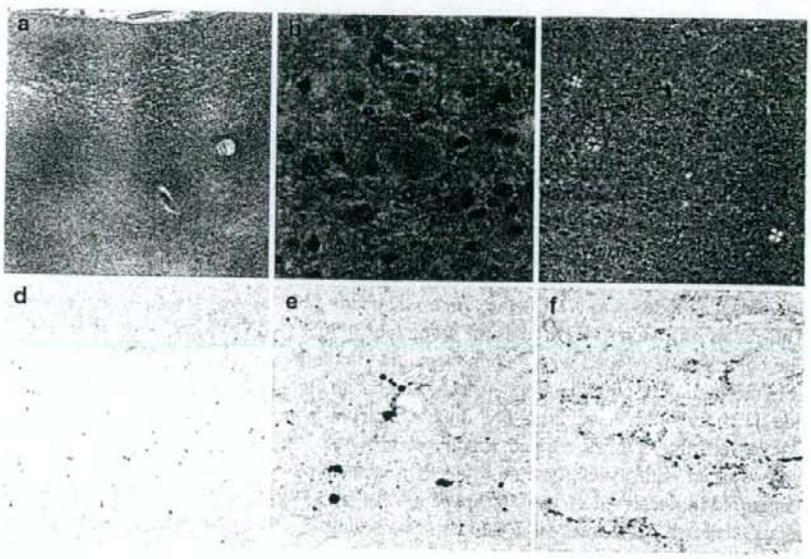

Fig. 3 **a** The cerebral cortex showed extensive neuronal loss and gliosis. The white matter was also severely damaged (frontal cortex of patient 2; H&E; ×20). **b** Amyloid plaque in the basal ganglia (patient 2; H&E; ×600). **c** Amyloid plaques in the white matter were stained with Congo red and showed green birefringence under polarized light (parahippocampal gyrus of patient 1; Congo red; ×200). **d–f** Immunohistochemistry for PrP revealed numerous plaque-type PrP deposits in the white matter of the frontal cortex (**d** patient 2; #71 antibody; ×40), parahippocampal gyrus (**e** patient 1; #71 antibody; ×200), and basal ganglia (**f** patient 2; #71 antibody, ×100)

Table 2 Distribution of PrP-amyloid plaques

Patient	PrP-amyloid plaques in the white matter[a]					
	Frontal cortex	Occipital cortex	Parahippocampus	Basal ganglia	Thalamus	Cerebellum
1	+	–	+++	+++	+	++
2	+++	+	+++	+++	++	++
3	–	–	++	–	–	–

[a] The sum of the number of PrP-positive plaques in 10 fields (×100 magnification)
– 0, + 1–10, ++ 11–100, +++ 100<

Discussion

Here we report three patients with sCJD-MM1 who showed prominent PrP-positive amyloid plaques in the cerebral and cerebellar white matter. PrP-amyloid plaques have been extremely rare in sCJD-MM1 cases, but the present three patients demonstrate that this could occur.

To date, CJD cases with the 129 M/M genotype and plaque-type PrP deposits have been mainly recognized in the infectious form of CJD, e.g., variant CJD (vCJD) or iatrogenic CJD. Only a single sCJD-MM1 case with amyloid plaques has been reported [4], but this case had a history of neurosurgery. Thus, the possibility of iatrogenic transmission of CJD other than sCJD-MM1 could not be excluded. In the present study, however, none of the three patients had any history of neurosurgery or hormone therapy. Moreover, the size of PrP^{res} in the brain was identical with that of type 1 PrP^{res} from a typical sCJD-MM1 case. Type 2 PrP^{res} or the intermediate type PrP^{res} [8], which is observed in vCJD or the plaque-type of dura graft-associated CJD (p-dCJD) cases, was not detected in the brains of the three patients. In addition, PrP-amyloid plaques were restricted within the white matter in the present cases. The distribution of the plaques differed from that observed in vCJD or p-dCJD cases. Therefore, it is certain that the three patients in this report should be classified into sCJD-MM1, but not the infectious form of CJD.

Co-accumulation of APP and PrP suggests that the axonal damage, i.e., impaired axonal transport, causes the deposition of PrP-amyloid plaques in the white matter of sCJD-MM1 cases. APP is transported by the fast anterograde component of axonal flow [9]. Under various pathological conditions, APP accumulates within the axonal swellings because of impaired axonal transport [13]. In the present study, PrP-amyloid plaques were identified exclusively in the white matter and most commonly in conjunction with APP. These findings indicate that PrP might accumulate within the axonal swellings in the damaged white matter and then form amyloid plaques. However, since plaque-type PrP deposits were more than APP deposits in all brain sections examined and APP accumulation was not observed in patient 3, we cannot rule out the reverse hypothesis that PrP-plaques in the white matter induce the axonal damage in case of long disease duration.

