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Case report

Visual impairment in Parkinson's disease treated with amantadine: Case report and review of the literature

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Abstract

A 61-year-old man with Parkinson's disease (PD) developed sudden-onset visual impairment after initiation of amantadine treatment. Ophthalmologic examination revealed corneal endothelial edema. Discontinuation of amantadine resulted in rapid improvement of visual acuity. A review of the literature indicated only a few reports of amantadine-associated corneal dysfunction in patients with neurological disorders as well as influenza syndrome, but none with PD. Amantadine-associated visual impairment in PD could be possibly overlooked, since PD mainly affects elderly people who often develop aging-related ocular changes. The present report alerts neurologists and physicians in general to the peculiar ophthalmologic side effect of amantadine.

Keywords: Amantadine; Corneal endothelial dysfunction; Side effects; Parkinson's disease

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease, affecting ~0.3% of the general population and 3% of people over the age of 65 [1]. The disease is characterized pathologically by loss of dopaminergic neurons in the substantia nigra pars compacta in the midbrain and phenomenologically by parkinsonism such as resting tremor, muscular rigidity, bradykinesia, and postural instability. Amantadine, an antagonist of *N*-methyl-D-aspartate (NMDA)–glutamate receptor [2], is widely used for the treatment of PD including levodopa-induced dyskinesias [3], since its anti-parkinsonian effects were reported in 1969 [4]. However, there have been few reports in the literature of ocular side effects of amantadine. We describe a rare case of a patient with PD who developed visual disturbance during treatment with amantadine.

2. Case report

A 61-year-old man presented with gradual left-sided shuffling gait. Twenty-five months after the onset, the patient noticed a mild resting tremor in the left hand and complained of a tendency for the left leg to stumble. He consulted a local neurologist 30 months after symptom onset and was diagnosed with PD. The patient was then started on treatment with amantadine (300 mg/day) and trihexyphenidyl (4 mg/day). At age 64, he was referred to the outpatient clinic of our hospital for a second opinion.

At that time, systemic examination was normal but neurological examination showed facial hypomimia, pill-rolling resting tremor of the left hand, and left-side bradykinesia. The patient dragged the left leg on walking with a slightly diminished left arm swing. He showed stooped posture, but postural reflex was preserved. He was alert and oriented, but was slightly slow to respond to questions. There were no cerebellar signs, the deep tendon reflexes were intact in all extremities, and the plantar responses were flexor. Sensory function was intact. The patient complained of constipation and impaired olfaction. Routine hematological and biochemical tests were normal. Magnetic resonance imaging of the brain showed no abnormality. Cardiac uptake of ^{123}I-metaiodobenzylguanidine was reduced. Based on these findings, the patient was diagnosed as having PD.

Eight months after commencement of amantadine treatment, the patient noticed sudden deterioration in

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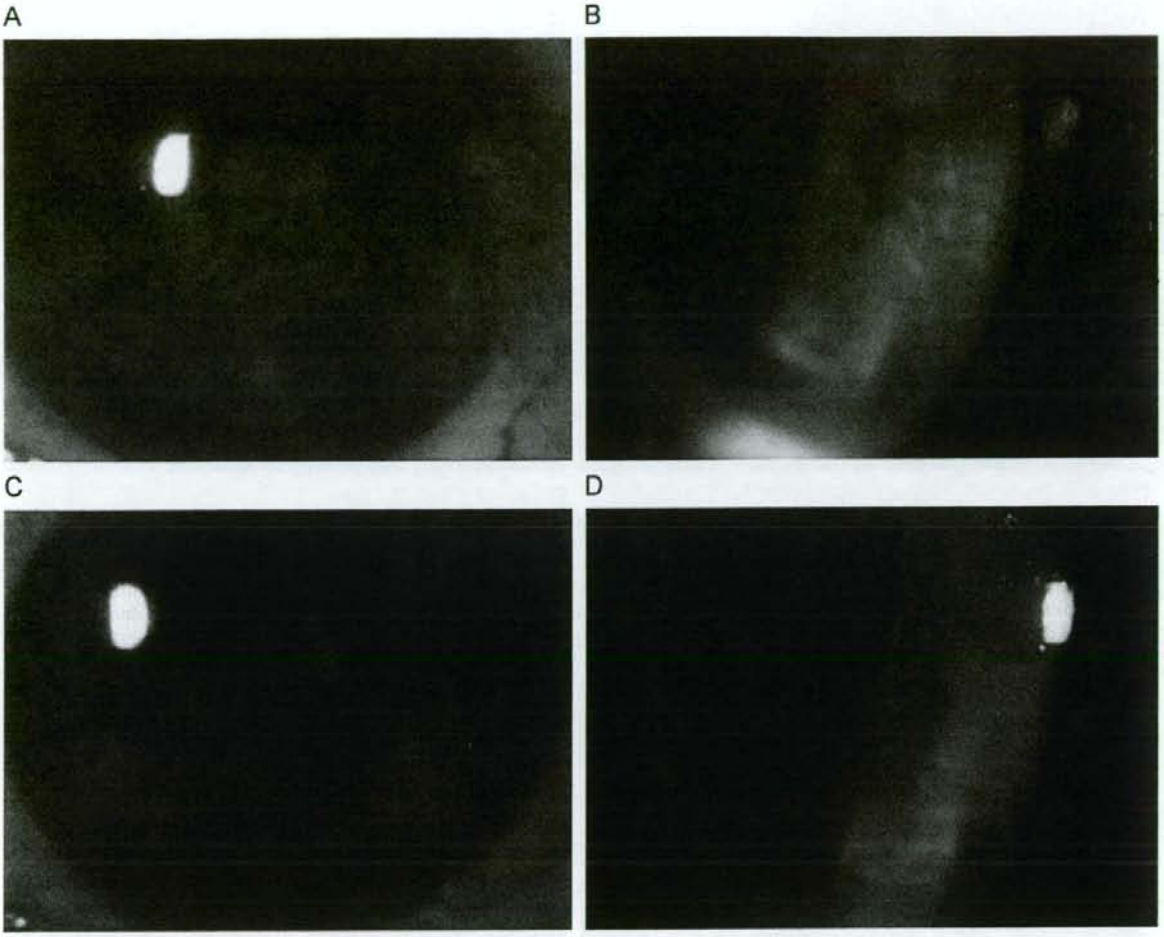

Fig. 1. Ophthalmologic examination conducted upon the complaint of sudden deterioration of visual acuity: (A) right cornea, (B) right cornea (slit-lamp examination). Note the presence of corneal edema and endothelial damage, (C) left cornea, and (D) left cornea (slit-lamp examination). Note corneal edema and endothelial damage.

visual acuity reflected by inability to read the small print of newspapers. He was referred urgently to the ophthalmology department at our hospital. Ophthalmologic examination revealed bilateral corneal endothelial damage and edema (Fig. 1A–D). Visual acuity for the left and right eye was 0.2 (OD) and 0.1 (OS), respectively. There were no signs of inflammation such as conjunctival redness, cells, or flare in the anterior chambers. Occlusion of the iridocorneal angles was ruled out, although the patient was taking trihexyphenidyl, an anti-cholinergic drug. The ophthalmologist indicated possible ocular side effect associated with amantadine. Accordingly, amantadine was tapered off in 3 days. Corneal endothelial damage and edema began to improve gradually and returned to normal with visual acuity of 1.0 (OD) and (1.2) (OS) at 8 days after discontinuation of amantadine (Fig. 2A–D), although resting tremor in the left hand slightly worsened.

3. Discussion

Amantadine, which was developed as a drug for influenza A virus in 1959, was later incidentally found to exhibit anti-parkinsonian effect in 1968 [4]. Since then, amantadine has been used in the management of PD worldwide. Notably, amantadine in addition to sulpiride [5] is pharmacologic therapy available for levodopa-induced dyskinesias, one of the motor complications in advanced stages of the disease [6]. Despite its frequent usage, there are only a few case reports of amantadine-associated visual impairment [7–11]. In these reported cases, the diseases associated with the development of amantadine-related visual impairment included essential tremor [7], influenza syndrome [8], vascular parkinsonism [10], and unknown neurological disorder presenting with tremor [11]. Surprisingly, there is no reported case with PD.

