ported in two families with genomic triplication and in three families with duplications.[11–13,19] These findings suggest that overproduction of α-synuclein is one of the most important factors in FPD. In general, unequal intrachromosomal crossovers that result from misalignment of two homologous flanking sequences may account for genomic multiplications as well as deletions. The *SNCA* multiplications mutations, triplications, and duplications found in five unrelated patients probands with FPD are de novo within each kindred.[11–13,19] Affected individuals within the Iowa kindred, with *SNCA* genomic triplication, have fulminant, early-onset disease with a phenotype ranging clinically and pathologically from PD to diffuse LB disease (DLBD).[20] In contrast, *SNCA* duplication families have later onset disease and a longer duration to death, and neither cognitive decline nor dementia are prominent. Therefore, overproduction of wild-type α-synuclein (*SNCA*) may result in phenotypes of PD, PD with dementia (PDD), and DLBD, suggesting that regulation of α-synuclein protein levels is central to the cause of these phenotypes. In summary, the phenotype may be dependent on copy numbers of *SNCA*.

In this study, to gain further insight into the role of this multiplication, we assessed a series of 113 PD patients with autosomal dominant mode of inheritance and 200 sporadic PD patients for multiplication at this locus.

Subjects and Methods

Patients

This study consisted of 113 patients with ADPD and 200 patients with sporadic PD. Diagnosis of PD was adopted by the participating neurologists and the diagnosis was established based on the United Kingdom Parkinson's Disease Society Brain Bank criteria.[21] The mean age at onset of the 56 male and 57 female index patients with ADPD was 66.0 ± 9.5 (±SD), and that of the 81 male and 119 female patients with sporadic PD was 64.7 ± 10.0 (±SD). All patients were of Japanese origin. The study was approved by the ethics review committee of Juntendo University. Blood samples for genetic analysis were collected after obtaining informed consent from each patient and 17 unaffected relatives. None had mutations in *parkin*, *PINK1*, or *DJ-1*. We could not detect heterozygous exon deletions of such recessive genes by quantitative analysis in the patients studied. In addition, none had mutations in exon 41 in *LRRK2*.

Gene Dosage Analysis for SNCA

DNA was prepared using standard methods. The mutation screening was performed as described previously.[22] Semiquantitative multiplex polymerase chain reaction (PCR) of genomic DNA samples was performed using a real-time PCR method to detect the dosage of *SNCA* (ABI Prism 7700 sequence detector; Applied Biosystems, Foster City, CA). As the first step, we targeted exon 3 of *SNCA* to screen the gene dosage of *SNCA*. β-*Globin* gene was amplified as an endogenous reference. In addition, we used a DNA sample from the Iowa family (patients had triplication of *SNCA*) as a positive control. The primer and TaqMan MGB probe sequences used in this study are described in Table 1. PCR was conformed with PCR universal master mix using 25ng of genomic DNA, 900nM primers, and 250nM probes (β-globin is 50–200nM) in a total reaction volume of 50μL. PCR cycling conditions were 95°C for 10 minutes, 95°C for 15 seconds, and 60°C for 1 minute (40 cycles). Values between 0.4 and 0.6 were considered as heterozygous deletion, between 0.8 and 1.2 as normal, between 1.3 and 1.7 as heterozygous duplication, and greater than 1.8 as triplication.

In the second step, we performed semiquantitative analysis on exons 1/2, 4, 6, and 7 for the patients found to carry multiplication of this gene in the first step. All the sequences of this gene are shown in Table 1.

Fluorescence In Situ Hybridization Analysis

We used two-color standard fluorescence in situ hybridization (FISH) and prophase FISH for metaphase and interphase. FISH analyses were performed as described previously,[23] using a BAC located around the region of interest. The location of each bacterial artificial chromosome (BAC) was archived by the database of UCSC (http://genome.ucsc.edu) or NCBI (http://www.ncbi.nlm.nih.gov). Two BAC contigs representing the region at 4q21-22. BACs RP11-17p8 and RP11-61407 were used as probes. BAC RP11-17p8 locates at site of centromere of chromosome 4, and BAC RP11-61407 locates at site of telomere of the same chromosome. PR11-61407 contains *SNCA*, suggesting that the signal of this clone shows the copy numbers of *SNCA*. The distance between the two BAC clones was approximately 1.4Mb. Probes were labeled with biotin-16-dUTP or digoxigenin-11-dUTP by nick-translation (Roche Diagnostics, Tokyo, Japan). The copy number of the region was assessed according to the hybridization patterns observed on both metaphase and interphase chromosomes. We established Epstein–Barr virus (EBV)–transformed lymphoblastoid cell line as described previously.[24]

Multiplication (duplication) Region Using Comparative Genomic Hybridization Array and Gene Dosage Technique

The triplication region in Iowa family is between 1.61 and 2.04Mb and contains 17 annotated or putative genes. A recently constructed high-density comparative genomic hybridization (CGH) array, designated MCG Whole Genome Array-4500,[25] which contains 4532 BAC/P1-artificial chromosome (PAC) clones covering the entire genome at intervals of approximately 0.7Mb, was used for CGH array analysis. This array is suitable for detecting the size of the multiplication if the size is greater than 0.7Mb. Hybridizations were performed as described previously with minor modifications.[26,27] In brief, test and reference genomic DNAs from the patient's lymphoblastoid cells and normal lymphocytes, respectively, were labeled with Cy3- and Cy5-dCTP (Amersham Biosciences, Tokyo), respectively, precipitated together with ethanol in the presence of Cot-1 DNA, redissolved in a hybridization mix (50% formamide, 10% dextran sulfate, 2 × standard saline citrate [SSC], and 4% sodium dodecyl sulfate [SDS], pH 7.0),