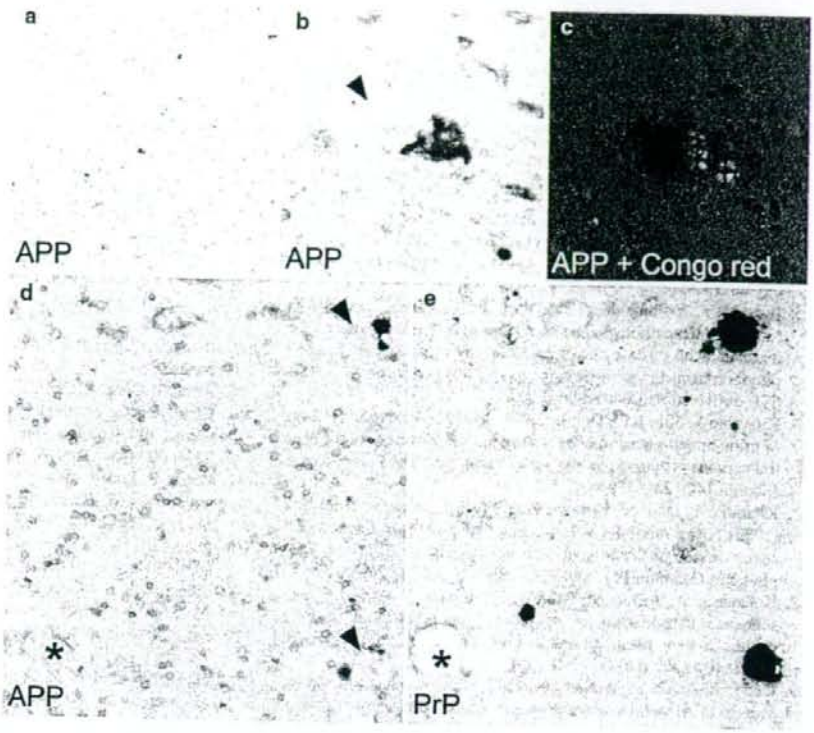

Fig. 4 **a** Immunohistochemistry using anti-APP antibody revealed many APP accumulations in the cerebral white matter (basal ganglia of patient 2; UT-18 antibody; ×100). **b** APP immunoreactivity adjacent to amyloid plaque-like structure (*arrowhead*) (basal ganglia of patient 2, UT-18 antibody; ×600). **c** Double staining with the APP immunohistochemistry and Congo red revealed co-localization of APP and amyloid plaques (basal ganglia of paeitnt 2; UT-18 antibody and Congo red; ×400). **d, e** Immunohistochemical analysis of the serial sections using anti-APP or anti-PrP antibody revealed that the APP immunoreactivities were co-localized with PrP-positive amyloid plaques (*arrowheads*) (parahippocampal gyrus of patient 1; **d** UT-18 antibody; **e** #71 antibody; ×400). *Asterisk* denotes a landmark vessel to indicate the serial sections

The most plausible explanation for the axonal damage is the prolonged disease duration. The three patients in this report showed clinical courses of long duration (2 years ≤). In sCJD-MM1 cases with long disease duration, the white matter is severely affected as the end-stage pathology of CJD [2, 5]. These findings lead us to surmise that PrP-amyloid plaques in the white matter may be a common feature of sCJD-MM1 cases with prolonged disease duration. However, it has been reported that four Japanese sCJD-MM1 cases with long disease duration of over 2 years showed no plaque-type PrP deposition in the white matter [5]. Therefore, not only the disease duration but also unidentified factors such as pharmacological treatment, minor trauma, or hypoxia during the clinical course might modify the pattern of PrP deposition.

As an explanation for PrP-amyloid plaques, we cannot rule out the possible existence of different prion strains in sCJD-MM1 prions. However, the biochemical properties of PrP^{Sc} in the brain are identical between the sCJD-MM1 cases with long and short disease duration [2]. In line with these findings, the three patients in this report had type 1 PrP^{res} identical in size and glycoform ratio to that from typical sCJD-MM1 cases. In addition, in contrast to the prolonged end-stage, the exacerbation periods of the three patients were as short as those of typical sCJD-MM1 cases. Thus, it is highly unlikely that an atypical prion strain caused PrP-amyloid plaques in the present cases. To exclude this possibility, it will be necessary to perform a transmission study using sCJD-MM1 prions from the present three patients.

In conclusion, this study shows that PrP-amyloid plaques can occur in the white matter of sCJD-MM1 cases with prolonged disease duration. Although plaque-type PrP deposition is a characteristic of other subtypes of CJD including sCJD-MV2, VV2, vCJD or p-dCJD, the plaques in the white matter of these cases might also have resulted from the axonal damage reflecting the prolonged disease duration rather than prion strain-dependent properties.

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NEUROLOGY

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

Y. P. Ning, K. Kanai, H. Tomiyama, Y. Li, M. Funayama, H. Yoshino, S. Sato, M. Asahina, S. Kuwabara, A. Takeda, T. Hattori, Y. Mizuno and N. Hattori

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Y.P. Ning, MD, PhD
K. Kanai, MD, PhD
H. Tomiyama, MD
Y. Li, MD
M. Funayama, PhD
H. Yoshino, BS
S. Sato, MD, PhD
M. Asahina, MD, PhD
S. Kuwabara, MD, PhD
A. Takeda, MD, PhD
T. Hattori, MD, PhD
Y. Mizuno, MD
N. Hattori, MD, PhD

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.

From the Department of Neurology (Y.P.N., H.T., Y.L., S.S., N.H.) and Research Institute for Diseases of Old Ages (M.F., H.Y., Y.M., N.H.), Juntendo University School of Medicine, Tokyo; Department of Neurology (K.K., M.A., S.K., T.H.), Graduate School of Medicine, Chiba University; and Department of Neurology (A.T.), Tohoku University School of Medicine, Miyagi, Japan.