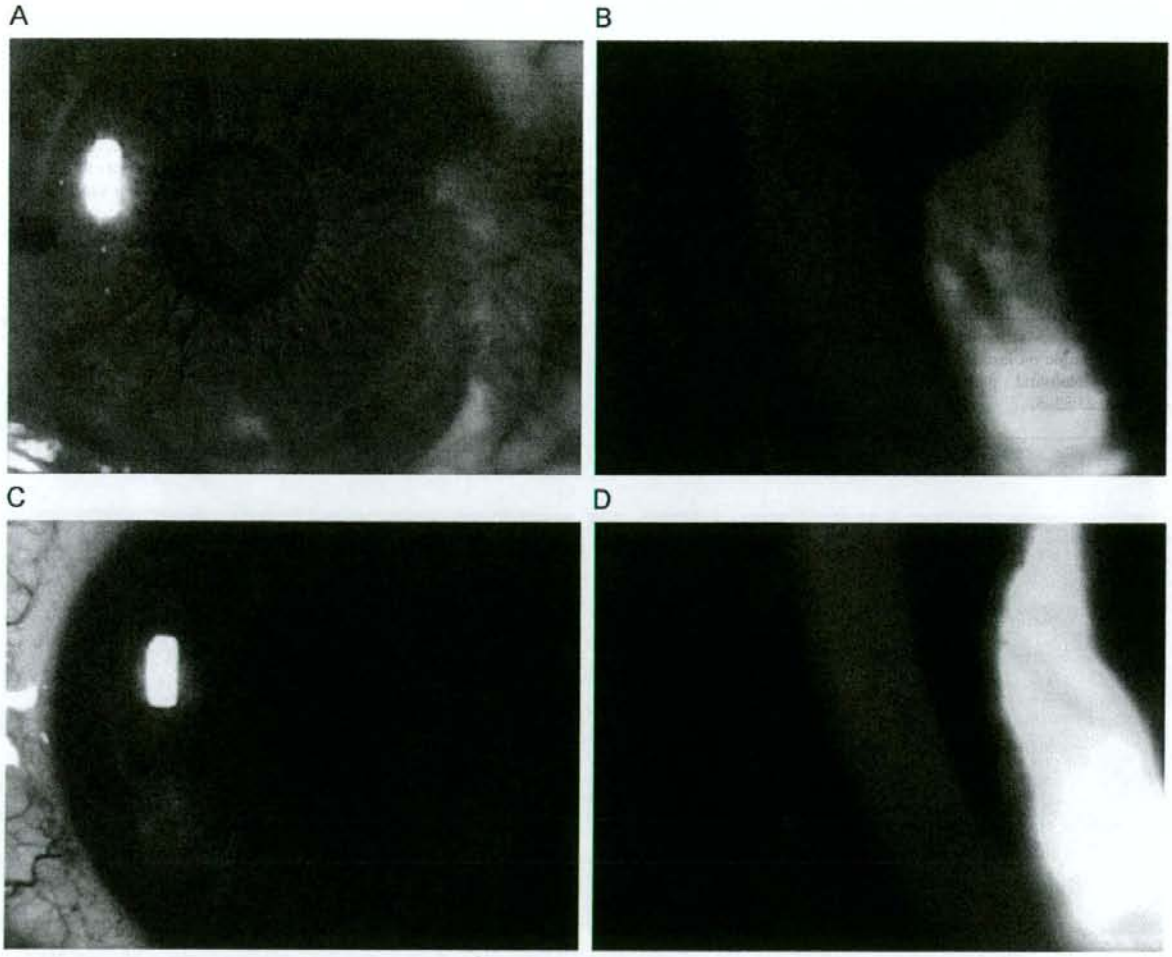

Fig. 2. Ophthalmologic examination conducted 8 days after discontinuation of amantadine: (A) right cornea, (B) right cornea (slit-lamp examination). Compare with Fig. 1 and note the disappearance of abnormal finding in the cornea, (C) left cornea, and (D) left cornea (slit-lamp examination). No abnormal finding is found in the cornea.

To our knowledge, our patient is the first reported PD case with visual impairment associated with amantadine. Based on the evidence that PD mainly affects elderly people, it is possible that PD patients with amantadine-associated visual impairment are misdiagnosed as aging-related ocular changes such as presbyopia and cataract.

Although the mechanism of amantadine-induced impaired vision remains poorly understood, there is no doubt in our case and in the reported cases about the relationship between amantadine and corneal dysfunction. In all cases, visual acuity recovered within a few weeks on cessation of the drug and such clinical improvement was associated with improvement in corneal lesions such as corneal endothelial or epithelial edema and superficial punctate keratitis [7–11]. Furthermore, resumption of amantadine was reported to result in recurrence of visual impairment [9,10], emphasizing such relationship. In previous reports, the dosage of amantadine was 100–400 mg/day comparable with that in our patient, and the interval between commencement of amantadine and appearance of visual symptoms was 1–3 weeks [7–11]. However, in the present case, the visual impairment developed 8 months after initiation of amantadine, suggesting that careful follow-up including ophthalmologic assessment is required whenever patients are under treatment of the drug. It is noteworthy that amantadine-associated visual impairment was of sudden onset and that amantadine did not cause permanent damage since such impairment disappeared within a few weeks after discontinuation of the drug therapy in our patient as well as reported cases.

In conclusion, amantadine can cause impairment of corneal endothelial function and needs to be considered in the differential diagnosis of visual impairment. As age is an important risk factor in PD, with the increasing age of the

general population and the prevalence of the disease, it is likely that the frequency of use of amantadine will increase steadily in the future. Neurologists and physicians in general should pay attention to amantadine, when they encounter sudden visual deterioration in patients with PD.

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ORIGINAL CONTRIBUTION

Mutation Analysis of the *PINK1* Gene in 391 Patients With Parkinson Disease

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Objectives: To determine the frequency, distribution, and clinical features of Parkinson disease (PD) with *PINK1* mutations.

Design: Retrospective clinical and genetic review.

Setting: University hospital.

Patients: We performed extensive mutation analyses of *PINK1* in 414 PD patients negative for *parkin* mutations (mean [SD] age at onset, 42.8 [14.3] years), including 391 unrelated patients (190 patients with sporadic PD and 201 probands of patients with familial PD) from 13 countries.

Results: We found 10 patients with PD from 9 families with *PINK1* mutations and identified 7 novel mutations (2 homozygous mutations [p.D297MfsX22 and p.W437R] and 5 single heterozygous mutations [p.A78V, p.P196QfsX25, p.M342V, p.W437R, and p.N542S]). No compound heterozygous mutations were found. The frequency of homozygous mutations was 4.26% (2 of 47) in families with autosomal recessive PD and 0.53% (1 of 190) in patients with sporadic PD. The frequency of heterozygous mutations was 1.89% (2 of 106) in families with potential autosomal dominant PD and 1.05% (2 of 190) in patients with sporadic PD. The mean (SD) age at onset in patients with single heterozygous mutations (53.6 [11.1] years; range, 39-69 years) was higher than that in patients with homozygous mutations (34.0 [20.3] years; range, 10-55 years). Myocardial iodine-123 metaiodobenzylguanidine uptake was low in patients with heterozygous mutations but not in those with homozygous mutations.

Conclusions: Our results suggest that homozygous *PINK1* mutations tend to be diagnosed as the early-onset autosomal recessive form of PD. Single heterozygous mutations may contribute to the development of sporadic PD and also could be an additional genetic predisposition for developing familial PD. The reduced myocardial iodine-123 metaiodobenzylguanidine uptake observed in patients with single heterozygous *PINK1* mutations is similar to that seen in patients with sporadic PD.

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PARKINSON DISEASE (PD) IS PREdominantly characterized by degeneration of midbrain dopaminergic neurons, eventually leading to various motor dysfunctions, such as rigidity, tremor, bradykinesia, and postural instability.[1] The etiology of PD is unknown but is presumably multifactorial, eg, perhaps having a genetic × environmental interaction.