Table 1. Sequences of Primer and TaqMan Probes Used in the This Study

		Forward Primer	Reverse Primer
β-globin	—	5′-TGGGCAACCCTAAGGTGAAG-3′	5′-GTGAGCCAGGCCATCACTAAA-3′
ABCG2	Exon 1	5′-GGAAGGTCCGGGTGACTCA-3′	5′-GGAGGCAGCGCTTTAACAAT-3′
—	Exon 2	5′-GTGTCACAAGGAAACACCAATGG-3′	5′-AGCTCCTTCAGTAAATGCCTTCAG-3′
DFKZ	Exon 7	5′-CTGGACCACTTACTGGTGAAAGC-3′	5′-CACTGTGCCTGGCCAAATT-3′
FAM13A1	Exon 12	5′-GAAGAGGACCTAACTCCCAGGAT-3′	5′-TTCTCAAGTTGGGAACCAAAACTCT-3′
LOC345278	Exon 8	5′-GTTGGCTGGGCCAATCTCT-3′	5′-TGGTCTTAGCTGAAGGCCAGTT-3′
SNCA	Exon s1/2	5′-CCTTCAAGCCTTCTGCCTTTC-3′	5′-CGAATGGCCACTCCCAGTT-3′
—	Exon 3	5′-TTCCAGTGTGGTGTAAAGAAATTCAT-3′	5′-CCTTGGCCTTTGAAAGTCCTT-3′
—	Exon 4	5′-CAGCAATTTAAGGCTAGCTTGAGACT-3′	5′-CCACTCCCTCCTTGGTTTTG-3′
—	Exon 6	5′-TATGCCTGTGGATCCTGACAAT-3′	5′-TCAGCTTGGACTCCTACCTCAGA-3′
—	Exon 7	5′-TCTTTGCTCCCAGTTTCTTGAGA-3′	5′-TGGAACTGAGCACTTGTACAGGAT-3′
MMRN1	Exon 1	5′-ATCAAACTCTCACATCCAC-3′	5′-CACCTGCTGAGGGTGTGAGA-3′
—	Exon 5	5′-CAGGCAATGAAACTGACTCTTCTG-3′	5′-CTTCTAGGGAGGAGTAAGTGTTCCT-3′
—	Exon 6	5′-GTTTCAATAGCAGCCCAGCAAAA-3′	5′-CAGTCAAAGTGGGCCGATTCT-3′
—	Exon 8	5′-GCTTCATATACCCCAAGAACTGGAA-3′	5′-GCACTAAATGACTCGATGGTGTACT-3′
KIAA1680	Exon 1	5′-TTAAATAACGCAGCTGGACTCTGT-3′	5′-TTAAATAACGCAGCTGGACTCTGT-3′
—	Exon 2	5′-GGCCACAATGATTCTACCTCTCA-3′	5′-CCGTAAGTTCTGTTGTTGTCTTTGT-3′
—	Exon 3	5′-AGCTCAGGTAGCACAGGTAAACG-3′	5′-TGGTGGAAGCTAATGGAAGGA-3′
—	Exon 4	5′-CCATTTCGTGAAGGAAGATTTATAGAG-3′	5′-TCCCTGCAGTGCCTTCTGA-3′
—		MGB probe	
β-globin	—	5′-CTCATGGCAAGAAAGTGCTCGGTGC-3′	
ABCG2	Exon 1	5′-CCCAACATTTACATCCTT-3′	
—	Exon 2	5′-CCGCGACAGCTTCCAA-3′	
DFKZ	Exon 7	5′-ACCATGCAAAAGAAAT-3′	
FAM13A1	Exon 12	5′-AAGCAACACACTCCCC-3′	
LOC345278	Exon 8	5′-CAGAAGCTGACTCTCA -3′	
SNCA	Exons 1/2	5′-ACCCTCGTGAGCGGA-3′	
—	Exon 3	5′-AGCCATGGATGTATTC-3′	
—	Exon 4	5′-TGTCTTGAATTTGTTTTTGTAGGC-3′	
—	Exon 6	5′-AGGCTTATGAAATGCC-3′	
—	Exon 7	5′-TGCTGACAGATGTTC-3′	
MMRN1	Exon 1	5′-ACTTGACCACTCCTTCTGCTTTCT-3′	
—	Exon 5	5′-CACAGTCAAAGAAATATTG-3′	
—	Exon 6	5′-CTTGCACCAAAACAAAC-3′	
—	Exon 8	5′-TCCAAGATACGGAATTCTA-3′	
KIAA1680	Exon 1	5′-TCCCCTTCTCGGCTGTTG-3′	
—	Exon 2	5′-ATGTCCCTCAATTCTG-3′	
—	Exon 3	5′-AGGAGCATATTCCG-3′	
—	Exon 4	5′-AGACTGCGATCCTC-3′	

and denatured at 75°C for 8 minutes. After 40-minute preincubation at 42°C, the mixture was applied to array slides and incubated at 50°C for 10 minutes, 46°C for 10 minutes, and 43°C for 60 hours in a hybridization machine, GeneTAC (Harvard Bioscience, Holliston, MA). After hybridization, the slides were washed once in a solution of 50% formamide, 2 × SSC (pH 7.0) for 10 minutes at 50°C and 1 × SSC for 10 minutes at 42°C, respectively, and then scanned with a GenePix 4000B (Axon Instruments, Foster City, CA). The acquired images were analyzed with GenePix Pro 4.1 imaging software (Axon Instruments). Fluorescence ratios were normalized so that the mean of the middle third of $\log_2$ ratios across the array was zero. The average values for each clone were within the thresholds of 0.2 and −0.2 (log2ratio), and the mean ± 2 SD values of all clones were within the range of 0.4 and −0.4 (log2ratio). The thresholds for copy number gain and loss were set at log2 ratios of 0.4 and −0.4, respectively.

We picked up the locus region between *ABCG* and *KIAA1680* of approximately 1.6 to 2.0 Mb. To identify the region of duplication spanning *SNCA*, we performed semiquantitative PCR on target genes including *ABCG*, *DFKZ*, *FAM13A1*, *LOC345278*, *MMRN*, and *KIAA1680* using the same methods. The sequences of all primer and probe sets are shown in Table 1.

Haplotype Analysis

To determine whether the same haplotype was shared between our probands with *SNCA* multiplication, we performed haplotype analysis in patients with *SNCA* duplication from unrelated families. We used four microsatellite markers including *D4S2361*, *D4S2505E* (located within *SNCA*), *D4S2380*, *D4S1647*, and *D4S421*.