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Address correspondence and reprint requests to Prof. Nobutaka Hattori, Department of Neurology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan; nhattori@med.juntendo.ac.jp

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E. Ohta, MD, PhD
T. Nagasaka, MD, PhD
K. Shindo, MD, PhD
S. Toma, MD, PhD
K. Nagasaka, MD
K. Ohta, MD, PhD
Z. Shiozawa, MD, PhD

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*

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Analysis of Lrrk2 R1628P as a Risk Factor for Parkinson's Disease

Owen A. Ross, PhD,[1] Yih-Ru Wu, MD,[2] Mei-Ching Lee, MD,[3] Manabu Funayama, PhD,[4] Meng-Ling Chen, MSc,[5] Alexandra I. Soto, BSc,[1] Ignacio F. Mata, PhD,[6] Guey-Jen Lee-Chen, PhD,[7] Chiung Mei Chen, MD, PhD,[2] Michelle Tang, BSc,[8] Yi Zhao, MD, PhD,[8] Nobutaka Hattori, MD, PhD,[4,9] Matthew J. Farrer, PhD,[1] Eng-King Tan, MD,[8,10] and Ruey-Meei Wu, MD, PhD[5]

Common genetic variants that increase the risk for Parkinson's disease may differentiate patient subgroups and influence future individualized therapeutic strategies. Herein we show evidence for *leucine-rich repeat kinase 2 (LRRK2)* c.4883G>C (R1628P) as a risk factor in ethnic Chinese populations. A study of 1,986 individuals from 3 independent centers in Taiwan and Singapore demonstrates that Lrrk2 R1628P increases risk for Parkinson's disease (odds ratio, 1.84; 95% confidence interval, 1.20–2.83; $p = 0.006$). Haplotype analysis suggests an ancestral founder for carriers approximately 2,500 years ago. These findings support the importance of *LRRK2* variants in sporadic Parkinson's disease.

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The discovery of *leucine-rich repeat kinase 2 (LRRK2)* mutations in both familial and sporadic forms of Parkinson's disease (PD) has caused a paradigm shift in the field. Six Lrrk2 substitutions have been proven to play a role in PD pathogenesis or susceptibility, and are distributed throughout the different protein domains, suggesting that each domain is critical for normal physiological Lrrk2 function (Roc, C-terminal of ROC [COR], mitogen-activated protein kinase kinase kinase, and WD40).[1] However, the *LRRK2* gene harbors numerous other nonsynonymous variants (>70), and the functional role of these variants, whether they are benign single nucleotide polymorphisms (SNPs) pathogenic mutations, or risk factors for disease, remains unresolved.

The recently identified genetic risk factor Lrrk2 G2385R is observed in approximately 5% of the healthy Asian population, increasing to approximately 10% in populations with sporadic, late-onset PD.[2–8] Lrrk2 G2385R is located in the WD40 domain and is hypothesized to impair Lrrk2 dimerization/scaffold formation and to promote apoptosis.[3,6] Herein we provide evidence to support Lrrk2 R1628P (rs33949390), within the COR domain, as the second genetic risk factor for PD identified in the ethnic Chinese population.

Subjects and Methods

Subjects

A total of 1,079 ethnic Han Chinese patients (average age at onset, 62 years) from Taiwan and Singapore have been examined clinically and are being longitudinally observed by neurologists at 3 centers (R.-M.W., Y.-R.W., C.M.C., and E.-K.T.) (Table 1). Of the 1,079 patients, 44 reported a family history of disease (defined as 1 or more relatives with parkinsonism within 3 meiosis of relationship), 179 presented with early-onset PD (<50 years), and 900 patients had typical late-onset PD (≥50 years). All patients fulfilled criteria for a clinical diagnosis of PD with at least two of three cardinal signs (tremor, rigidity, and bradykinesia) and a positive response to L-dopa therapy.[9] A total of 907 ethnically matched Han Chinese control subjects (average age, 57 years) without evidence of neurological disorder were also recruited from participating centers. Population stratification is minimized because these study participants are all of Han Chinese descent. In addition, 151 PD patients and 95 control subjects from the Japanese population, diagnosed at Juntendo University (by M.F. or N.H.), were included in the study. Research protocols were reviewed by the institutional ethics board committee of each center, and all subjects gave informed consent.

Genetic Analysis

LRRK2 c.4883G>C (R1628P; rs33949390) was genotyped by restriction fragment length polymorphism (RFLP) or ABI Taqman (Applied Biosystems, Foster City, CA) "by-design" oligonucleotide probes and positives confirmed by direct DNA sequencing of exon 34, as described previously.[10]

Haplotype analysis was performed on 32 Lrrk2 R1628P carriers with chromosome 12q12 polymorphic markers amplified by polymerase chain reaction using fluorescently la-

From the [1]Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL; [2]Department of Neurology, Chang Gung Memorial Hospital and Chang Gung University, College of Medicine; [3]Department of Neurology, Cathay General Hospital, Taipei, Taiwan; [4]Research Institute for Diseases of Old Ages, Juntendo University School of Medicine, Bunkyo, Tokyo, Japan; [5]Department of Neurology, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan; [6]Department of Neurology, University of Washington School of Medicine, Seattle, WA; [7]Department of Life Science, National Taiwan Normal University, Taipei, Taiwan; [8]Department of Neurology, Singapore General Hospital, National Neuroscience Institute of Singapore; [9]Department of Neurology, Juntendo University School of Medicine, Bunkyo, Tokyo, Japan; [10]Duke-NUS Graduate Medical School, Singapore.

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Address correspondence to Dr Wu, Department of Neurology, National Taiwan University Hospital, No. 7, Chung-Shan South Road, Taipei 100, Taiwan. E-mail: robinwu@ntu.edu.tw

Table 1. Allele and Genotype Frequencies of *LRRK2* c.4883G>C (R1628P; rs33949390)

Series	Affection Status	Genotype GG (n)	Genotype GC (n)	Genotype CC (n)	Carrier Frequency	G Allele (n)	C Allele Frequency	Allelic p	OR (95% CI)
1. R.-M. Wu	Patients (n = 484)	452	31	1	6.6%	935	33 (3.4%)	0.025	2.15 (1.08–4.29)
	Control Subjects (n = 341)	330	11	0	3.2%	671	11 (1.6%)		
2. Y.-R. Wu	Patients (n = 345)	324	21	0	6.1%	669	21 (3.0%)	0.179	1.39 (0.70–2.75)
	Control Subjects (n = 316)	302	14	0	4.4%	618	14 (2.2%)		
3. E.-K. Tan	Patients (n = 250)	237	13	0	5.2%	487	13 (2.6%)	0.163	2.20 (0.83–5.83)
	Control Subjects (n = 250)	244	6	0	3.0%	494	6 (1.2%)		
Overall	Patients (n = 1,079)	1,013	65	1	6.1%	2091	67 (3.1%)	0.006	1.84 (1.20–2.83)
	Control Subjects (n = 907)	876	31	0	3.4%	1783	31 (1.7%)		