Although most PD cases are sporadic, several causative genes have been identified in recent years in familial forms of PD. For example, *alpha-synuclein* (loci, *PARK1* and *PARK4*), *UCH-L1* (*PARK5*), and *LRRK2/dardarin* (*PARK8*) are reported to be the causative genes for autosomal dominant PD (ADPD)[2-6]; and *parkin* (*PARK2*), *DJ-1* (*PARK7*), and *PINK1* (OMIM 608309) (*PARK6*) are reported to be the causative genes for autosomal recessive PD (ARPD).[7-9] Mutations in *parkin* are the major cause of ARPD, and the frequency of such mutations in families with ARPD is approximately 50%.[10] In contrast, mutations in *DJ-1* are rare (≤ 1%) in ARPD.[11] Increasing numbers of patients with *PINK1* mutations are being reported; however, there are no sufficiently large studies to define the frequency, age distribution, or clinical features of patients with PD associated with *PINK1* mutations worldwide, especially not in Asia. Moreover, no association between PD and coding single nucleotide polymorphisms within *PINK1* has been reported.[12] The role of a single heterozygous *PINK1* mutation in the clinical manifestation of parkinsonism, such as age at onset, is not clear at present, mainly because previous reports have not identified substantial num-

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Table 1. Characteristics of 414 Analyzed Patients With Parkinson Disease

Type of Disease	No. of Patients	Mean (SD) Age at Onset, Range, y
Sporadic Parkinson disease	190 (105 males, 85 females)	37.2 (10.4), 7-81
Familial Parkinson disease	224 (201 probands, 23 relatives; 100 males, 124 females)	47.6 (15.5), 10-85
ARPD	55 (47 probands)	52.8 (13.8)
ADPD	121 (106 probands)	47.1 (15.8)
Unclear hereditary information	48	43.1 (16.0)
Total	414 (391 unrelated patients [190 patients with sporadic disease and 201 probands])	42.8 (14.3)

Abbreviations: ADPD, autosomal dominant Parkinson disease; ARPD, autosomal recessive Parkinson disease.

bers of *PINK1* mutations. To clarify these aspects, we performed extensive mutation analysis in a large number of patients with PD in 13 countries.

METHODS

PATIENTS

We studied 414 *parkin*-negative PD patients (391 unrelated patients and 23 relatives) from 13 countries (249 Japanese, 55 Korean, 28 Israeli, 27 Taiwanese, 27 Chinese, 14 Tunisian, 5 Turkish, 3 Greek, 2 Moroccan, 1 Filipino, 1 Bulgarian, 1 Brazilian, and 1 Australian individual). Patients received clinical diagnoses of PD[13] regardless of their familial history. The distribution of age at onset was as follows: younger than 50 years (early-onset) (n=287 [69.3%]), 50 years or older (late-onset) (n=117 [28.3%]), and unknown (n=10 [2.4%]). Hereditary information and the mean ages at onset of patients with PD are provided in **Table 1**. In this study, we defined mode of inheritance as autosomal recessive (≥2 affected siblings in only 1 generation) and autosomal dominant (≥1 affected member in 2 consecutive generations). All participants in the control cohort were Japanese individuals. The study was approved by the ethics review committee of Juntendo University. Blood samples for genetic analysis and clinical information were collected by local neurologists after obtaining informed consent from the patients.

GENETIC ANALYSIS

Genomic DNA was isolated from peripheral blood using standard protocols. For direct sequence analysis, DNA was amplified by polymerase chain reaction of each exon, using standard methods and published primers.[14] Dideoxy sequencing was performed with Big Dye Terminator Chemistry (Applied Biosystems, Foster City, California). These products were loaded on ABI 377, ABI 310, and ABI 3130 automated DNA sequence analyzers (Applied Biosystems) and analyzed with DNA Sequence Analysis software (Applied Biosystems). *Parkin* mutations were examined by polymerase chain reaction, direct sequencing, and quantitative assays based on real-time polymerase chain reaction with TaqMan probes (Applied Biosystems) in each exon. We ruled out *parkin* mutations including exonic deletions or multiplications by dosage studies before analysis of *PINK1*. For extensive screening of substitutions and to determine whether or not the novel *PINK1* mutations were pathogenic, we performed direct sequencing in 300 chromosomes of healthy control DNA samples for all coding exons. All controls were evaluated by neurologists to ensure none of them had parkinsonism.

GENE DOSAGE STUDIES

We performed polymerase chain reaction–based exon dosage assay using TaqMan Chemistry and an ABI PRISM 7700 sequence detection system (Applied Biosystems) in the 5 patients who had a heterozygous *PINK1* mutation (patients E, F, G, H, and J) to rule out compound heterozygous mutations with other heterozygous exonic deletion or multiplication. We used the primer and the probe of Assay by Design (Applied Biosystems) according to a previously published report.[14]

MYOCARDIAL IODINE-123 METAIODOBENZYLGUANIDINE SCINTIGRAPHY

Myocardial iodine-123 metaiodobenzylguanidine (^{123}I-MIBG) scintigraphy was performed in 5 *PINK1* mutation–positive patients (from different hospitals) with an intravenous injection of 111 MBq of ^{123}I-MIBG (Daiichi Radioisotope Laboratories, Tokyo, Japan). Early images were obtained 15 minutes and delayed images were obtained 3 to 4 hours after injection. Whole myocardial ^{123}I-MIBG uptake was measured on a planar image as the early and delayed heart to mediastinum activity ratio.

STATISTICAL ANALYSIS

Data are expressed as mean (SD). For continuous variables, such as age at onset, the *t* test was used to test for significant differences between the 2 groups. Categorical data, such as individual responses to each question on the diagnosis checklist and frequencies, were compared with the χ^2 test, with Yates correction when appropriate.

RESULTS

GENETIC ANALYSIS

We identified 10 patients with PD from 9 families with *PINK1* mutations, including 7 novel mutations (**Table 2**). Three homozygous missense mutations were found in 4 patients: p.T313M, p.C388R, and a novel p.W437R. Previously, p.T313M and p.C388R had been reported.[15,16] In addition, 3 novel single heterozygous missense mutations were found in 4 patients: p.A78V, p.M342V, and p.N542S. We also identified 1 novel homozygous deletion (p.D297MfsX22) and 1 novel single heterozygous deletion (p.P196QfsX25). We also found 1 patient with familial PD (without clear mode of inheritance) with a novel single heterozygous variant (p.V482M).

Table 2. Clinical Features of Study Patients With *PINK1* Mutations

Measure	Missense Mutations								Homozygous Deletion	Heterozygous Deletion
	Homozygous				Heterozygous					
Patient	A	B	C	D	E	F	G	H	I	J
Nucleotide change	c.938C>T	c.1162T>C	c.1162T>C	c.1309T>C	c.233C>T	c.1024A>G	c.1024A>G	c.1625A>G	c.889delG	c.585delC
Amino acid change	p.T313M	p.C388R	p.C388R	p.W437R	p.A78V	p.M342V	p.M342V	p.N542S	p.D297MfsX22	p.P196QfsX25
Exon	4	6	6	7	1	5	5	8	4	2
Heredity form	SPD	ARPD	ARPD	FPD	SPD	SPD	ADPD	ADPD	ARPD	FPD
Country of residence	Japan	Japan	Japan	Turkey	Japan	Japan	Japan	Japan	Greece	Japan
Consanguinity	–	+	+	+	–	–	–	–	+	+
Age at onset, y	32	55	54	19	39	49	53	69	10	58
Disease duration, y	2	10	2	21	21	12	4	7	15	16
Sex	M	F	F	F	F	F	M	F	F	F
Resting tremor	+	+	+	+	+	+	+	+	+	+
Rigidity	+	+	+	+	+	+	+	+	+	+
Bradykinesia	–	–	–	+	+	+	+	+	+	+
Postural instability	–	–	–	–	+	+	+	–	+	–
Gait disturbance	+	–	+	+	+	+	+	+	+	+
Frozen gait	+	–	–	+	–	–	–	–	+	+
Wearing off	+	–	–	+	–	+	–	+	+	–
On/off states	+	–	–	+	–	+	–	–	+	NA
Asymmetry at onset	–	–	+	+	–	+	–	+	–	+
Orthostatic hypotension	–	–	–	–	+	–	–	–	–	+
Incontinence	–	–	+	–	–	–	–	+	–	+
Urinary urgency	+	–	–	+	–	+	–	+	–	–
Levodopa-induced dyskinesia	–	–	–	+	–	+	–	–	+	–
Sleep benefit	–	–	+	–	–	–	–	–	–	–
Dystonia at onset	–	–	–	–	–	–	–	–	+	–
Hyperreflexia	–	–	–	–	–	–	–	–	+	+
Dementia	–	–	–	–	–	–	–	+	–	–
Depression	–	–	–	–	–	–	–	–	–	–
Hallucinations	–	–	–	–	–	+	–	+	–	–
Other psychosis	–	–	–	–	–	–	–	–	–	–
UPDRS score, on/off states	19/39	18/NA	NA/6	16/22	NA	NA	NA	NA	77/NA	64/259
Hoehn-Yahr stage										
On state	2	2	1	1.5	2.5	4	3	2	2	3
Off state	3	NA	NA	2.5	NA	NA	NA	3	4	NA
Myocardial ^{123}I-MIBG uptake	NA	Not decreased	Not decreased	NA	Decreased	NA	NA	Decreased	NA	Decreased
Early H:M activity ratio (standard value)	NA	1.82 (> 1.45)	1.97 (> 1.45)	NA	1.49 (> 1.45)	NA	NA	1.4 (> 1.84)	NA	1.64 (> 2.2)
Delayed H:M activity ratio (standard value)	NA	2.93 (> 1.45)	1.97 (> 1.45)	NA	1.25 (> 1.45)	NA	NA	1.18 (> 1.78)	NA	1.28 (> 2.2)

