質に強い変性がみられたが，更に残存神経細胞にtrans-active-response(TAR)DNA-binding protein of 43 kDa(TDP-43)陽性の封入体がみられることを報告した[13]．この封入体は黒質，淡蒼球など基底核および脳幹部にみられ，核内(neuronal intranuclear inclusions: NII)，細胞質(neuronal cytoplasmic inclusions: NCI)，神経突起，およびグリア細胞質(GCI)も認められた．これら封入体はユビキチン抗体で陽性で，タウおよびαシヌクレインの免疫組織では染まらなかった．

Perry症候群は新しいTDP-43-proteinopathyの一つと考えられるが，封入体の出現する部位は黒質，淡蒼球，脳幹部が主で，大脳皮質，海馬あるいは運動ニューロンには認められない．このような特異は分布を呈した病理所見を示すTDP-43-proteinopathyはほかになく，病理学的にも独立した疾患であることが示された．

Perry症候群の病理所見で必ずみられる強い黒質の神経細胞脱落は，臨床上進行性のパーキンソニズムの原因病理として説明される．一方で無気力，うつ，体重減少と中枢性換気障害の病理学的背景に関しての検討では，剖検脳の延髄腹外側の呼吸中枢に注目し，neurokinin-1 receptors, tyrosine hydroxylase, およびtryptophan hydroxylaseの免疫染色が陽性となる神経細胞の密度がコントロール脳に比べて有意に減少していることを示した[14]．それ以外にも呼吸に関連する中枢である縫線核(セロトニン作動性ニューロン)の細胞減少も認めた．その他，青斑核や腹側被蓋野のアミン作動性ニューロンの減少もみられ，うつや無気力に関連する可能性がある．体重減少を説明できる特定の病理学的異常(例えば視床下部)は今後の課題である．

5. 診断と鑑別診断

Perry症候群は病初期には孤発性PDと区別がつかないことが多いが，発症2年以内に強い体重減少と呼吸障害がみられることから，この疾患が疑われる．呼吸障害は頻呼吸，睡眠中の不規則呼吸，呼吸停止など様々であり，呼吸苦は訴えない．睡眠ポリグラフでは不規則呼吸と，時に中枢性の呼吸停止がみられ，診断の根拠となるので重要な所見である．頭部MRIでは正常例と前頭葉に軽度萎縮がみられる例が報告されている．脳血流SPECTでは前頭葉血流の低下が特徴である(**図1**)．また家族歴が重要で，通常常染色体優性遺伝を呈する．しかし，必ずしもPDとは診断されず，突然死，自殺などの家族歴に遭遇する場合もある．診断は原因遺伝子変異の同定をもってなされる．