Results

Gene Dosage Analysis for α-Synuclein

Using semiquantitative PCR to detect gene dosage, we did not find patients harboring *SNCA* multiplication

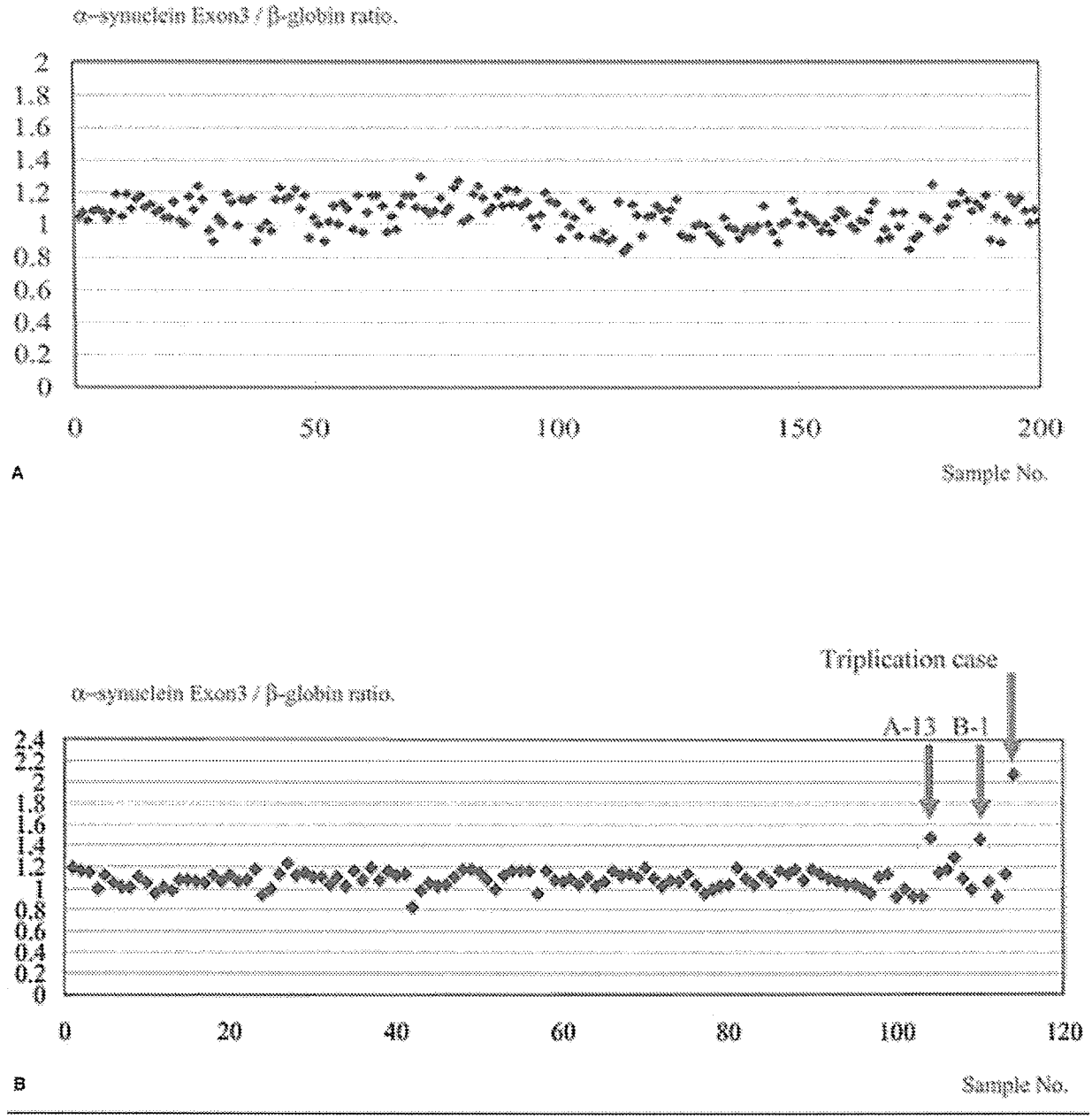

Fig 1. The ratio of α-synuclein exon 3, used as a target gene, to β-globin, used as a reference gene, as determined by semiquantitative real-time polymerase chain reaction in: (A) 200 patients with sporadic PD (the ratio ranged from 0.8 to 1.3, suggesting that single SNCA *copy exists in one allele, and (B) 113 patients with autosomal dominant hereditary Parkinson's disease. Note the two cases of duplication ratio (the ratio is 1.46 in one patient and 1.48 in the other), and the single Iowa family triplication case with a ratio of 2.07.*

among 200 sporadic cases (Fig 1A) but detected two index patients (A-13 and B-1) with potential *SNCA* duplications among 113 autosomal dominant pedigrees using exon 3 of *SNCA* (Fig 1B). To confirm the entire region of the α-synuclein gene was multiplied, we performed the exon dosage analysis including exons 1/2, 4, 6, and 7. We confirmed duplication of this gene in two patients. Thus, we were able to confirm that two families (Families A and B) were ADPD with *SNCA* duplication. In Family A, two patients with duplication had typical PD whereas five carriers were asymptomatic (Fig 2A). In Family B, one patient had duplication of the *SNCA* gene; two members were carriers (see Fig 2B).

FISH analysis also confirmed the *SNCA* duplication in the two index patients (Fig 3A, B). Figure 3 shows