Displays the frequencies observed for the *leucine-rich repeat kinase 2 (LRRK2)* c.4883G>C (R1628P; rs33949390) variant in each of the three series. Series 1 and 2 are from Taiwan, an island of the east coast of China, and Series 3 uses subjects from Singapore, an island of the south coast of Malaysia. p values are calculated by χ^2 with Yates correction. Power calculations suggest that for replication studies in the ethnic Chinese population given a disease allele frequency in cases of 0.061 and odds ratio (OR) of 1.84, a sample size of 614 patients and an equal number of matched control subjects would be required to have 80% power to observe a statistically significant difference ($p < 0.05$).

beled primers (sequences are available on request). DNA products were run on an ABI3730 and analyzed using GeneMapper software (Applied Biosystems, Foster City, CA) alongside standard controls (CEPH 1331-01 and -02). Physical map positions are given with reference to the March 2006 human reference sequence (National Center for Biotechnology Information Build 36.1). Using marker allele frequencies in the putative, mutation-bearing ancestral haplotype in comparison with the noncarrier population, we estimated the age of the Lrrk2 R1628P variant. In brief, under the assumption of an ancestral haplotype, marker frequencies were referenced as 0.99 in carriers and empirically determined in noncarriers (n = 80). Linkage disequilibrium index (δ) between each marker and mutation was calculated.[11] Average genetic distances and recombination fractions (θ) were estimated between each marker and *LRRK2* using the Marshfield recombination map. The age of the mutation in generations (g) was derived from the equation $g = \ln \delta / \ln(1 - \theta)$ for each marker.[12]

Results

The Lrrk2 R1628P variant is approximately twice as frequent in affected individuals as control subjects (odds ratio, 1.84; 95% confidence interval, 1.20–2.83; $p = 0.006$) (see Table 1). Independently the same trend was observed in each ethnic Chinese series although statistical significance was not reached in two cohorts given their size and relatively low frequency of the 1628P allele (see Table 1). Unaffected carriers in Series 2 (Y.-R.W.) are approximately 10 years younger than affected carriers (51 vs 61 years of age), which may affect statistical significance. The Lrrk2 R1628P variant was not observed in our 246 Japanese subjects.

One Lrrk2 R1628P carrier was sequenced for all exons and exon-intron boundaries of *LRRK2* in our previous study.[10] No other variant was observed that could account for the associated risk. Even though all Lrrk2 R1628P carriers also harbor Lrrk2 S1647T and two additional synonymous changes (G1624G and K1637K) in exon 34, their relatively high allele frequency, global dispersion, and lack of significance in previous PD association studies indicates they are unlikely to influence disease risk.[13,14] These data support the hypothesis that the Lrrk2 R1628P substitution is the functional risk factor in carriers.

Haplotype Analysis

Our haplotype results suggest that Lrrk2 R1628P carriers are related to a single common founder (Table 2). We observed SNP alleles located in exon 34 adjacent to *LRRK2* c.4883G>C (R1628P; rs33949390), which cosegregates with the mutation. Population stratification does not appear to influence our association because these shared SNP alleles are present in both affected and unaffected carriers demonstrating a shared genetic background. Data from adjacent microsatellite

Table 2. **Chromosome 12q12 Haplotype Analysis of *LRRK2* c.4883G>C (R1628P; rs33949390) Carriers**

Marker Name	Position[a]	A	B	C	D	E	F	G	H	I	J	Shared Alleles
D12S2080	33,305,718	184/188	196/196	196/200	184/196	184/188	192/196	188/196	188/188	184/188	192/196	
D12S2194	38,738,008	249/253	249	249	249/253	249	249	249	249	249/257	245/249	249
rs11175964	38,989,254	GG	GG	GA	GG	GG	GG	GG	GG	GG	GA	G
D12S2516	38,989,339	252/254	254	254	252/254	252/254	252/254	252/254	252/254	252/254	254	254
rs1896252	39,000,026	TC	CC	CC	TC	TC	TC	TC	TC	TC	CC	C
rs1427263	39,000,101	CA	AA	AA	CA	CA	CA	CA	CA	CA	AA	A
rs33949390	39,000,112	GC	GC	GC	GC	GC	GC	GC	GC	GC	GC	C
rs11176013	39,000,140	GA	GG	GG	GA	GA	GA	GA	GA	GA	GG	G
rs11564148	39,000,168	TA	AA	TA	TA	TA	TA	TA	TA	TA	TA	A
rs10878405	39,028,521	GA	AA	GA	GA	GA	GA	GA	GA	GA	GA	A
rs11176143	39,028,630	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	G
D12S2519	39,116,885	132/138	132	132	132	134/140	132/138	134/138	132/140	132/134	132/140	132
D12S2521	39,128,754	323/351	351/367	351/367	351/363	319/375	323/351	327/379	319/351	363/379	319/351	351
D12S2522	39,132,267	281/297	297	297/299	297	283/297	281/297	281/297	283/297	297	285/297	297
D12S2517	39,282,898	188	182/188	188/192	182/188	188/190	188/190	186/188	188/190	182/188	188/202	188
D12S1301	42,348,809	116/120	100/116	100/116	112/124	112/116	100/120	104/120	104/116	116/116	116/120	

We examined 32 carriers for haplotype analysis on the surrounding chromosome 12q12 region with results indicative of a common ancestral founder in carriers.