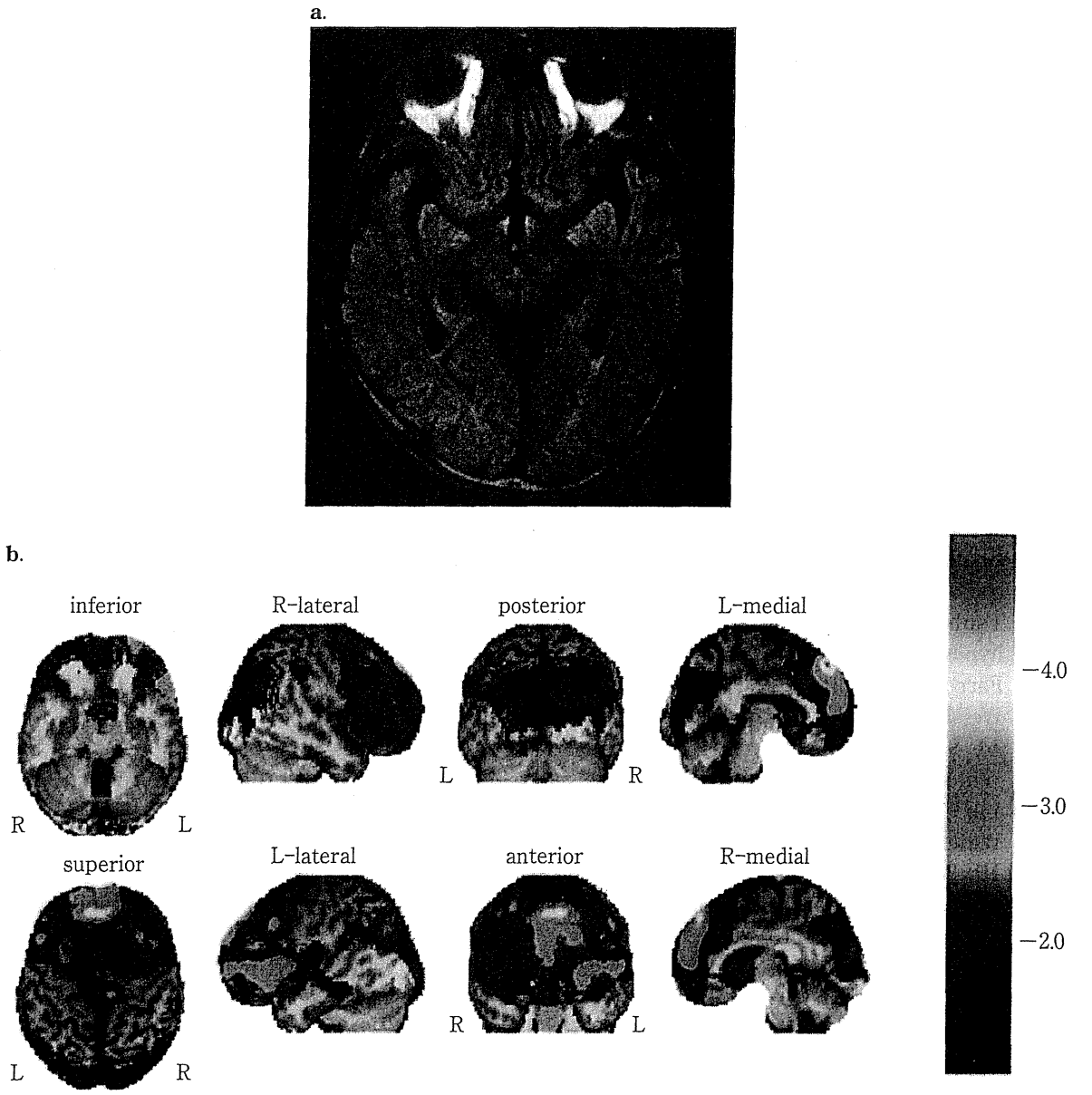

図1 Perry症候群の画像所見

a. Perry症候群の頭部MRI所見(FLAIR画像):軽度の前頭側頭葉萎縮がみられる.

b. Perry症候群の脳血流SPECT所見(easy Z-score imaging system: eZIS解析画像):前頭葉,後頭葉に脳血流の低下がみられる.

6. 治療と予後

病初期のパーキンソニズムに関して,L-DOPAの反応性は良いが,進行も早く日常生活動作の低下は避けられない.ドパミンアゴニストも効果を示すが,一部に衝動制御障害が出現することがあり注意を要する.L-DOPA関連のウエアリングオフ,ジスキネジアの発現も多い.抗精神病薬に対する副作用が強く,うつの治療中にパーキンソニズムの悪化が提示の症例のように,時にみられる.非運動症状として,一番重要なのは呼吸不全に対する治療であり,これまでの経験では持続陽圧呼吸療法(CPAP)の効果は一時的で,気管切開,人工呼吸器の使用が必要となる.予防的に横隔神経刺激療法が試みられている.平均予後は3-5年で肺炎,敗血症などの合併症によるものが一番多いが,突然死,自殺もみられる.

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DCTN1/Perry症候群

DCTN1/Perry syndrome

坪井義夫(写真)　三嶋崇靖
Yoshio Tsuboi and Takayasu Mishima
福岡大学医学部神経内科学教室

◎Perry症候群は常染色体優性遺伝型の家族性パーキンソン病で，急速に進行するパーキンソニズム，うつ，体重減少，睡眠困難と中枢性換気障害を特徴とする．これまで世界で16家系，うちわが国では5家系(未報告も含む)確認されているたいへんまれな疾患であるが，2009年にPerry症候群の原因が2番染色体短腕上にあるダイナクチン遺伝子(DCTN1)内の点変異であったことが報告されて以降，注目を集めている．DCTN1内で別の点変異が家族性運動ニューロン疾患(HMN7B)の原因となっており，ひとつの遺伝子の非常に近い部位に存在する点変異が運動ニューロン疾患とパーキンソン病という異なる変性疾患を引き起こすことが判明した．DCTN1はダイナクチン複合体のp150gluedをコードし，この蛋白はダイナクチン複合体が直接微小管に結合する部位である．同遺伝子の機能分析から複数の神経変性疾患にまたがる病態が解明される可能性がある．