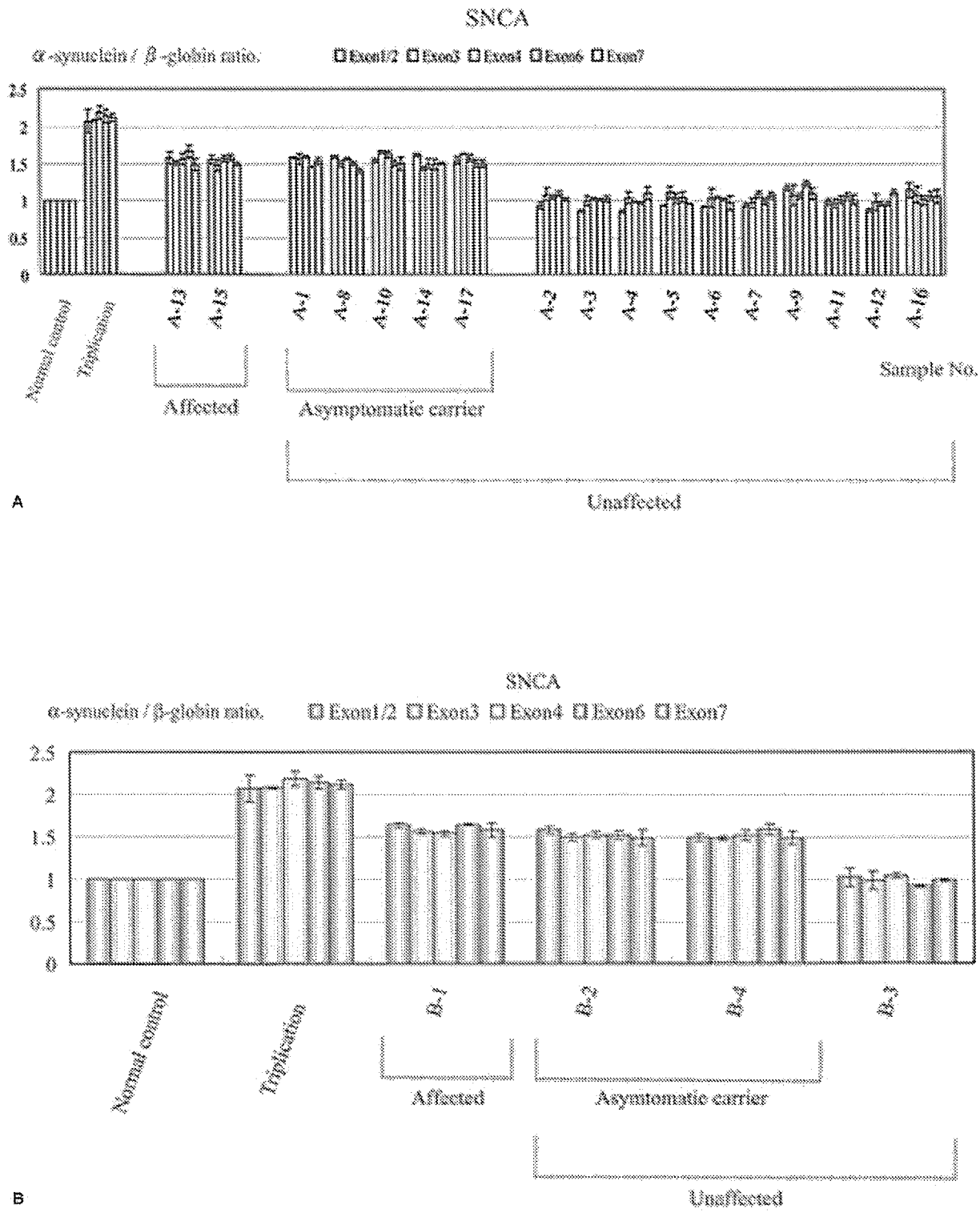

Fig 2. Results of screening for SNCA *multiplications for exons 1 to 7 in Family A (A). We detected two patients with* SNCA *duplication and five asymptomatic carriers in this family (a penetrance ratio of 33.3%) and (B) Family B. We detected three patients with* SNCA *duplication in four family members.*

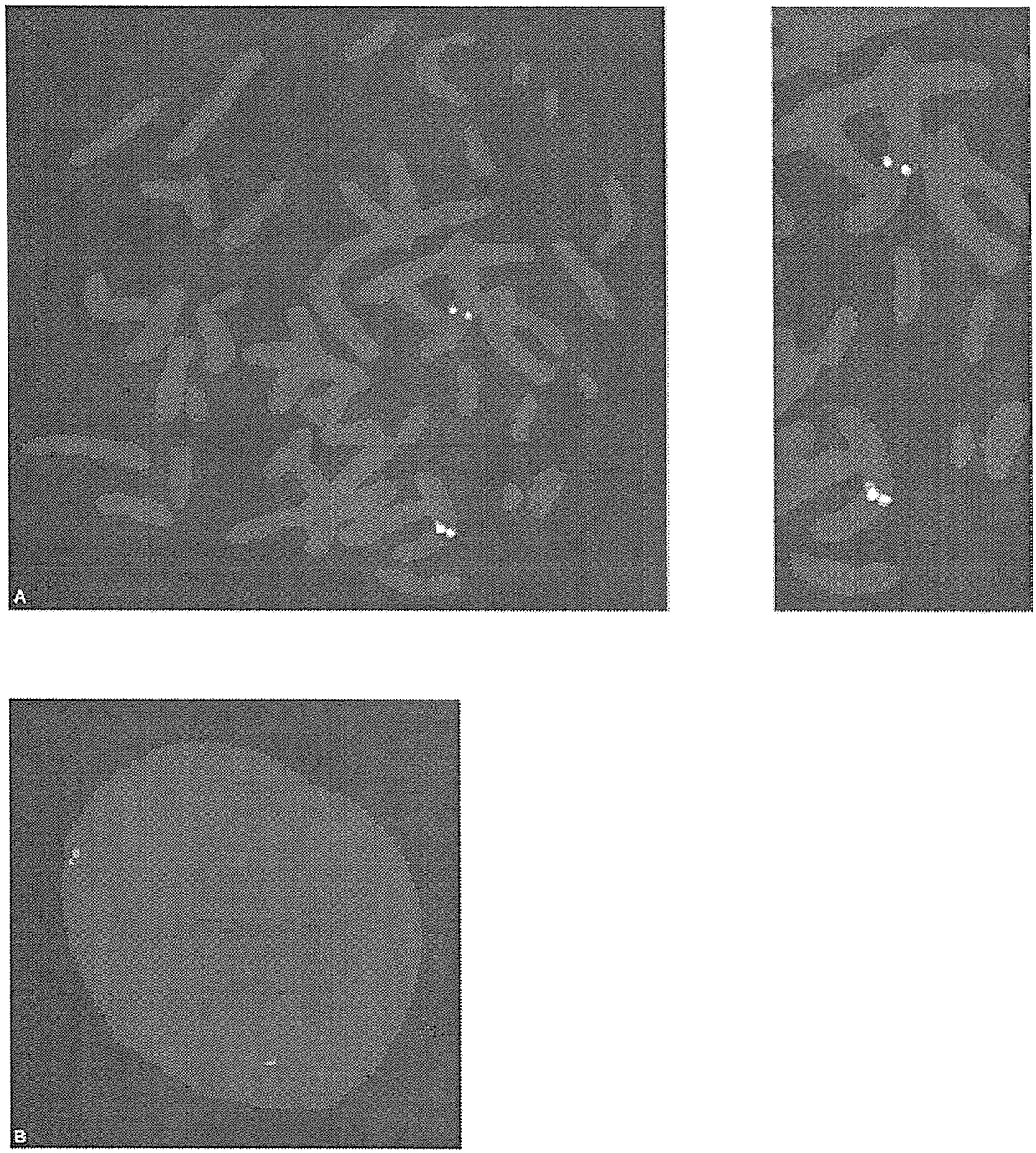

Fig 3. (A) Schematic representation of fluorescence in situ hybridization assay of metaphase chromosomes from Epstein–Barr virus (EBV)–transformed lymphocytes derived from Patients A-13 and B-1. We used BACs RP11-17p8 for normal control sample (shown in green and located 1.4Mb centromeric to SNCA, *left panel) and RP11-61407, which included the* SNCA *shown in red on chromosome region 4q21-22 (right panel). These pictures show clearly disproportional segregations compared with the normal control. (B) Standard one-color FISH of the interphase, using BACs RP11-61407. Note the two disproportional signals.*

the representative results of FISH analysis of interphase and metaphase chromosomes from EBV-transformed lymphocytes derived from Patients A-13 and B-1. We detected tight apposition of the metaphase chromatids compared with signals of BAC RP11-17P8 located 1.4Mb centromeric to *SNCA*. The intensity of the sig-