Abbreviations: ADPD, autosomal dominant Parkinson disease; ARPD, autosomal recessive Parkinson disease; FPD, familial Parkinson disease (definite information on mode of inheritance not available, though some family members had parkinsonism); H:M, heart to mediastinum; NA, not applicable or no information available; SPD, sporadic Parkinson disease; UPDRS, Unified Parkinson Disease Rating Scale; +, present; –, absent; ^{123}I-MIBG, Iodine-123 metaiodobenzylguanidine.

We did not find any of these mutations or variants in 300 chromosomes in a healthy Japanese population, and we did not detect exonic deletion or multiplication by gene dosage study. The aforementioned novel missense mutations and variant have not been reported as polymorphisms. In addition, we examined the homology regarding the *PINK1* protein. The site of p.W437R mutation was highly conserved among various species. On the other hand, the p.V482M variant was not highly conserved (data not shown).

The affected relatives of patients G, H, and J could not be tested for cosegregation of the same heterozygous mutation that was found in the probands. Thus, we could not exclude that the mutation does not cosegregate in 1 or more of these families. No cosegregation of the p.V482M variant was observed among patients in the same family. Therefore, the role of this variant in this family was not clear.

The frequency of homozygous *PINK1*-positive patients was 1.02% (4 of 391 [1 patient with sporadic PD + 3 familial PD probands]/[190 patients with sporadic PD + 201 familial PD probands]) among the entire group of PD patients. Furthermore, the frequency of homozygous *PINK1*-positive patients was 4.26% (2 of 47) in ARPD families and 0.53% (1 of 190) in patients with sporadic PD. Homozygous mutations were not detected in patients with ADPD. However, the frequency of single heterozygous *PINK1*-positive patients was 1.28% (5 of 391) among the entire group of PD patients, 1.89% (2 of 106) in ADPD families, and 1.05% (2 of 190) among patients with sporadic PD. No single heterozygous mutations were detected in patients with ARPD.

CLINICAL ANALYSIS

Table 2 lists the clinical features of 10 *PINK1*-positive patients and the **Figure** shows the pedigree of families with the *PINK1* mutation. In this study, the family with no cosegregation of p.V482M was excluded from Table 2 and the Figure, because the role of the V482M variant in this

family was not clear. Among the *PINK1*-positive families, consanguineous marriages were noted in 5 patients (patients B, C, D [pedigree not available], I, and J).

The mean age at onset of patients with homozygous *PINK1* mutations was 34.0 (20.3) years (range, 10-55 years), and that of patients with a single heterozygous *PINK1* mutation was 53.6 (11.1) years (range, 39-69 years). The age at onset was significantly lower in the homozygous *PINK1*-positive patients compared with the single heterozygous *PINK1*-positive and *PINK1*-negative patients.

As presented in Table 2, motor dysfunction was comparatively mild in many *PINK1*-positive patients. The mean Hoehn-Yahr stage of homozygous *PINK1*-positive patients was 1.7 (0.4) in the on state and 3.3 (0.6) in the off state. In contrast, the average Hoehn-Yahr stage of patients with a single heterozygous *PINK1* mutation was 2.9 (0.5) in the on state and 3.0 (0.0) in the off state. Even in patient E, who had had PD for 21 years, the Hoehn-Yahr stage was 2.5. None of the patients had a Hoehn-Yahr stage of 5.0.

Patient I had a homozygous 1-base deletion mutation and patient J had a single heterozygous 1-base deletion mutation. These 2 patients had similar deletion mutations that caused stop codons within the serine/threonine kinase domain of *PINK1*, but age at onset was clearly different: 58 years for patient J (the latest) and 10 years for patient I (the earliest among *PINK1*-positive patients). Although both patients had hyperreflexia, patient J did not have dystonia at onset, while patient I had dystonia at onset. To date, none of the *PINK1*-positive patients in this study were investigated pathologically.

MYOCARDIAL ^{123}I-MIBG SCINTIGRAPHY

Myocardial ^{123}I-MIBG scintigraphy was performed in 5 *PINK1*-positive patients (patients B, C, E, H, and J). The early and delayed heart to mediastinum ratios of these patients are listed together with the age-matched standard values in Table 2. Myocardial ^{123}I-MIBG uptake was normal in patients with homozygous *PINK1* mutations (patients B and C), whereas it was decreased in patients with single heterozygous *PINK1* mutations (patients E, H, and J).

COMMENT

Combining the results of our previous studies[14,15,17] and this study, the frequency of *PINK1*-positive families with 2 allele mutations (homozygous mutations and compound heterozygous mutations) among *parkin*-negative ARPD was 11.5% (10 of 87). Among heterozygous mutations, many were single heterozygous rather than compound heterozygous. Our results showed that not only a Japanese individual but 1 Greek and 1 Turkish individual had *PINK1* mutations (Table 2), which suggests that the mutation is possibly distributed worldwide, similar to *parkin* mutations.[10,11] Considering previous reports on the frequencies of *parkin*[10,11] and *DJ-1*[18,19] mutations, we propose that we should first screen patients with PD for *parkin* mutations, including gene dosage

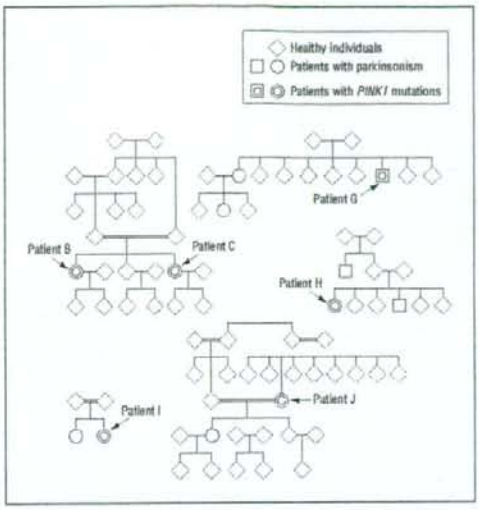

Figure. Pedigrees of patients with *PINK1* mutations. Patients B and C had the same homozygous missense mutation (p.C388R) in exon 6. Patient G had a single heterozygous mutation (p.M342V) in exon 5. Patient H had a single heterozygous mutation (p.N542S) in exon 8. Patient I had a homozygous deletion mutation (p.D297MfsX22) in exon 4, and patient J had a single heterozygous deletion mutation (p.P196QfsX25) in exon 2. The sexes are concealed to safeguard the confidentiality of the family members.

study, then screen for *PINK1* mutations, and finally screen for *DJ-1* in ARPD.

In the present study, we did not screen fully for heterozygous *PINK1* deletion mutations and multiplications by the gene dosage study using TaqMan assay to save time in screening all patients. Homozygous *PINK1* deletion mutation of more than 1 exon structure had been reported in only 1 case so far.[15] *PINK1* and *DJ-1* deletion mutations seem to be less frequent than *parkin* deletion mutations even if these heterozygous deletion mutations are to be included. In this regard, we think that gene dosage study of *PINK1* may not be as important as that of *parkin*.

Although the prevalence was rare, our study and others[20-22] showed that homozygous mutations as well as single heterozygous *PINK1* mutations are found not only in ARPD but also in ADPD families and patients with sporadic PD. These results suggest that screening for *PINK1* mutations may also be necessary in patients with potential ADPD and sporadic PD.