Key word：Perry症候群，家族性パーキンソン病，ダイナクチン1遺伝子，TDP-43蓄積病

Perry症候群とダイナクチン

“Perry症候群”は1975年，カナダのPerryにより報告された，常染色体優性遺伝を呈し，急速に進行するパーキンソニズム，うつ，体重減少，睡眠困難と中枢性換気障害を特徴とした症候群である[1,2]．その後，同症候群がカナダ，アメリカ，イギリス，フランス，トルコの家系から報告され[3-7]，2002年にはわが国からFUK-1家系が報告された[8](図1)．FUK-1家系の発端者は41歳時に無気力で発症し，パーキンソン症候群を呈するとほぼ同時期に1年に約15 kgの体重減少を認めた．その後中枢性換気障害でCO_2ナルコーシスのエピソードを繰り返し，人工呼吸器管理を必要とした．この症例のようにPerry症候群は例外なく，重度の呼吸障害が出現し，それが原因で呼吸不全あるいは突然死で亡くなることが多い．このように進行性のパーキンソニズムと進行期の呼吸不全はこの症候群の共通した特徴といえる．

2009年に著者らを含めた国際共同研究で，Perry症候群の発症者に2番染色体短腕上にあるダイナクチン遺伝子(*DCTN1*)内に変異を発見した[9](図2)．検討した8つの家族のすべてに変異がみられ，*DCTN1*のエクソン2上で，非常に近接した部位に5つの突然変異(p.G71A/E/R，p.T72Pとp.Q74P)が認められた．これらの変異はコントロールおよび他の家族性パーキンソン症候群患者では認められず，原因遺伝子であることが確認された．

Perry症候群の病理所見
——新しいTDP-43-proteinopathy

Perry症候群の病理学的報告は乏しく，FUK-1家系の病理において詳細に検討された．変性の強い黒質をはじめ他の部位でもLewy小体はみられず，これはαシヌクレイン抗体による免疫染色でも確認した[8]．Widerらは，著者らの症例を含めた8例のPerry症候群の神経病理を検討し，黒質等病変の強い部位の残存神経細胞にtransactive-response DNA-binding protein of 43kDa(TDP-43)陽性の封入体がみられることを報告した[10]．

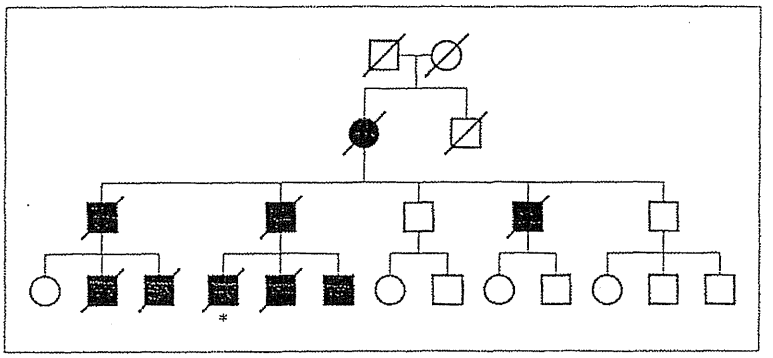

図 1　Perry症候群FUK-1の家系図
□は男性，○は女性，斜線は亡くなった家族で黒色が発症者．＊は発端者．

家系		トルコ	カナダ	フランス	英国	米国ハワイ	日本FUK-1	米国	日本FUK-4
Ex 2	Q74P(c.221A>C)	A	A	A	A	A	A	A	C
Ex 2	T72P(c.214A>C)	A	A	A	A	A	A	C	A
Ex 2	G71A(c.212G>C)	G	G	G	C	C	C	G	G
Ex 2	G71E(c.212G>A)	G	G	A	G	G	G	G	G
Ex 2	G71R(c.211G>A)	A	A	G	G	G	G	G	G