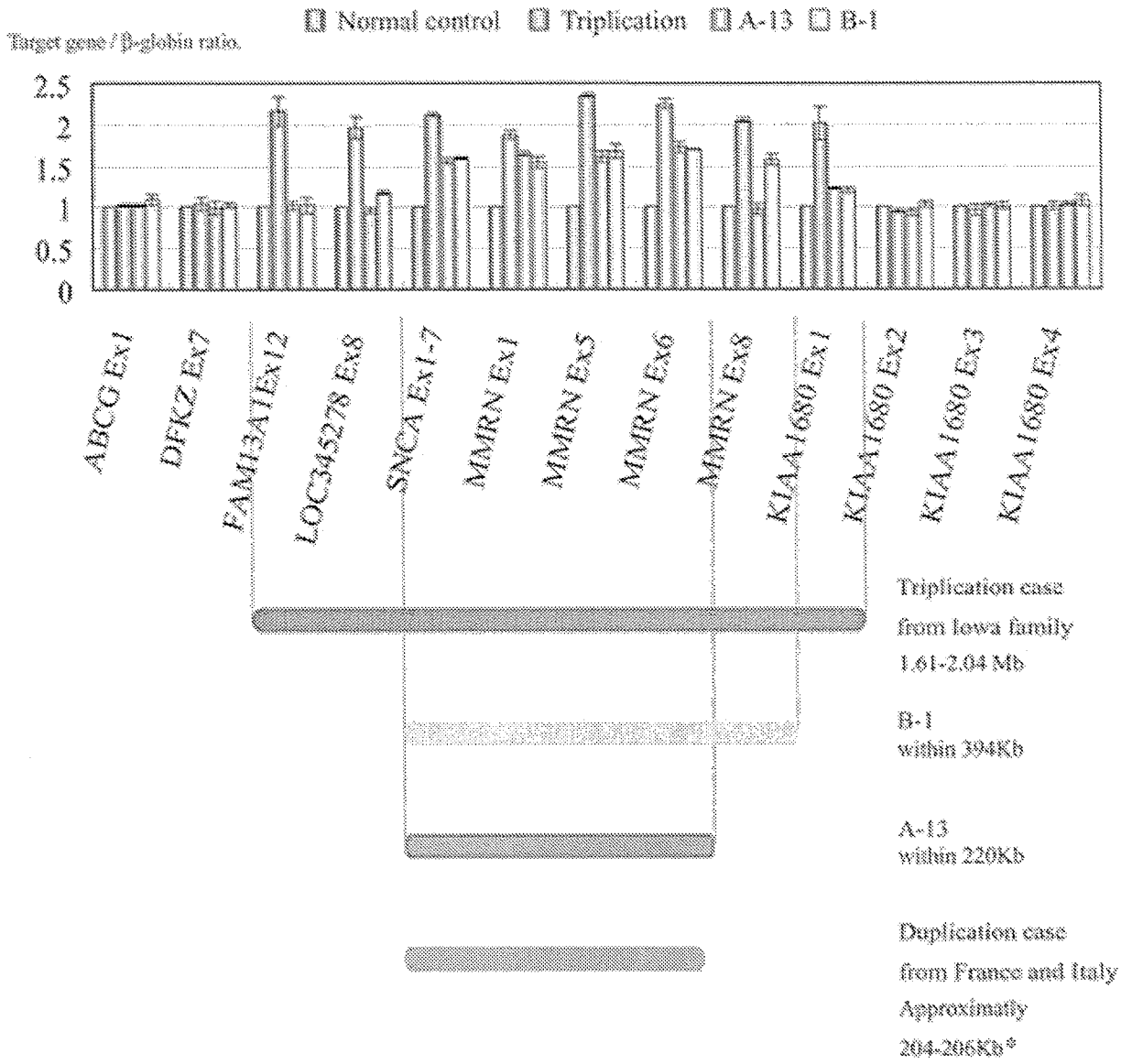

Fig 4. Identification of the region of SNCA *duplication between* ABCG *to* KIAA1680 *by using real-time semiquantitative polymerase chain reaction method. The different duplication region appears on* MMRN1 *Exon 8. *Duplication case as reported in Ibanez et al.*[13]

nal suggests *SNCA* duplication in these two patients. When considered together with the results of gene dosage analysis, we were able to confirm *SNCA* duplication. We did not observe two separate signals between BACs RP11-17P8 and PR11-61407, suggesting that the size of the duplication region is less than 1.4Mb. CGH array analysis showed that the specific elevation ratio could not be detected because the *SNCA* region could not be directly included in BAC probes used in MCG Whole Genome Array-4500. However, this BAC-based array contains BACs RP11-49M7 and RP11-17p8 that are close to 5′ or 3′ sites of *SNCA*, respectively. Alternatively, this finding indicates that the *SNCA* duplication region is less than 0.7Mb based on information archived by the database of UCSC (http://genome.ucsc.edu) and NCBI (http://www.ncbi.nlm.nih.gov). Although MCG Whole Genome Array-4500 covers the entire genome, no specific multiplication or deletions existed in other regions apart from 4q21-22. Identification of the *SNCA* duplication region was carefully assessed by gene dosage analysis for flanking genes around *SNCA* (Fig 4). The length of *SNCA* duplication of Patient A-13 spanned all of *SNCA* and part of *MMRN1* such as exons 1 to 6. In