[a]Microsatellite allele sizes were normalized using CEPH-control DNA (1331-01 and 1331-02), and approximate positions are determined from the National Center for Biotechnology Information (NCBI) March 2006 human genome assembly. The shared alleles between markers D12S2194 and D12S2517 indicate a minimum ancestral haplotype of approximately 500kb and are highlighted in the last column. Allele 351 for marker D12S2521 is found in 74% (n = 32) of Lrrk2 R1628P carriers and is rare in noncarriers (n = 80; 1%). Nonsharing was observed for markers D12S2519 (n = 3; 9.3%) and D12S2521 (n = 8; 25%). However, given the distance from the mutation of approximately 100Kb and the shared single nucleotide polymorphism (SNP) data, these are most likely due to recombination events.

markers are in good agreement; allele 351 of D12S2521 is rare in the general population (n = 1/80; 1%) but frequent in Lrrk2 R1628P carriers (n = 23/31; 74%), consistent with one ancestral haplotype. However, for a number of carriers, historical recombination may have occurred between markers D12S2519 and D12S2522. Allele sharing between markers D12S2194 to D12S2517 indicates a minimum ancestral haplotype of approximately 500kb.

It is possible to generate an estimate of the age of the mutational event using the allele frequencies of the markers in Lrrk2 R1628P carriers and noncarriers, and the excess of linkage disequilibrium. However, low sample numbers and unphased haplotypes are two caveats of this approach. It should also be noted that this calculation is under the assumption of a major ancestral haplotype, and that nonallele sharing is due to recombination and not independent founders, thus biasing the estimate toward a more recent event. From our calculations assuming each generation to approximate 30 years, *LRRK2* c.4883G>C (R1628P; rs33949390) occurred 89 generations ago (95% confidence interval, 85–92) or approximately 2,500 years ago.

Discussion

Lrrk2 R1628P is the second major risk variant identified in the ethnic Chinese populations of Taiwan and Singapore. The clinical phenotype of affected Lrrk2 R1628P carriers is typical late-onset L-dopa–responsive PD. The average age at onset in our affected Lrrk2 R1628P carriers is 60 years. Of note, the average age in our unaffected carriers is 5 years younger (55 years) and suggests some may yet develop PD symptoms.

Lrrk2 R1628P appears to be restricted to the ethnic Chinese population. We did not observe the Lrrk2 R1628P in 246 Japanese subjects, and this absence is supported by a *LRRK2* sequencing project that did not observe the variant in 36 probands with familial PD of Japanese descent (Dr Cyrus Zabetian, personal communication). Our estimation of the mutation's age (approximately 2,500 years) coupled with population-specific mutation frequencies in Taiwan, Singapore, and Japan provides evidence that the Lrrk2 R1628P substitution occurred some 2,000 years later than Lrrk2 G2385R. Given the global ethnic Chinese Diaspora, it is likely both variants will be observed in communities outside of the Asian continent. Of note, the dbSNP database does record one carrier of European

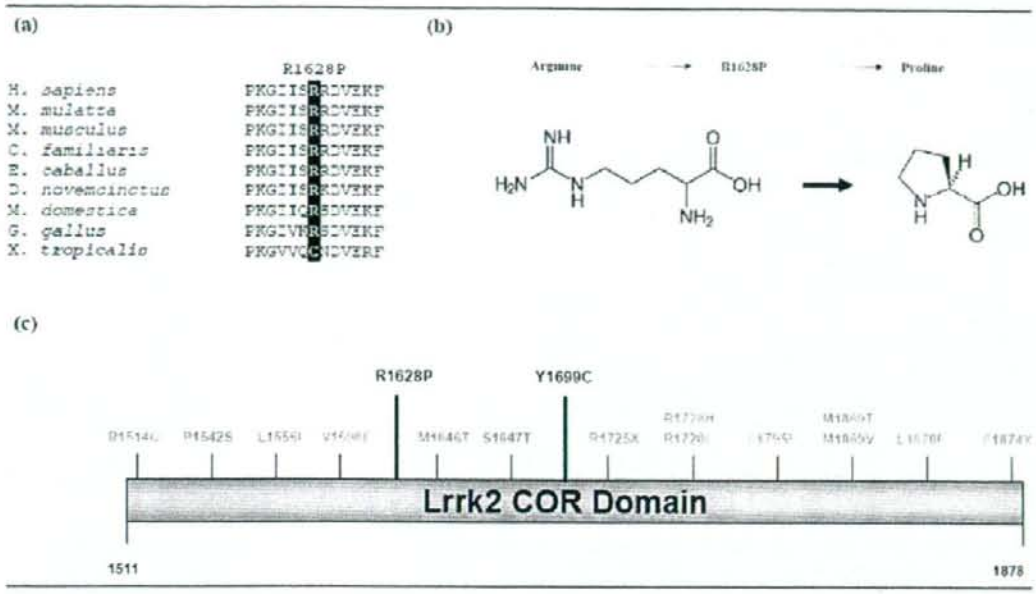

Fig. The COR domain (C) extends from amino acid 1511 to 1878 and contains 16 reported nonsynonymous changes (pathogenic variant Lrrk2 Y1699C is highlighted).[1] The Lrrk2 R1628P substitution (B) results in the replacement of an arginine with a cyclic proline residue. Given the conservation (A) at this amino acid position across species, this substitution may disrupt an important protein-protein interaction or the observed dimerization of the Lrrk2 protein.

descent; this may be a rare independent event, but previous studies have failed to identify any non-Asian carriers ($n > 2,500$), and it is equally likely this individual has some Asian genetic background.