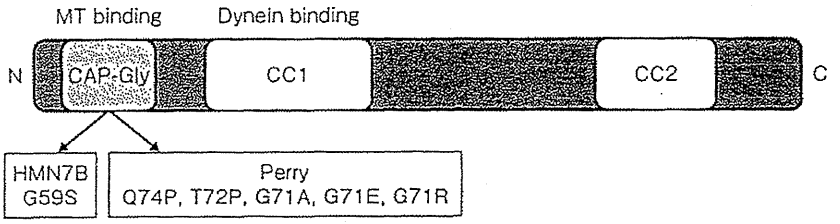

図 2　Perry症候群にみられたDCTN1エクソン2の遺伝子変異（上）とp150glued分子とCAP-Gly領域の位置（下）

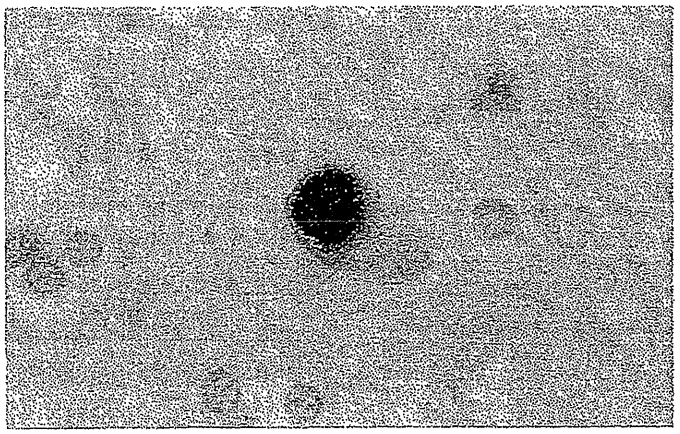

図 3　Perry症候群の黒質にみられたTDP-43免疫組織陽性細胞
茶色に染色されているのが細胞質内封入体．

この封入体の分布は黒質，淡蒼球など基底核および脳幹部にみられ，核内(intranuclear inclusions：NII)，細胞質(cytoplasmic inclusions：NCI)，神経突起，およびグリア細胞質(GCI)も混

在して認められた(図3)．これら封入体はユビキチン抗体陽性で，タウおよびαシヌクレインの免疫組織では染まらなかった．

TDP-43陽性封入体はユビキチン陽性の前頭側頭葉変性(FTLD-U)，前頭側頭型認知症-運動ニューロン疾患(FTD-MND)および筋萎縮性側索硬化症(ALS)において知られている[11,12]．Perry症候群は新しいTDP-43-proteinopathyのひとつと考えられるが，封入体がみられる部位は黒質，淡蒼球，脳幹部が主で，大脳皮質，海馬あるいは運動ニューロンには認められない．このような特異な分布を呈した病理所見を示すTDP-43-proteinopathyは他に存在せず，病理的にも独立した疾患であることが示された．

TDP-43陽性封入体は，FTLD-U，FTD-MNDあるいはALSに特異的な所見と考えられていたが，Perry症候群をはじめ，Lewy小体病，グアムALS/パーキンソン認知症，Alzheimer病や海馬硬化[10,13]においてもTDP-43封入体が出現することが知られてきた．これらから多くの変性疾患における共通の細胞反応と考えられ，その病的意義がより注目されている．さらに最近，TDP-43の直接的な病態への関与を示す証拠として，TDP-43遺伝子(TARDBP)の変異が家族性ALSの原因であることが示された[14]．いまではPerry症候群，FTLD-U，FTD-MNDとALSではTDP-43蓄積という共通の病態がかかわっている可能性がある．αシヌクレインあるいはタウ陽性封入体を有する疾患とは異なる細胞反応と考えられるが，Perry症候群の臨床症状は孤発性パーキンソン病とオーバーラップする点はたいへん興味深い．

*DCTN1*遺伝子と2つの疾患：家族性運動ニューロン疾患(HMN7B)とPerry症候群

Perry症候群の遺伝子変異発見以前に，家族性運動ニューロン疾患(HMN7B)の家系から*DCTN1*の点変異(p.G59S)が報告されていた[15,16]．その後さらに別の変異との関連が疑われる家族性ALSとFTLDの家系ら報告されている[17,18]．*DCTN1*はダイナクチン蛋白質複合体の主要なサブユニットp150gluedをコードする[19]．二量体として存在して，p150gluedはダイナクチン複合体が直接微小管結合する部位となる．ダイナクチンは小胞体，Golgi装置など細胞小器官の間における物質輸送にかかわることがわかっているが，ダイナクチンを含む複合多重結合物質は微小管とp150gluedサブユニットを介してダイニンと作用し，さらに他のサブユニットとともに細胞内物質輸送に関与する．p150gluedはそのN末端部位に細胞骨格関連蛋白Cytoskeleton-associated protein-glycine-rich(CAP-Gly)領域が存在する[20]．興味深いことに，Perry症候群にみられた5つの変異も家族性運動ニューロン疾患(HMN7B)でみられたp.G59S変異もともに，CAP-Gly領域内に存在する．実験的に，HMN7B変異体p.G59SとPerry症候群変異体(p.G71Rとp.Q74P)はp150gluedの微小管との結合能が低下していることが示され，*DCTN1* p.G71R，p.Q74P，p.G59Sの遺伝子導入細胞は野生株と比較してダイナクチン蛋白の細胞内分布が異なっていた[9]．