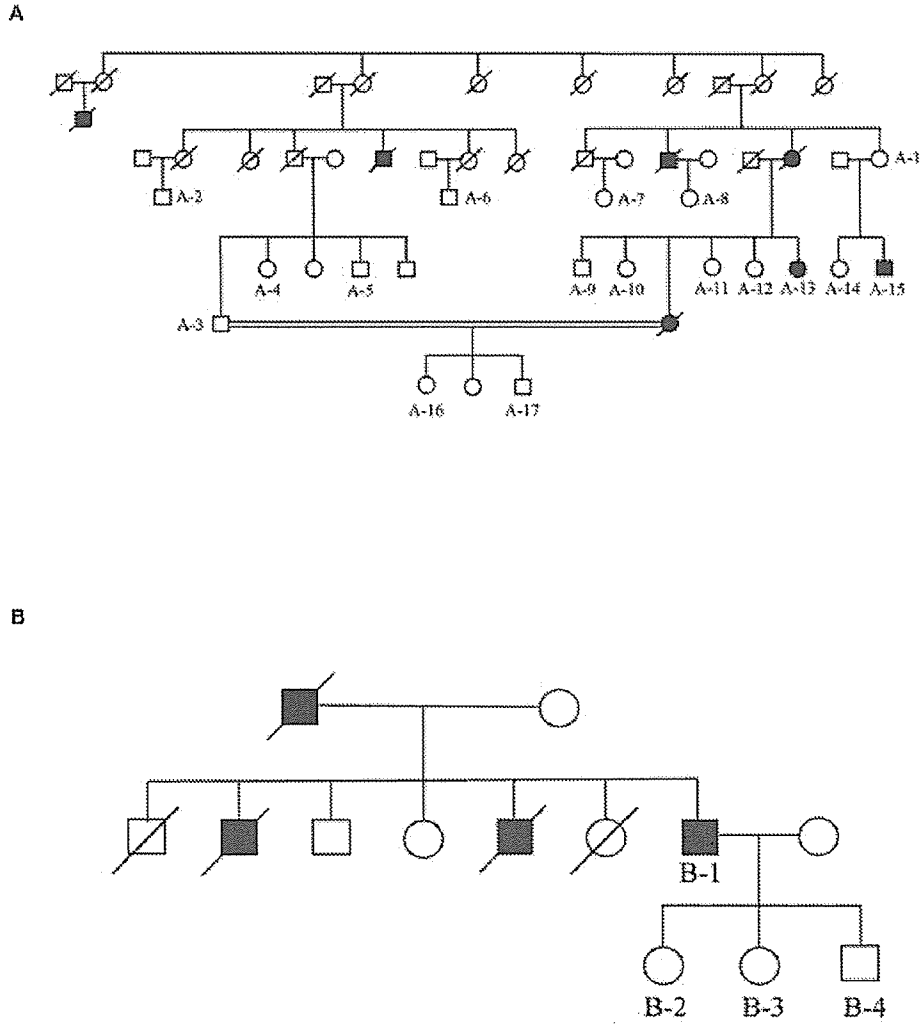

Fig 5. (A) Pedigrees of Patient A-1 with Parkinson's disease (PD) showing four generations. Black boxes represent affected patients. Symbols with numbers represent family members who were examined clinically by neurologists and from whom blood samples were collected. In 17 members, two patients were affected and five members (A-1, A-8, A-10, A-14, A-17) were carriers. Among seven carriers with SNCA *duplication, the ages of all carriers except for A-17 were beyond the mean age at onset of patients with* SNCA *duplication. Thus, the penetrance ratio was 33.3% (two patients/six asymptomatic carriers). (B) Pedigree of Patient B-1 with PD showing three generations. Symbols are as for Figure 6A. In four members, one patient was affected, and two members were carriers.*

contrast, the duplication region of Patient B-1 spanned all of *SNCA* and *MMRN1*. In addition, the regions of both patients did not span *LOC345278* and in Patient B-1, no duplication of *KIAA1680* was observed. Thus, the length of the duplication of Patient A-13 was shorter than that of Patient B-1, suggesting that the different lengths of the duplications differ by approximately 100 to 200kb. Furthermore, these two families have different allele sizes in microsatellite markers, suggesting that SNCA duplication is also de novo (data not shown). Clinical data, including the results of neuroimaging such as magnetic resonance imaging (MRI) and single-photon emission computed tomography (SPECT) and [^{123}I] meta-iodobenzylguanidine (MIBG) myocardial scintigraphy, are described below.

Family A

We collected DNA samples from 17 members of this family, including three affected and 14 unaffected members (Fig 5A). Among the three affected members, one patient (A-2) had no *SNCA* duplication. In addition, the age at onset of parkinsonism was 74 years. Moreover, L-dopa responsiveness was not excellent. Although MRI examination was not available, we considered that the cause of PD in this patient was not duplication but rather vascular parkinsonism based on

neurological findings. The mean age at onset of the disease was 43 years. The parents of A-16 and A-17 were close relatives. Five asymptomatic carriers were recognized by genomic analysis. No parkinsonism was observed in these asymptomatic carriers based on clinical neurological examination by two expert neurologists (K.N. and N.H.). The youngest age at onset was 38 years including the deceased patient (50 years old at onset). Thus, age 43 years was the cutoff age in this family. Considering this point, the penetrance ratio was 33.3% (2/6).

Patient A-13

The age of onset was 48 years. The initial symptom in Patient A-13 was rigidity and bradykinesia. She responded well to L-dopa. Six years after commencement of treatment with L-dopa, she developed drug-induced dyskinesia, which subsequently showed marked resolution. No tremor at rest has yet been noted. During the day, clinical assessment indicated Hohen and Yahr stage III. No dementia has developed yet and she has no symptoms related to autonomic nervous system dysfunction. Brain MRI study showed no abnormal mass or ischemic changes (Fig 6A) and ^{123}I-IMP SPECT study showed no evidence of hypoperfusion. However, the H/M ratio of MIBG myocardial scintigraphy was less than that of the normal control (A-13; early: 1.4, late: 1.24; see Fig 6D, E).

Patient A-15

The age at onset was 38 years. This patient was the cousin of Patient A-13. The initial symptom was gait disturbance with frequent falls. Tremor and autonomic nervous dysfunction were not seen. He was diagnosed with depression during the course of the disease, but neither dementia nor cognitive deterioration was prominent. The clinical course of this patient was similar to that of Patient A-13. Although this patient responded to L-dopa, he showed excellent response to anticholinergic agents such as trihexyphenidyl hydrochloride rather than L-dopa. In addition, the patient developed psychosis at 43 years of age.

Family B

DNA samples were collected from four members of Family B (see Fig 5B). Among the two generations, the number of affected member was four including three deceased members, and the unaffected members were three including two carriers with *SNCA* duplication. The age of asymptomatic carriers (B-2, B-3, and B-4) was younger than 35 years at the time of collection of DNA samples. Thus, it is difficult to speculate whether these carriers will develop PD in the future.