To date, genome-wide association studies have not found such risk factors in US PD patients. A question remains whether multiple variants with small effect sizes contribute to complex disorders such as PD. Lrrk2 R1628P and G2385R in ethnic Chinese samples provide support for this hypothesis. Although no subjects with Lrrk2 R1628P and G2385R were observed in our study, no doubt carriers with digenic inheritance will be identified, and it will be interesting to assess whether a potential increased level of susceptibility exists in such individuals. However, it should be noted that homozygous Lrrk2 G2019S carriers do not appear to present with a more severe phenotype than heterozygous carriers.[15]

Lrrk2 R1628P is located in the COR domain and is evolutionarily conserved across species highlighting the importance of the residue to protein function (Fig). Indeed, the substitution of a highly basic polar arginine (R) with a neutral nonpolar proline (P) is likely to cause a conformational change in Lrrk2 secondary structure; proline is considered an α-helix breaker that introduces a β-hairpin turn. We postulate this substitution affects the dynamic interaction among the Roc, COR, and mitogen-activated protein kinase kinase kinase domains critical for activity, and may disrupt Lrrk2 dimerization.

Herein we present the first evidence to support Lrrk2 R1628P as the second common genetic risk factor for PD in the ethnic Chinese population. Moreover, we have reproduced the risk effect in a multicenter approach with combined pooled analysis of other ethnic Chinese series (see Table 1). This collaborative approach will be crucial in determining the pathogenicity of other *LRRK2* variants. Future therapeutic interventions will most likely be determined by the genomic background of the individual; thus, identification of common risk factors in PD (odds ratio, ≤2) will have a profound effect on diagnosis and treatment.

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Gene Expression Study on Peripheral Blood Identifies Progranulin Mutations

Giovanni Coppola, MD,[1] Anna Karyda, BA,[2] Rosa Rademakers, PhD,[3] Qing Wang, PhD,[1] Matt Baker, BSc,[3] Mike Hutton, PhD,[3] Bruce L. Miller, MD,[2] and Daniel H. Geschwind, MD, PhD[1]

Peripheral blood is a readily available tissue source allowing relatively noninvasive screening for a host of medical conditions. We screened total-blood progranulin *(PGRN)* levels in 107 patients with neurodegenerative dementias and related conditions, and 36 control subjects, and report the following findings: (1) confirmation of high progranulin expression levels in peripheral blood; (2) two subjects with reduced progranulin levels and mutations in the *PGRN* gene confirmed by direct sequencing; and (3) greater *PGRN* messenger RNA levels in patients with clinical diagnosis of Alzheimer's disease. This proof-of-principle report supports the use of gene quantification as diagnostic screen for *PGRN* mutations and suggests a potential role for progranulin in Alzheimer's disease.

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Frontotemporal lobar degeneration (FTLD) comprises a group of dementias with related clinical and neuropathological characteristics.[1] FTLD is the second most common cause of presenile dementia after Alzheimer's disease (AD)[1–3] and accounts for 5 to 10% of neurodegenerative dementias in epidemiological samples and between 9 and 16% in autopsy series.[4] Clinical subtypes of FTLD include (1) a behavioral variant with predominant frontotemporal involvement, (2) semantic dementia, and (3) primary progressive aphasia. A family history is present in about 40% of the FTLD patients, and four genes have been discovered as genetic causes. Mutations in *MAPT* have been identified in more than 100 families, and 2 other causative genes (*VCP*[4] and *CHMP2B*[5])

From the [1]Department of Neurology, Program in Neurogenetics, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles; [2]Department of Neurology, Memory and Aging Center, University of California, San Francisco, San Francisco, CA; and [3]Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL.

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Address correspondence to Dr Geschwind, Department of Neurology, David Geffen School of Medicine at UCLA, 695 Charles E. Young Drive South, Los Angeles, CA 90095. E-mail: dhg@ucla.edu

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

Manabu Funayama, PhD,[1] Yuanzhe Li, MD,[2] Tak-Hong Tsoi, FHKAM, FRCP,[3] Ching-Wan Lam, MBChB, PhD,[4] Takekazu Ohi, MD, PhD,[5] Shogo Yazawa, MD, PhD,[6] Eiichiro Uyama, MD, PhD,[7] Ruth Djaldetti, MD,[8] Eldad Melamed, MD,[8] Hiroyo Yoshino, BS,[1] Yoko Imamichi,[2] Hiroshi Takashima, MD, PhD,[9] Kenya Nishioka, MD, PhD,[2] Kenichi Sato, MD, PhD,[10] Hiroyuki Tomiyama, MD,[2] Shin-Ichiro Kubo, MD, PhD,[2] Yoshikuni Mizuno, MD,[1] and Nobutaka Hattori, MD, PhD[1,2]*

[1]Research Institute for Diseases of Old Age, Juntendo University School of Medicine, Bunkyo-Ku, Tokyo, Japan; [2]Department of Neurology, Juntendo University School of Medicine, Bunkyo-Ku, Tokyo, Japan; [3]Department of Medicine, Pamela Youde Nethersole Eastern Hospital, Hong Kong, China; [4]Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, China; [5]Department of Neurology, Kurashiki Central Hospital, Kurashiki, Okayama, Japan; [6]Department of Neurology, Miyazaki Prefectural Hospital of Nobeoka, Nobeoka, Miyazaki, Japan; [7]Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Kumamoto, Japan; [8]Department of Neurology, Rabin Medical Center, Beilinson Campus, Petah Tiqva, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; [9]Department of Neurology and Geriatrics, Kagoshima University School of Medicine, Kagoshima, Japan; [10]Department of Neurology, Juntendo University Nerima Hospital, Nerima-Ku, Tokyo, Japan