軸索の物質輸送は，キネシンがかかわる順行性輸送とダイニンがかかわる逆行性輸送がある．逆行性輸送は変性蛋白を細胞質に輸送し分解する役割と，シナプスからのシグナルを細胞質に伝え，遺伝子発現の調節にかかわるとされている．逆行性輸送機能はダイニン単独ではなく，ダイナクチンと共同で行われる．ダイナクチンは輸送する物質とダイニンを接合する役割をもち，さらに，その最大のサブユニットであるp150gluedは微小管と結合する部位である．

ダイナクチンの機能とPerry症候群にみられる変異

ダイナクチンは神経細胞内の物質輸送にかかわり，ダイナクチン複合蛋白のなかで最大のポリペプチドがp150gluedで，微小管およびダイニン蛋白に直接接着する．p150gluedは，CAP-Glyドメインを含み，この機能が失われると逆行性の軸索輸送が障害される[21]．p150gluedのG59S変異体を遺伝子導入したマウスでは，運動神経の進行性変性が観察され，家族性運動ニューロン疾患(HMN7B)のモデルとして報告された[22]．この場合，その蛋白は微小管結合能が低下し，遺伝子導入細胞では

細胞質に封入体も形成される．遺伝子導入細胞で変異蛋白がダイナクチン複合体の機能異常を呈し細胞障害を呈するのか，あるいは封入体形成が細胞毒性を有するのかの病態はまだ不明である．

Perry 症候群の遺伝子変異は HMN7B と同じく p150glued の CAP-Gly 領域内に存在し，遺伝子導入細胞において HMN7B 変異と同じように，細胞内に凝集体を形成し，さらに微小管結合能も同様に低下することが知られている．

CAP-Gly はこれまで微小管の上を滑る，あるいはブレーキをかける機能が考えられていた．p150glued をノックアウトしたマウスでは逆行性軸索輸送が障害されるが，そこに野生型 p150glued を加えるとその機能が回復する．つぎに CAP-Gly を欠いた p150glued でも同様に機能回復することから，物質輸送中は微小管上の滑動には関与していない可能性がある[21]．p150glued は神経突起の先端でダイニンを引き連れて，逆行性の軸索輸送を開始する役割を果たしているといわれている．近い遺伝子変異が 2 つの異なる疾患を引き起こすメカニズムはまだ不明であるが，遺伝子導入細胞に形成される蛋白凝集は HMN7B 変異のほうが強い．また，生化学的実験では HMN7B 変異ではダイナクチン複合蛋白がダイニンに結合することを阻害するのに対し，Perry 変異はダイニン結合を阻害はしないことから Perry 変異は逆行性輸送の開始を選択的に阻害すると考えられる[23]．

もうひとつの機能として，小胞体や Golgi 装置間の小胞輸送にダイニンが関与しており，ダイニンの機能異常でオートファジーによる変性蛋白分解系が障害されることが知られている．

オートファジーは細胞機能のひとつであり，多くの生理作用や病態に関与する．オートファジーの役割は飢餓などで誘導されて細胞内の蛋白などを分解し，アミノ酸の産生，再利用することである．オルガネラや細胞質の一部を隔離して二重膜からなるオートファゴゾームがリソソームに融合し，内容物がリソソーム酵素により蛋白分解される．オートファジー関連蛋白の欠損で神経変性疾患が誘導され，変異体ダイニンの発現によりダイニン機能に異常が生じるとオートファゴゾーム・リソソーム融合が障害され，神経変性が起こることから，ダイナクチンダイニン複合体がオートファジーシステムの障害をきたす機序が推測される[11]．HMN7B 変異導入マウスでは運動神経内にオートファゴゾームなどの小胞が増加しており，この仮説を裏付ける．

おわりに

Perry 症候群と HMN7B は同じ遺伝子変異で異なる神経変性疾患を呈することで，DCTN1 の分子機能の解明が神経変性における変性機序と障害神経の選択性という 2 つの課題に示唆を与える．現時点では両者の遺伝子変異がもたらす病態の違いは明らかではないが，DCTN1/Perry 症候群の研究から得られる知見は，同時に他の孤発性あるいは家族性神経変性疾患（パーキンソン病，多系統萎縮症，FTLD-U あるいは ALS）の病態の解明に寄与する可能性があると思われる．