Although heterozygous carriers are clinically unaffected in most autosomal recessive disorders, higher preponderance of heterozygous *PINK1* mutations in patients with sporadic PD, compared with matched controls, has been reported.[21-23] Accordingly, although it is difficult to make a firm conclusion about the frequencies of heterozygous *PINK1* mutations in patients vs controls, all the single heterozygous *PINK1* mutations were found only in Japanese patients with PD but not in Japanese controls. Moreover, in the positron-emission tomographic study, carriers of heterozygous *PINK1* mutations showed significant reductions in caudal and putaminal fludeoxyglucose F18 uptake (mean of 20%-30% lower than the controls), indicating increased susceptibility for the de-

Table 3. Clinical Features of 23 Patients With *PINK1* Mutations in Current and Past Studies

Measure	No. of Patients					*P* Value		
		PINK1-Mutation Positive						
	No *PINK1* Mutation (n=404)	Homozygous (n=16)	Compound Heterozygous (n=2)	Heterozygous (n=5)	All (n=23)	2 Mutations vs 1 Mutation	2 Mutations vs No Mutation	Mutation Positive vs No Mutation
Sporadic PD	187	1	0	2	3			
ARPD[a]	52	14	2	0	16			
ADPD	119	0	0	3	3			
Patients with familial PD, unclear hereditary information	46	1	0	0	1			
Age at onset, mean (SD), y	42.8 (14.3)	32.6 (8.5)	18.5 (0.7)	53.6 (11.1)	35.9 (15.0)	< .001	< .001	.03
Resting tremor	293	11	2	5	18	.47	.83	.72
Rigidity	366	13	2	5	20	.82	.54	.83
Bradykinesia	368	12	2	5	19	.62	.14	.32
Postural instability	244	9	0	4	13	.49	.53	.88
Gait disturbance	268	15	1	5	21	.91	.08	.02
Frozen gait	NA	9	0	1	10	.49	NA	NA
Wearing off	227	11	2	2	15	.42	.27	.53
On/off states	NA	9	0	1	10	.49	NA	NA
Asymmetry at onset	293	9	1	3	13[b]	.64	.34	.26
Orthostatic hypotension	43	2	0	2	4[c]	.57	.99	.30
Incontinence	30	1	0	2	3[b]	.23	.81	.52
Urinary urgency	63	2	0	2	4[b]	.44	.93	.98
Levodopa-induced dyskinesia	170	6	2	1	9	.51	.96	.95
Sleep benefit	112	6	2	0	8	.14	.20	.62
Dystonia at onset	53	3	1	0	4	.62	.45	.79
Hyperreflexia	51	8	0	1	9	.50	.001	.001
Dementia	41	2	0	0	2	.91	.79	.90
Depression	NA	2	0	0	2	.91	NA	NA
Hallucinations	62	2	0	2	4	.40	.88	.97
Other psychosis	26	1	0	0	1	.48	.73	.97
Hoehn-Yahr stage, mean (SD)								
On state	2.5 (1.0)	2.3 (0.7)	NA	2.9 (0.5)	2.5 (0.7)	.06	.29	.48
Off state	3.4 (1.1)	2.9 (0.9)	NA	3.0 (0.0)	3.3 (1.1)	NA	.27	.24

Abbreviations: ADPD, autosomal dominant Parkinson disease; ARPD, autosomal recessive Parkinson disease; NA, not applicable; PD, Parkinson disease.
[a] Thirteen of the patients with ARPD were reported previously by our group.[12,15,17]
[b] n=22.
[c] n=19.

velopment of parkinsonism.[24] In addition, our data showed that the age at onset of patients with heterozygous *PINK1* mutations was higher than that of patients with homozygous *PINK1* mutations and was similar to that of classic sporadic PD. Thus, the previous findings and our data emphasize the importance of heterozygous *PINK1* mutations as a possible risk factor for developing the common classic form of sporadic PD. However, we could not exclude other possibilities, eg, that these mutations could be coincidental findings or even be a cause of ADPD, because we did not perform the genetic tests in the relatives of the patient with a single heterozygous mutation or in controls outside of the Japanese population. In addition, we could not exclude the possibility of digenic inheritance or technical limitations in detecting all possible mutations (eg, in the introns and promoter).

Table 3 lists the clinical symptoms of the patients in this study and patients reported previously by our group.[14,15,17] Thus, we could compare 23 *PINK1*-positive patients with 404 *PINK1*-negative patients and compare 18 patients with 2 allele *PINK1* mutations (16 patients with homozygous *PINK1* mutations and 2 patients with compound heterozygous mutations) with 5 patients with 1 allele *PINK1* mutation. The data in Table 3 show that most *PINK1*-positive patients develop early-onset parkinsonism. Moreover, the mean age at onset of patients with 1 allele *PINK1* mutation was higher than that of patients with 2 allele mutations.

Age at onset, hyperreflexia, and gait disturbances were significantly more frequent in homozygous *PINK1*-positive patients than in *PINK1*-negative patients. Indeed, these symptoms were also significantly different in patients with or without *PINK1* mutations. However, there were no statistical differences in pathognomonic symptoms between patients with 1 or 2 allele *PINK1* mutations, except for age at onset. These data indicate that the phenotypes of patients with a single heterozygous *PINK1* mutation are more likely to be similar to those of homozygous *PINK1*-positive patients, except for age at onset.

Myocardial ^{123}I-MIBG scintigraphy is one of the most supportive diagnostic tools used in differentiating PD from

conditions such as essential tremor, progressive supranuclear palsy, and multiple system atrophy.[25,26] In this regard, some patients with 2 allele *parkin* mutations without Lewy bodies were reported to have normal ^{123}I-MIBG uptake.[27-29] Another study demonstrated markedly low heart to mediastinum ratios in patients with classic PD with Lewy bodies and in incidental Lewy body disease, suggesting that Lewy body pathology itself may be responsible for low ^{123}I-MIBG uptake.[30] Although a single case with a homozygous *PINK1* mutation was reported to have a very mild decrease in ^{123}I-MIBG uptake,[31] our data showed that 2 patients with homozygous *PINK1* mutations (patient B with disease duration of 10 years and patient C with disease duration of 2 years) had normal myocardial ^{123}I-MIBG uptake. In contrast, 3 patients with single heterozygous *PINK1* mutations (patients E, H, and J) had low myocardial ^{123}I-MIBG uptake. These findings suggest that patients with a single heterozygous mutation are more likely to have cardiac sympathetic denervation than those with homozygous *PINK1* mutations, which accounts for the low ^{123}I-MIBG uptake. One can further speculate that patients with heterozygous *PINK1* mutations may have Lewy body pathology, whereas those with homozygous *PINK1* mutations have no Lewy body pathology, similar to patients with *parkin* mutations,[10,32] though no pathologic study of patients with 2 allele *PINK1* mutations has been reported to date. Additional studies of cardiac scintigraphy in a larger number of *PINK1*-positive patients with PD are required to clarify these points.

In summary, we assume that homozygous *PINK1* mutations may manifest in an early-onset autosomal recessive form of PD. We can also speculate that single heterozygous mutations may be 1 of the risk factors in developing the sporadic or autosomal dominant form of PD. Additional studies are necessary to clarify the etiopathogenic roles of 1 allele *PINK1* mutation in developing various forms of PD.

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In conclusion, COMT inhibition combined with LD/DDI improves absorption of a coadministered salt probably due to a COMT inhibition induced basic environment in gastrointestinal membranes. This improves dissolution and absorption of acids and salts. Thus it may enhance absorption of LD itself.[2,4,5]

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Familial Parkinsonism with Digenic *Parkin* and *PINK1* Mutations

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Abstract: **To clarify the genetic correlation between *parkin* and *PINK1*, we screened for *PINK1* mutations in 175 parkinsonism patients with *parkin* mutations. We detected two sibling pairs and one sporadic patient carrying both *parkin* and *PINK1* mutations. The age at onset of Parkinsonism of patients with the digenic mutations was lower than that of patients with the same *parkin* mutation alone. In addition, two of three patients carrying both *parkin* and *PINK1* mutations had schizophrenia. These findings indicate that *PINK1* mutation might modify *parkin* mutation-positive Parkinsonism, and *PINK1* mutations might**

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be associated withpsychiatric disorders. © 2008 Movement Disorder Society

Key words: Parkinson's disease; *parkin*; *PINK1*; digenic; psychiatric disorder

Parkinson's disease (PD) is one of the most frequent neurodegenerative disorders caused by loss of dopaminergic neurons in the substantia nigra, which results in decreased dopamine availability in the striatum. Although most cases with PD are sporadic, several genes are associated with the monogenic forms of Parkinsonism and related disorders. Identification of the causative genes and their functions in these rare forms of the disease can provide tremendous insights into the pathogenesis of PD and opens up new areas of medical research on this disease.