Patient B-1

The age at onset was 47 years. In the early stage, he responded to L-dopa; however, at 58 years of age, the disease was evaluated as stage III. Moreover, the gait disturbance and bradykinesia worsened and he suffered from cognitive dysfunction a few years later. Since 61 years of age, he has found it difficult to communicate with others and started gradually to develop abnormal behavior. Mini-Mental State Examination score was 17/30 at 61 years of age. At 62 years, his gait disturbance and hallucination worsened. At 64 years, he spent most of the day on the bed and required tracheostomy because of repeated episodes of aspiration pneumonia. Brain MRI showed moderate dilation of Sylvian fissure and atrophic changes in the temporal lobe on both sides. There was no evidence of ischemic changes or abnormal mass (see Fig 6B). A 99m-Tc-ECD SPECT study showed hypoperfusion predominantly on both frontotemporal lobes (see Fig 6C). The H/M ratio of MIBG myocardial scintigraphy was reduced (B-1; early: 1.40, late: 1.24).

Subject B-4

Subject B-4 was mentally retarded and had autism and generalized seizure. Since 1 year of age, he could not speak and was diagnosed with mental retardation by a pediatrician. At 12 years of age, he started to speak a few words and was sometimes observed to have sudden outburst of rage. At 15 years, he developed generalized seizure. EEG showed spiking waves predominantly localized to the right frontal lobe. Brain computed tomography scan showed no abnormal densities or other signs. No parkinsonism has been noted so far.

Table 2 summarizes the clinical features of these cases, including the results of neuroimaging and MIBG scintigraphy.

Discussion

Several recent studies suggest *SNCA* multiplications are a rare cause of PD, PDD, and DLBD.[22,28,29] In this study, we detected *SNCA* duplication in PD patients from 2 of 113 unrelated Japanese families with autosomal dominant parkinsonism. Thus, the incidence of *SNCA* multiplication may be more frequent than previously estimated. To our knowledge, the Iowa family and a single family of Swedish-American descent have been reported previously to have *SNCA* triplication.[11,19] In addition, two French families and one Italian family with *SNCA* duplication have been reported.[12,13] Taken together with this study, a total of seven families with *SNCA* multiplication, including triple and double *SNCA* copies, have been reported worldwide.

For all patients with *SNCA* duplication reported here, including patients of Family A, the phenotype was indistinguishable from idiopathic PD and no other

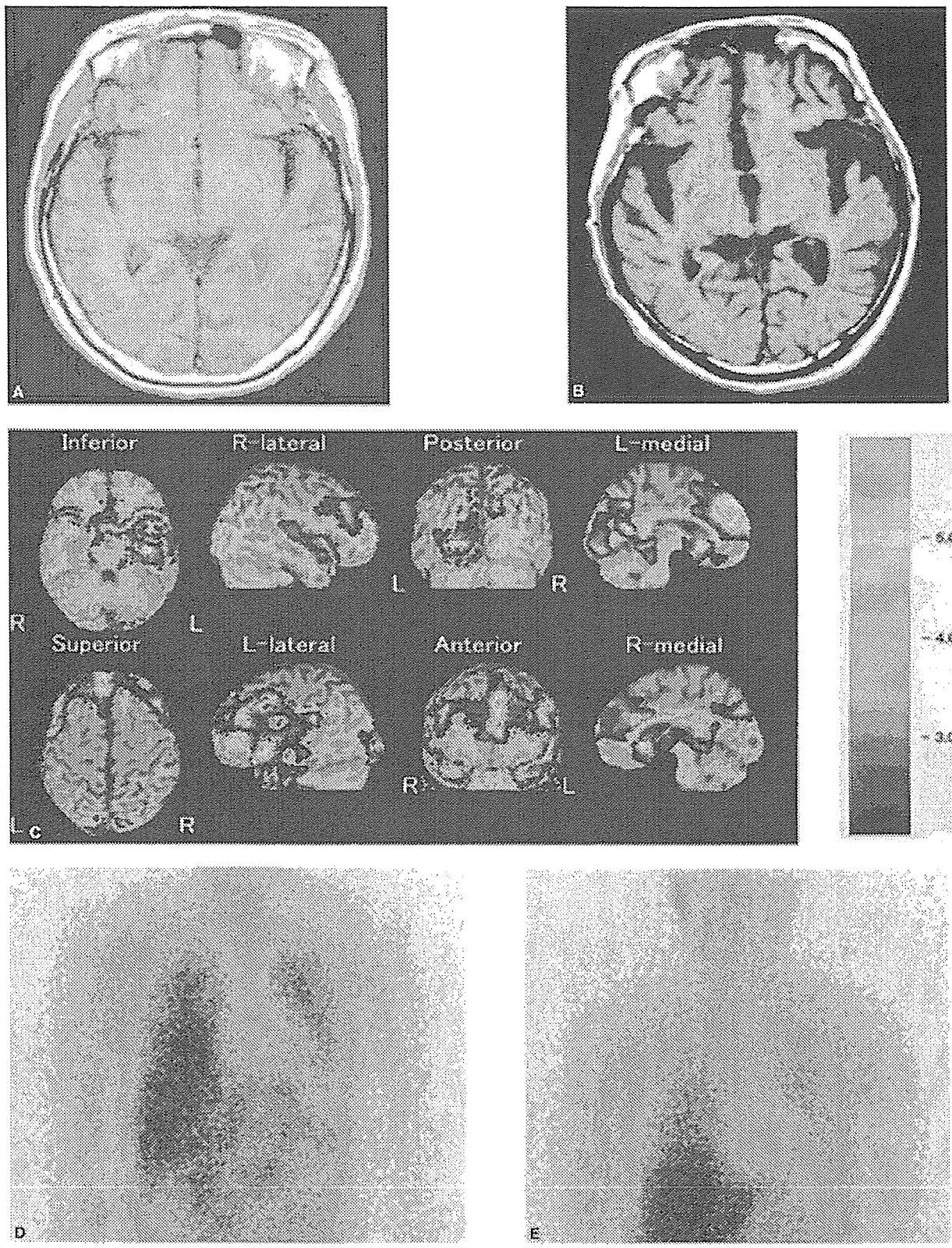

Fig 6. (A) Brain magnetic resonance imaging (MRI) T1 wedge study of Patient A-13. No abnormal masses or ischemic changes were evident. (B) Brain MRI T1 wedge study of Patient B-1. Note the dilation of Sylvian fissure and atrophic changes in both temporal lobes. (C) ^{123}I-IMP SPECT study of Patient B-1. Note the hypoperfusion of both frontotemporal lobes and medial-occipital lobes. (D, E) [^{123}I]meta-iodobenzylguanidine (MIBG) myocardial scintigraphy (D; early, E; late) of Patient A-13. The H/M ratio was reduced in this patient.