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**

*Correspondence to: Dr. Nobutaka Hattori, Department of Neurology, Juntendo University Graduate School of Medicine and Dental Sciences, Bunkyo-Ku, Tokyo 113-8421, Japan.
E-mail: nhattori@med.juntend.ac.jp

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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

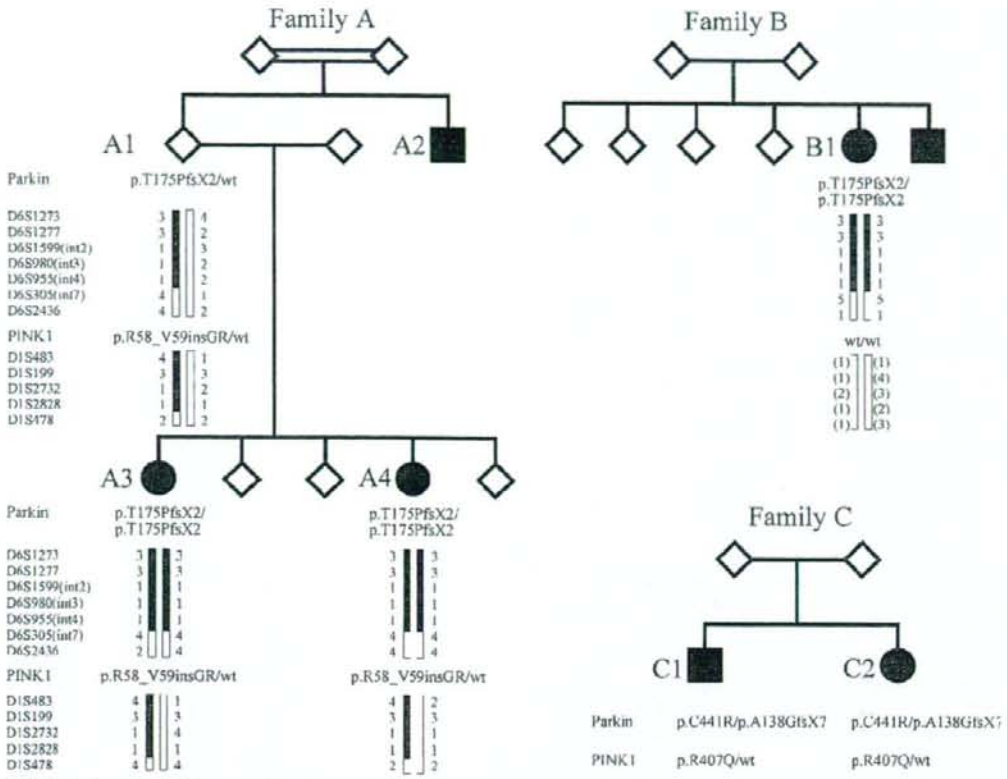

FIG. 1. Pedigrees of families analyzed in this study. Solid bars indicate shared disease haplotype. DNA of Patient A2 was not available. The haplotypes with undetermined phases in proband B-B1 are shown in parentheses. Int, intron.

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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Mutation Analyses in Amyotrophic Lateral Sclerosis/ Parkinsonism–Dementia Complex of the Kii Peninsula, Japan

Hiroyuki Tomiyama, MD,[1] Yasumasa Kokubo, MD,[2] Ryogen Sasaki, MD,[2] Yuanzhe Li, MD,[1] Yoko Imamichi,[1] Manabu Funayama, PhD,[3] Yoshikuni Mizuno, MD,[3] Nobutaka Hattori, MD, PhD,[1] and Shigeki Kuzuhara, MD[2,4]*

[1]*Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan*
[2]*Department of Neurology, Mie University School of Medicine, Tsu, Japan*
[3]*Research Institute for Diseases of Old Ages, Juntendo University School of Medicine, Tokyo, Japan*
[4]*Department of Neurology, National Center Hospital of Neurology and Psychiatry, Tokyo, Japan*

Abstract: To clarify the genetic background of amyotrophic lateral sclerosis (ALS)/parkinsonism–dementia complex (PDC) of the Kii peninsula, Japan (Kii ALS/PDC), we performed extended mutation analyses of three patients with pathologically diagnosed Kii ALS/PDC. Direct sequencing analyses were performed in 19 genes, including ALS/frontotemporal lobar degeneration (FTLD)-related genes (*SOD2, SOD3, ALS2/alsin, SMN1, PGRN, ANG, VEGF, VCP, VAPB, DCTN1, CHMP2B,* and *TARDBP* or *TDP-43*), tauopathy-related gene (*GSK3*β), and parkinsonism-related genes (*alpha-synuclein, LRRK2, parkin, DJ-1, PINK1,* and *ATP13A2*). Gene dosage analyses were conducted in screening of *MAPT, alpha-synuclein, TDP-43* (or *TARDBP*), *GSK3*β, and *parkin*. We found no mutation in the 19 genes. We found a homozygous nonsynonymous SNP (*ALS2/alsin* V368M) shared by all the three patients. Gene dosage was normal in *MAPT, alpha-synuclein, TDP-43, GSK3*β, and *parkin*. The present findings, together with a previous negative study on *MAPT* and *SOD1* mutation, further elucidated the lack of causative mutations in all exons, exon–intron boundaries, or some rearrangements of the reported major causative or susceptible genes related to ALS, FTLD, parkinsonism, synucleinopathy, TDP-43 proteinopathy, and tauopathy. However, the familial aggregation and lack of any environment factors suggest that Kii ALS/PDC is caused by other yet unidentified genetic factors. © 2008 Movement Disorder Society

Key words: Kii ALS/PDC; amyotrophic lateral sclerosis; parkinsonism; dementia; genetics