Parkin [MIM 602544; *PARK2*] and *PTEN-induced putative kinase 1* (*PINK1*) [MIM 608309; *PARK6*] have been reported as the causative genes of *PARK2*- and *PARK6*-linked autosomal recessive parkinsonism (ARP), respectively.[1] Intriguingly, several lines of evidence suggest that heterozygous mutations of *parkin* and *PINK1* could play a role in the development of parkinsonism despite the fact that they were originally identified as the responsible genes for ARP.[2,3] In addition, *parkin* and *PINK1* mutations might be associated with psychiatric disorders.[1,4,5] Thus, these results suggest the importance of these genes in sporadic PD as well as psychiatric disorders, in addition to ARP.

Recent biochemical and morphological studies using *Drosophila melanogaster* suggest that Parkin and PINK1 are involved, through a common pathway, in maintenance of mitochondrial function and that PINK1 acts upstream of Parkin.[6,7] Thus, it is possible that reduced activities of both gene products significantly lower the threshold of nigral degeneration compared with loss of activity of either Parkin or PINK1 alone.

In the present study, we screened for *PINK1* mutations in Parkinsonism patients with *parkin* mutations and detected patients with both *PINK1* and *parkin* mutations. Clinicogenetic analysis revealed that the presence of *PINK1* mutation in addition to *parkin* mutation could hasten the disease process.

PATIENTS AND METHODS

Subjects

This study was approved by the ethics review committee of Juntendo University School of Medicine. All subjects gave informed and written consent before participation. We selected patients with one- (single heterozygous, n = 19; 19 probands), and two- (homozygous or compound heterozygous, n = 156; 119 probands) *parkin* mutation(s). All patients were screened for *parkin* mutations by PCR, direct sequencing, and gene dosage analyses of all exons. The mean age at onset was 40.6 ± 17.6 years (±SD, range 18–75; one *parkin* mutation) and 27.9 ± 9.9 years (range 6–61; two *parkin* mutations). Among the total of 175 patients, 130 (74.3%) had family histories of Parkinsonism, and 149 (85.1%) were Asian (133 Japanese, 6 Chinese, 6 Korean, and 4 Taiwanese). The remaining were 15 Israelis, 3 Americans, 2 Tunisians, 2 Greeks, 1 Canadian, 1 German, 1 Iraqi, and 1 Moroccan.

Genetic Analyses

Genomic DNA samples were sequenced for all exons and splice junctions of *PINK1* using BigDye Terminator v1.1 Cycle Sequencing kit and 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Only patients with heterozygous *PINK1* mutation were also screened by gene dosage analyses of all exons of *PINK1* by real-time PCR using TaqMan probes and ABI PRISM 7700 Sequence Detector (Applied Biosystems). Microsatellite markers flanking PARK2 and PARK6 loci were genotyped by PCR using fluorescence labeled primers, 3130 Genetic Analyzer, and GeneMapper software (Applied Biosystems). PCR, sequencing, and real-time PCR were used standard methods and published primers and probes.[8]

RESULTS

We identified a novel heterozygous mutation (p.R58-V59insGR) in exon 1 of *PINK1* in a pair of Japanese siblings with homozygous *parkin* mutations (p.T175PfsX2: Fig. 1; Family A, A3 and A4). These mutations were absent in 300 Japanese normal chromosomes, indicating that the mutations might be pathogenic. We also detected the same heterozygous *PINK1* mutation in one of the unaffected parents who had heterozygous *parkin* p.T175PfsX2 mutation (Fig. 1; Family A, A1). Another heterozygous *PINK1* mutation (p.R407Q) in exon 6 was detected in a pair of Chinese siblings with compound heterozygous *parkin* mutations (p.C441R and p.A138GfsX7: Fig. 1; Family C). The p.R407Q mutation of *PINK1* was reported previously in one Taiwanese patient with PD, but was absent in 188 Taiwanese control chromosomes.[9] We

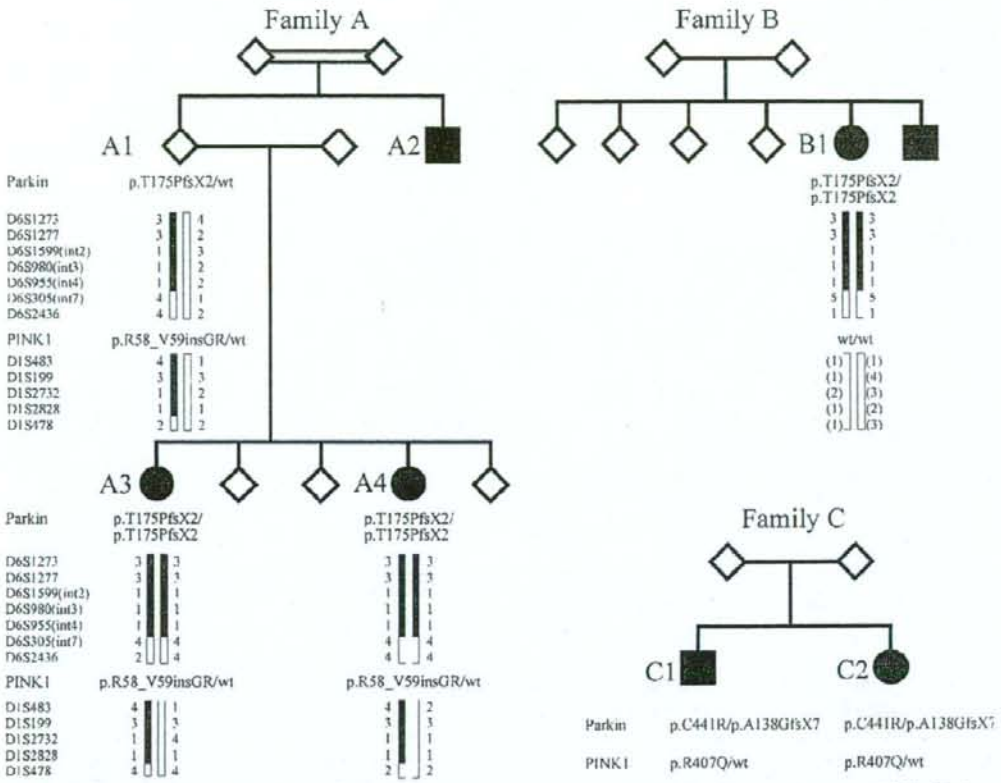

FIG. 1. Pedigrees of families analyzed in this study. Solid bars indicate shared disease haplotype. DNA of Patient A2 was not available. The haplotypes with undetermined phases in proband B-B1 are shown in parentheses. Int, intron.

did not detect this mutation in 300 Japanese normal chromosomes.

Next, we screened mutations of *PINK1* in patients who had heterozygous *parkin* mutation. We detected a patient with sporadic PD with heterozygous *PINK1* mutation (p.E476K) and heterozygous *parkin* mutation (p.P437L: Table 1; Patient D), which were absent in 300 Japanese normal chromosomes. In addition, we performed gene dosage analyses of *PINK1* for subjects who were identified with a single heterozygous mutation of the gene. No exonic rearrangements in *PINK1* were detected in any of the subjects.