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BRIEF REPORT

A Novel *DCTN1* Mutation With Late-Onset Parkinsonism and Frontotemporal Atrophy

Eiichi Araki, MD,[1] Yoshio Tsuboi, MD,[2*] Justus Daechsel, PhD,[3] Austen Milnerwood, PhD,[4] Carles Vilarino-Guell, PhD,[4] Naoki Fujii, MD,[1] Takayasu Mishima, MD,[2] Takayuki Oka, MD,[5] Hideo Hara, MD,[5] Jiro Fukae, MD[2] and Matthew J. Farrer, PhD[4]

[1]Department of Neurology, National Omuta Hospital, Fukuoka, Japan [2]Department of Neurology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan [3]Department of Cancer Cell Biology, Mayo Clinic, Jacksonville, Florida, USA [4]Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada [5]Division of Neurology, Department of Internal Medicine, Saga University Faculty of Medicine, Saga, Japan

ABSTRACT

Background: Depression, parkinsonism, and hypoventilation (Perry syndrome) or familial motor neuron disease have been linked to mutations in dynactin P150^{Glued} (*DCTN1*).

Methods: We employed genealogic, clinical, neurologic, and MRI investigations, as well as analysis of genes implicated in parkinsonism. Cellular transfection, immunocytochemistry, and immunoprecipitation analysis of wild-type (WT) and mutant *DCTN1* were also performed.

Results: A novel heterozygous mutation, *DCTN1* c.156T>G, encoding p.Phe52Leu, segregates with parkinsonism in a Japanese family. The substitution was not observed in affected probands with familial parkinsonism or control subjects and is evolutionarily conserved. In contrast to Perry syndrome, affected carriers have late-onset disease and slower progression, with frontotemporal atrophy revealed by MRI. *In vitro* studies suggest the mutant protein has impaired microtubule binding, compared to WT dynactin p150^{Glued}.

Conclusions: *DCTN1* mutations may contribute to disparate neurodegenerative diagnoses, including familial motor neuron disease, parkinsonism, and frontotemporal atrophy, and further studies of dynactin-mediated cargo transport may prove insightful. © 2014 International Parkinson and Movement Disorder Society

Key Words: Parkinson's disease; genetics; parkinsonism; Perry syndrome

Missense mutations in the p150^{Glued} subunit of dynactin (*DCTN1*) have been linked to Perry syndrome associated with depression, rapidly progressive parkinsonism, weight loss, sleep difficulties and central hypoventilation.[1] Pathogenic p150^{Glued} mutations, including p.Gly71Arg/Ala/Glu, p.Thr72Pro, and p.Gln74Pro, have been described within, or adjacent to, the highly conserved *N*-terminal cytoskeleton-associated protein, glycine-rich (CAP-Gly) domain.[2] In families with Perry syndrome, mean onset is 48 years (range, 35-61), with a 5-year (range, 2-10) duration to death attributed to respiratory failure or suicide.[3] Brain autopsy reveals a pallidonigral TDP-43 proteinopathy affecting the ventrolateral medulla respiratory center but sparing the cortex, hippocampus, and motor neurons.[2,4,5] Decreased metaiodobenzylguanidine (MIBG) cardiac scintigraphy is also observed suggesting that postganglionic sympathetic nerve degeneration is not specific to Lewy body diseases.[6] Hereditary motor neuropathy with a distinct vocal fold paresis (laryngeal dysfunction) was previously linked to another p150^{Glued} mutation, p.Gly59Ser[7,8] with distal spinal and bulbar muscular atrophy and a mean onset of 34 years (range, 23-39) and 17-year duration (range, 7-31). Herein, we describe a Japanese family with later-onset autosomal dominant parkinsonism, hypoventilation and frontotemporal atrophy with a novel heterozygous mutation in *DCTN1*.

Patients and Methods

Clinical Studies

The Fukuoka University (Fukuoka, Japan) and National Omuta Hospital (Fukuoka, Japan) Research Ethics Committee approved this protocol. Family members >18 years gave written informed consent. Detailed medical histories were obtained for patients, nearest relatives or both; when possible, neurological and neuropsychiatric examinations were performed.

*Correspondence to: Dr. Yoshio Tsuboi, Department of Neurology, Faculty of Medicine, Fukuoka University, 45-1, 7-chome Nanakuma, Jonan-Ku, Fukuoka 814-0180, Japan; tsuboi@cis.fukuoka-u.ac.jp

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