The Western Pacific amyotrophic lateral sclerosis (ALS)/parkinsonism–dementia complex (PDC) is a progressive and fatal neurodegenerative disorder with high incidence among the indigenous people of three areas on the Pacific volcanic belt; Chamorros on Guam and Mariana Islands, Papuans in the coastal plain of West New Guinea, and Japanese in the Kii peninsula of Japan.[1] Clinically, ALS and PDC occur in isolation or in combination. Neuropathologically, ALS and PDC on Guam and Kii are characterized by abundant neurofibrillary tangles (NFTs) throughout the entire central nervous system, most markedly in the brainstem and temporal lobe, together with selective involvement of the upper and lower motor neurons.[2,3] Most, but not all, investigators consider ALS and PDC to be different manifestations of a single disease entity (ALS/PDC).[1,4]

In the 1980s, the disappearance of high incidence of ALS and marked decline in PDC were reported in Guam possibly related to changes in the environment and westernization of the lifestyle of Chamorros.[5] Although various environment factors, such as consumption of cycad and fruit bats, and deficiency of various minerals, have been suspected in Chamorros of Guam,[6–8] none has been experimentally verified so far. With regard to genetic factors, although *Tau (MAPT)*[9]

H. Tomiyama and Y. Kokubo contributed equally to this work.

*Correspondence to: Dr. Shigeki Kuzuhara, Department of Neurology, National Center Hospital of Neurology and Psychiatry, 4-1-1 Ogawahigashimachi, Kodaira, Tokyo 187-8551, Japan.
E-mail: kuzuhara@ncnp.go.jp

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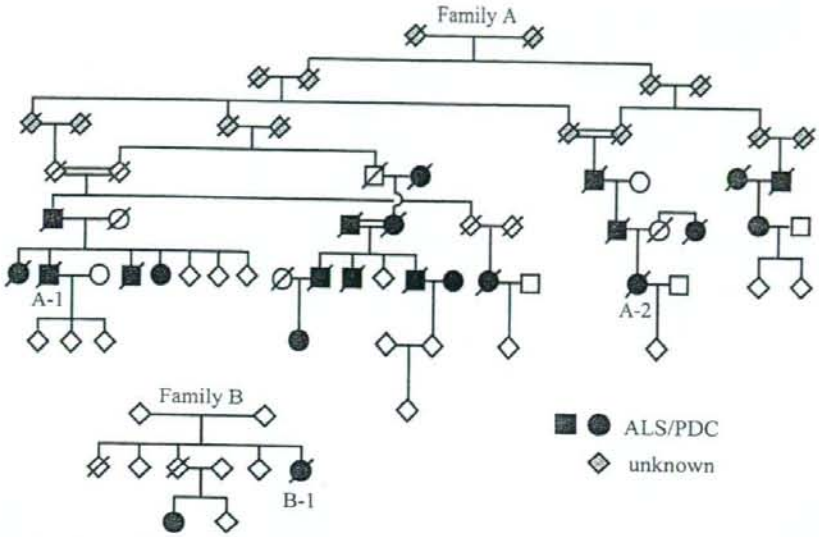

FIG. 1. Family trees (Family A and Family B) of patients with ALS/PDC from the Kii peninsula. Squares, men; circles, women; solid symbols, patients with amyotrophic lateral sclerosis/parkinsonism-dementia complex (ALS/PDC); open symbols, healthy individuals; gray symbols, unknown (precise information was not available); /(slash mark), deceased. The sexes are concealed (diamond symbols) to safeguard the confidentiality of the family members.

might be a modifier gene that increases the risk for Guam ALS, Guam PDC, and Guam neurodegenerative disorders in the presence of other unidentified gene(s) or by regulating *Tau* expression,[10,11] no causative mutation in *Tau* was detected in both Guam and Kii ALS/PDC with abundant NFTs pathology.[4] In addition, a previous genome-wide association study could not identify a single gene locus for Guam PDC, suggesting a geographic disease isolate with a complex genetic, genetic/environmental, or purely environmental etiology.[12] On the other hand, other studies proposed a mixture of other factors in the pathogenesis of ALS/PDC on Guam, including prolonged exposure to an environment severely deficient in Ca^{2+} and Mg^{2+} concurrent with a susceptibility genotype of *TRPM7* T1482I allele,[13] as well as neurotoxicity associated with β-methylamino-L-alanine in the cycads.[6]

In contrast to Guam ALS/PDC, high average annual incidence rates (417.9/100,000 in 1995–1998, unpublished data) of ALS/PDC in Hohara area of Kii continuing even after dramatic changes in foods and drinking water, and the much higher aggregation in the same family with a family history of approximately 80% in patients with Kii (40% in Guam ALS/PDC) strongly suggest major contribution of genetic factors.[11,14,15] And no customs of eating cycad or fruit bats exist in Japanese people living in Kii. Thus, further genetic analyses for Kii ALS/PDC might help disclose the pathogenesis of ALS/PDC.[4,16] Backed with this background, we performed mutation analysis of genes related to ALS, frontotemporal lobar degeneration (FTLD), tauopathy, and parkinsonism, and gene dosage analyses of *MAPT*, *alpha-synuclein*, *TDP-43* (or *TARDBP*), *GSK3*β, and *parkin* in three Kii patients with neuropathologically verified ALS/PDC.

METHODS

Patients

The study was approved by the Ethics Review Committees of Mie and Juntendo Universities. We analyzed DNA samples of three patients with Kii ALS/PDC from two families in the Kii peninsula. Consanguinity was seen in Family A but not in Family B (Fig. 1). The clinical diagnoses were based on features of typical ALS and PDC occurring singularly or in combination as shown in Table 1. The clinical diagnosis was verified as ALS/PDC in each patient at postmortem examination. All patients showed cardinal neuropathological findings of ALS/PDC including abundant NFTs associated with loss of nerve cells in the cerebral cor-