We found one patient (Patient B1) from the original sample series who had homozygous *parkin* p.T175 PfsX2 mutation (the same mutation in Patients A3 and A4) but no *PINK1* mutation. Haplotype analyses of PARK2 and PARK6 loci in families A and B revealed a common haplotype in PARK2, but not in PARK6 locus (Fig. 1). The p.T175PfsX2 mutation was absent in 108 normal chromosomes from the Kyusyu region in Japan (families A and B originated from Kyusyu region). These results suggest that p.T175PfsX2 mutation of *parkin* spread from a single founder. With regard to the clinical features, the age at onset in patients of family A who had both homozygous *parkin* mutation (p.T175PfsX2) and heterozygous *PINK1* mutation (p.R58-V59insGR) was more than 10 years earlier than that in Patient B1 who had only homozygous *parkin* mutation (Table 1). In addition, the age at onset was significantly lower in patients with both two *parkin* and one *PINK1* mutations (Patients A3, A4, C1, and C2) compared with the only two *parkin* mutations ($P = 0.025$, Student's *t*-test). Interestingly, two of the three patients with PD of family A had nondrug-

TABLE 1. *Clinical features of patients*

Patient	A2	A3	A4	B1	C1	C2	D
Origin	Japan	Japan	Japan	Japan	Hong Kong	Hong Kong	Morocco
Age at onset	15	12	20	30	18	18	35
Disease duration	38	25	9	36	22	17	18
Sex	M	F	F	F	M	F	M
Resting tremor	+	+	+	−	−	−	+
Rigidity	+	+	+	NA	+	+	+
Bradykinesia	+	+	−	+	+	+	+
Postural instability	+	+	−	−	+	+	−
Frozen gait	−	+	−	+	−	−	+
Clinical response to levodopa	+	+	+	+	+	+	+
Wearing off	−	+	−	+	+	+	+
On off	−	+	−	+	+	+	+
Asymmetry at onset	+	+	+	−	−	−	+
Incontinence	−	−	−	−	−	−	−
Urinary urgency	−	−	−	NA	−	−	+
Levodopa-induced dyskinesia	+	+	−	+	NA	+	+
Sleep benefit	−	−	−	+	+	+	NA
Dystonia at onset	−	+	−	−	+	+	+
Hyperreflexia	−	−	−	−	+	+	−
Dementia	−	+	−	NA	−	−	−
Depression	−	−	−	−	−	−	+
Hallucination	+	+	−	+	−	−	−
UPDRS III (on/off)	20/NA	32/NA	NA	15/34	NA	NA	NA
Other psychosis	sch	sch	−	−	−	−	−
Special comment	−	−	−	−	−	−	RLS, RBD, facial dyskinesia with grimacing, severe dysarthria from onset

sch, schizophrenia; UPDRS, unified Parkinson's disease rating scale (motor score) in on and off condition; NA, not applicable or not available; RLS, restless legs syndrome; RBD, REM sleep behavior disorder; +, present; −, absent.

induced schizophrenia with hallucination. None of the patients in this cohort other than family A had schizophrenia. In addition, Patient B1 had hallucination and Patient D had depression.

DISCUSSION

In the present study, we set out to investigate whether Parkin and PINK1 could influence each other in patients with PD, based on the reports that Parkin and PINK1 share a common pathway using *Drosophila* models.[6,7] We identified digenic mutations of *parkin* and *PINK1* and found that *PINK1* mutation could modify the clinical course of *parkin* mutation-positive parkinsonism. Our results suggest that a single heterozygous mutation of *PINK1* might act not only as a susceptibility gene[3] but also as a modifier gene, in the pathogenesis of PD.

The relatively high frequency of *PINK1* heterozygous mutation identified in the present study (2.2% in PD vs. 0% in controls) is similar to that reported in a recent study (1.2% in PD vs. 0.4% in controls).[3] These results suggest that *PINK1* heterozygous mutation might also increase the risk of development of PD in patients who have mutations in other PD genes. Considering Patient D (Table 1), heterozygous *PINK1* p.E476K mutation was reported previously in three patients and two control subjects.[3,10] In addition, heterozygous p.P437L of *parkin* was found at the same frequency in patients and control subjects,[11] whereas none of Japanese 300 normal chromosomes harbored these mutations in the present study. This could represent differences based on ethnicity. Observation of patients carrying single nucleotide polymorphisms in both *parkin* and *PINK1* might be somewhat related to the position of mutated amino acids, the type of mutation, and one or more of the other gene mutations. On the other hand, the presence of asymptomatic carrier with the digenic mutations (family A-A1) also indicates the role of heterozygous mutation of *PINK1* in disease modification and suggests that other factors such as aging and environment are required for the development of the disease.

Based on recent reports, asymptomatic carriers of heterozygous *parkin* or *PINK1* mutations exhibit low ^{18}F-dopa uptake in the putamen on positron emission tomography.[12,13] These studies suggest that heterozygous mutation of *parkin* or *PINK1* gradually impairs the function of dopaminergic neurons. Interestingly, our patients of Family A, B, and D also developed

psychiatric disorders. Previous studies also reported that some *parkin* and *PINK1* mutations, even though heterozygous mutations, could be related to levodopa-responsive parkinsonism and psychiatric clinical pictures.[1,4,5] In this regard, our results might further indicate that *parkin* and *PINK1* mutations could be involved in psychiatric disorders not only singularly but also in combination. Furthermore, additional heterozygous *PINK1* mutation could hasten the age at onset of the disease. Combining the previous reports, our results emphasize that some heterozygous *PINK1* mutations might be related to the development of PD.[3,10] However, further genetic and functional analyses are required before one can make definite conclusions.

Intriguingly, digenic mutations of *PINK1-DJ-1* and *parkin-LRRK2* have recently been reported.[14,15] Screening for digenic or more mutations in responsible genes for familial PD could lead to the elucidation of the molecular pathway involved in nigral degeneration. In this regard, the mitochondrion is a good target for elucidating the pathogenesis of PD since Parkin, PINK1, and DJ-1 could be related to the mitochondrial function/dysfunction. Indeed, several studies highlighted the role of ARP gene products in maintaining mitochondrial function and in the pathogenesis of PD. Our results and these findings suggest that, multigenic mutation screening and analyses for interactions among related gene products could help enhance our understanding of the pathogenesis of PD.

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PARK9-LINKED PARKINSONISM IN EASTERN ASIA: MUTATION DETECTION IN *ATP13A2* AND CLINICAL PHENOTYPE

PARK9, a form of autosomal recessive parkinsonism, or Kufor-Rakeb syndrome (KRS), is characterized by subacute or slowly progressive, juvenile-onset, levodopa-responsive parkinsonism, pyramidal signs, dementia, and supranuclear gaze palsy.[1-5] Recently, *ATP13A2* was identified as the causative gene for *PARK9* in Chilean and Jordanian families.[4] This gene contains 29 exons encoding a lysosomal type 5 P-type ATPase. Six mutations have been reported in only five probands so far.[4,5] Here, we describe a Japanese patient with KRS with a novel mutation who developed early onset parkinsonism, dementia, and other features. We also describe PET findings of *PARK9*-linked parkinsonism.

Methods. Haplotype analysis was conducted in 117 (mainly Japanese) patients with early onset (≤50, 26.8 ± 11.7 years, mean ± SD) parkinsonism. Among them, 14 patients had dementia. Patients who exhibited homozygosity on *PARK9* locus by haplotype analysis underwent direct sequencing for all 29 exons (e-Methods on the *Neurology*® Web site at www.neurology.org); the remaining patients underwent direct sequencing for exons 13, 16, and 26, in which mutations have been identified.[4] The methods of direct sequencing, sequences of the primers, and PCR conditions are available (table e-1). The study was approved by the ethics committee of Juntendo University and all subjects gave informed consent.

Results. Twenty-eight of 117 patients exhibited homozygosity on *PARK9* locus. Among them, we found a Japanese proband (Family A) with a novel homozygous F182L (c.546C>A) mutation (figure e-1A). The consanguineous parents and the other two unaffected siblings had heterozygous F182L mutation. This mutation was not detected by direct sequencing of exon 6 in 300 chromosomes of normal controls.

Haplotype analysis showed homozygosity spanning the *PARK7* and *PARK9* regions (figure e-1B) in the proband and heterozygosity in her parents and the other two unaffected siblings. No causative mutation was detected in *DJ-1* and *PINK1* in all patients.

Supplemental data at www.neurology.org

The clinical features of the proband, a 43-year-old woman, are described in the table, the e-Case report, the video, and figure e-2. Neuroimaging showed several interesting findings: MRI showed diffuse brain and spinal cord atrophy, and ^{18}F-dopa PET study revealed reduced uptake in the striatum bilaterally (figure e-2).

Discussion. The cardinal features and diffuse brain atrophy of the proband closely resembled previously reported ones.[1-5] Therefore, it was possible that this patient was given a diagnosis of KRS clinically. Genetically, phenylalanine-182 is highly conserved throughout most species (figure e-1C). It has been reported that missense mutations in the loop between the transmembrane segment of the membrane protein (including *ATP13A2*) could affect disease phenotype significantly.[5] These findings and absence of F182L in normal controls support that the homozygous F182L mutation causes KRS.

Our findings of *ATP13A2* mutation in a Japanese family together with the reported Jordanian, Chilean, Brazilian, and Italian cases suggest that *PARK9* exists worldwide though rearrangements could not be excluded.[1-5] The role of a single heterozygous mutation remains unclear, although two symptomatic Italians and two asymptomatic Brazilian and four asymptomatic Japanese carriers have been reported.[5]

The clinical symptoms of our patient were similar to those reported previously.[1-5] However, there were also some different findings. Our patient was comparatively older at onset (22 years), without subacute onset like the Brazilian with homozygous missense mutation,[5] a slower progression rate compared with the Jordanian family (time of progression to bed-ridden state = 20 years vs 12 months),[1,3,4] and no apparent motor fluctuation. Our patient also showed inconsistent levodopa responsiveness with severe drug-induced psychosis and amyotrophy. These differences might be due to the different mutation types (such as missense/truncation mutations) or the different mutation localization.

A new interesting aspect of our report is neuroimaging in KRS. Although peripheral neuropathy was not apparent (e-Case report), our patient had generalized brain and spinal cord atrophy on MRI, which might reflect pyramidal tract degeneration and also multisystemic neurodegeneration in KRS by *ATP13A2* mutation. The pyramidal symptoms and weakness of the lower limbs, described previously in patients with KRS,[1] also could be caused by spinal cord atrophy.

PET findings of patients with levodopa-responsive autosomal recessive parkinsonism with *parkin*, *PINK1*, or *GBA* single heterozygous mutation indicate presynaptic dopaminergic dysfunction

Table Clinical features of patients with Kufor-Rakeb syndrome[1-5]

Origin of family	Japanese	Chilean				Jordanian				Brazilian	Italian	Italian
Patient	A	II-8	II-9	II-10	II-11	V44	V48	V49	V53	BR-3042	VE-29	PK-69-01
Zygosity	Homo	Comp hetero				Homo				Homo	Hetero	Hetero
Mutation	F182L	1019GfsX1021/1306+5G→A				552LfsX788				G504R	T12M	G533R
Age at onset, y	22	18	17	15	12	12	15	13	12	12	30	40
Disease duration, y	21	27	26	26	26	24	19	18	11	10	5	16
Initial symptoms	G	B, M	B, R	B, M	D	B, M, R	B, R	M, R	B, R	B	N/A	N/A
Clinical signs												
Increased muscle tone	+	+	+	+	+	+	+	+	+	+	+	+
Babinski sign	+	+	−	+	+	+	+	+	+	−	−	−
Palmomental reflex	+	+	−	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Tremor	+	+	+	+	+	−	−	−	−	−	+	−
Rigidity	+	+	+	+	+	+	+	+	+	+	+	+
Bradykinesia	+	+	+	+	+	+	+	+	+	+	+	+
Slowed saccade eye movement												
Vertical	+	N/A	−	+	+	+	+	+	+	N/A	N/A	N/A
Horizontal	−	N/A	−	+	+	+	+	−	−	N/A	N/A	N/A
Supranuclear upgaze palsy	+	+	−	+	+	+	+	+	+	+	−	−
FFF mini-myoclonus	+	+	−	+	+	+	+	+	+	−	N/A	N/A
Hallucination	+	+	+	−	−	+	+	+	+	+	−	+
Dementia (MMSE)	15/30	N/A	19/30	15/30	9/28	14/30	2/30	13/30	2/30	−	−	−
Response to anti-PD drugs												
Trihexyphenidyl	N/A	+	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Levodopa	+	N/A	No tolerance	No tolerance	N/A	+	+	+	+	+	+	+

Homo = homozygous; Comp hetero = compound heterozygous; Hetero = heterozygous; B = bradykinesia; M = mental retardation; R = rigidity; D = developmental disturbance; G = gait disturbance; FFF mini-myoclonus = facial-faucial-finger mini-myoclonus; MMSE = Mini-Mental State Examination; PD = Parkinson disease; − = absent; + = present; N/A = not assessed.

in striatonigral system, in contrast to postsynaptic dysfunction in multiple system atrophy and progressive supranuclear palsy without levodopa responsiveness.[6] ^{18}F-dopa PET scan of our patient with levodopa-responsive parkinsonism with homozygous *ATP13A2* mutation also showed a presynaptic pattern often observed in idiopathic PD.

Intriguingly, the *GBA* gene encoding lysosomal enzyme was reported to be associated with synucleinopathies such as Lewy body diseases. Since the lysosomal degradation pathway can clear α-synuclein aggregates,[7] lysosomal dysfunction by *ATP13A2* or *GBA* mutation could be important in the pathogenesis of parkinsonism.

Altogether, our findings expand the phenotypic spectrum associated with *PARK9*-linked parkinsonism into multiple-system disorders. Furthermore, functional analysis of *ATP13A2* could open a new therapeutic window in widespread neurodegenerative disorders.

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NEUROFERRITINOPATHY IN A JAPANESE FAMILY WITH A DUPLICATION IN THE FERRITIN LIGHT CHAIN GENE

Neuroferritinopathy is a rare autosomal dominant movement disorder with the deposition of iron and ferritin within the basal ganglia. Four different pathogenic mutations in the ferritin light polypeptide (FTL) gene have been reported.[1-4] The variety of its clinical features makes the diagnosis of neuroferritinopathy difficult. In this study we investigated a Japanese family with neuroferritinopathy to clarify the phenotypic and genetic spectrum of neuroferritinopathy.

Proband. A 42-year-old Japanese man first developed hand tremors in his middle teens. He noticed his right foot dragging at age 35, and generalized hypotonia, hyperextensibility, aphonia, micrographia, hyperreflexia, and cognitive impairment (IQ = 66) at age 42. His unsteady gait with long steps, with his arms and legs dangling, seemed to be due mainly to hypotonus. Rigidity, spasticity, dystonia, or chorea were not observed. His serum ferritin concentration was 5 μg/L (normal = 33 to 330). A brain MRI revealed bilateral symmetric cystic changes of the pallidum and the striatum. Hyperintense lesions in the T2-weighted imaging involved the thalamus, dentate nucleus, and substantia nigra.

The proband's mother had developed hand tremors at age 10. She presented with difficulty walking at age 35 and developed cognitive impairment and akinetic mutism, and died at age 64. Her CT imaging showed cystic changes of the pallidum and the striatum. None of the proband's relatives, except for his mother, had any neurologic symptoms.

Methods. After informed consent was obtained, genomic DNA was extracted from a blood sample of the proband and was amplified by PCR. The entire coding region of the FTL gene was sequenced using a BigDye Terminator Cycle Sequencing Kit according to the manufacturer's protocol. In order to confirm the mutation, the PCR-RFLP assay was developed with AciI. We have not performed genetic testing in any asymptomatic family member because informed consent was not obtained.

Results. In exon 4 of the *FTL* gene, duplication of the 469–484 sequence was found (figure, A). The mutation replaces the C-terminal 14 amino acid residues with a novel 23 amino acid sequence (figure, B). This mutation is described as c.469_484dup16nt (p.Leu162Arg*fs*X185) in standard genetic nomenclature. The mutation was not found in 20 control chromosomes and after BLASTN searching of International Nucleotide Sequence Database Collaboration (INSDC). The mutation creates the gain of an AciI restriction site, proven by PCR–restriction fragment length polymorphism analysis (figure, C).

Discussion. Neuroferritinopathy was first reported in 2001. The original mutation, an insertion of adenine in position 460–461 (460InsA), has been found mainly in cases of neuroferritinopathy of the north of England.[1] The insertion of a dinucleotide, thymine and cytosine, in position 498–499 was detected in a French family.[2] The insertion of a cytosine in position 646–647 was reported in a family of French Canadian and Dutch ancestry.[3] A missense mutation in position 474 of guanine to adenine was found in a family of Gypsy ancestry.[4] In this study, we found a novel mutation, the duplication of the 469–484 sequence of the *FTL* gene in a Japanese family. This is the first family with neuroferritinopathy of non-European origin. The deceased proband's mother was undoubtedly affected by neuroferritinopathy based on her clinical features and CT findings. All of her relatives, except for the proband, had no neurologic symptoms. Considering the high penetration of neuroferritinopathy,[5] we suspect that a new genetic mutation in the *FTL*