Table 2. Clinical Features of Four Affected Patients in Two Unrelated Pedigrees

	A Family			B Family
Feature	A-13	A-15	A-2	B-1
Age (yr)	57	43	77	65
Age at onset (yr)	48	38	74	47
Disease duration (yr)	10	6	4	19
Initial symptom	Rigidity	Rigidity	Bradykinesia	Bradykinesia
Bradykinesia	+	+++	++	+++
Rigidity	+++	+++	++	+++
Resting tremor	−	−	−	−
Postural instability	−	+	+	−
UPDRS	10/108	32/108	27/108	−
MMSE	30/30	30/30	17/30	17/30
L-Dopa response	+++	+	−	+
SNCA duplication	+	+	−	+

UPDRS = Unified Parkinson's disease rating scale; MMSE = Mini-Mental Status Examination.

clinical features such as dementia were present, in contrast with families with *SNCA* triplication. Notably, dementia was observed in one patient of Family B. Therefore, it is important to screen PDD or DLB for *SNCA* multiplications. However, the age of onset of PD in the patient with dementia was older than that of Iowa patients (36.0 ± 10.5 years) and the patient of Swedish-American family (31 years).[19] Moreover, the age at onset of Japanese patients was similar to those of other families with *SNCA* duplication (48.4 ± 15.0 years). In addition, the asymptomatic carrier, B-2, had epilepsy, which has been reported in one French PD patient.[13] In addition, autism was observed in the same patient, although no clear parkinsonism was evident. Patient B-1 had dementia, in contrast with previously reported cases with *SNCA* duplication, although the duration of the disease was longer (18 years) compared with reported cases of *SNCA* duplication. In addition, dementia only appeared after 14 years of diagnosis of parkinsonism. Therefore, *SNCA* duplication may be a risk factor for development of dementia.

Within each kindred the SNCA multiplication is a de novo mutation. The 4q21 genomic duplication in Patient B-1 included all of *SNCA* and *MMRN1*, whereas the duplicated region in Patient A-13 contained all of *SNCA* but only part of *MMRN1*. The *SNCA* triplication in the Iowa family also contains *MMRN1*, suggesting that overexpression of MMRN1 plays a role in cognitive deficit.

However, northern blotting analysis indicates a paucity of expression for *MMRN1* in neurons.[30] It therefore is unlikely that the effects of *MMNR1* are related to the development of dementia. *MMRN1* more likely plays a role in hemostasis and if vasogenic factors, including platelets and endothelial cells, are involved in dementia, *MMRN1* overexpression may still contribute to the dementia phenotype.

Previous studies reported the association of cardiac denervation and parkinsonism caused by *SNCA* gene triplication.[31] Low H/M ratios by [^{123}I]MIBG myocardial scintigraphy were reported in patients with sporadic PD.[32,33] In contrast, the H/M ratio was not decreased in patients with *parkin* mutations who lacked LBs in the autopsied brains.[34] In this regard, this finding is similar in patients with *SNCA* multiplication.

This study showed that the disease penetrance of Family A was 33.3%. The current ages of the asymptomatic carriers in this family are beyond the mean age at onset of patients. Thus, the difference may be caused by the *SNCA* expression levels between patients and asymptomatic carriers. Considering the multiple copies of *SNCA*, the expression level could be important. Indeed, double expression level of this protein compared with the normal brain was identified in Iowa family with *SNCA* triplication.[19] In addition, several haplotypes in the promoter region of SNCA including the sequence repeat element Rep1 were shown to associate with increased risk for sporadic PD.[35,36] However, whether the promoter alleles are risk factors for the development of PD is currently controversial.

Recently, Mueller and colleagues reported that single nucleotide polymorphisms located within the 3′side of exons 5 and 6, but not promoter polymorphism, correlated significantly with PD.[35] However, the functional association between PD and the associated region of SNCA remains unclear. In our study, the presence of asymptomatic carriers indicated that not only *SNCA* dosage but also another genetic variability in *SNCA* may be a risk factor for the development of PD.

References

1. Bonifati V, Rizzu P, van Baren MJ, et al. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 2003;21:256–259.

2. Gasser T, Muller-Myhsok B, Wszolek ZK, et al. A susceptibility locus for Parkinson's disease maps to chromosome 2p13. Nat Genet 1998;18:262–265.
3. Hicks AA, Petursson H, Jonsson T, et al. A susceptibility gene for late-onset idiopathic Parkinson's disease. Ann Neurol 2002; 52:549–555.
4. Leroy E, Boyer R, Auburger G, et al. The ubiquitin pathway in Parkinson's disease. Nature 1998;395:451–452.
5. Kitada T, Asakawa S, Hattori N, et al., Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 1998;392:605–608.
6. Le WD, Xu P, Jankovic J, et al. Mutations in NR4A2 associated with familial Parkinson disease. Nat Genet 2003;33: 85–89.
7. Paisan-Ruiz C, Jain S, Evans EW, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 2004;44:595–600.
8. Pankratz N, Nichols WC, Uniacke SK, et al. Significant linkage of Parkinson disease to chromosome 2q36–37. Am J Hum Genet 2003;72:1053–1057.
9. Polymeropoulos MH, Lavedan C, Leroy E, et al. Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science 1997;276: 2045–2047.
10. Valente EM, Abou-Sleiman PM, Caputo V, et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 2004;21:1158–1160.
11. Singleton AB, Farrer M, Johnson J, et al. α-Synuclein locus triplication causes Parkinson's disease. Science 2003;302: 841.
12. Chartier-Harlin MC, Kachergus J, Roumier C, et al. α-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet 2004;364:1167–1169.
13. Ibanez P, Bonnet AM, Debarges B, et al., Causal relation between alpha-synuclein gene duplication and familial Parkinson's disease. Lancet 2004;25:1169–1171.
14. Zimprich A, Biskup S, Leitner P, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 2004;44:601–607.
15. Kruger R, Kuhn W, Leenders KL, et al. Familial parkinsonism with synuclein pathology: clinical and PET studies of A30P mutation carriers. Neurology 2001;22:1355–1362.
16. Zarranz JJ, Alegre J, Gomez-Esteban JC, et al. The new mutation, E46K, of α-synuclein causes Parkinson and Lewy body dementia. Ann Neurol 2004;55:164–173.
17. Spillantini MG, Schmidt ML, Lee VM, et al. α-Synuclein in Lewy bodies. Nature 1997;28:839–840.
18. Vaughan J, Durr A, Tassin J, et al. The alpha-synuclein Ala53Thr mutation is not a common cause of familial Parkinson's disease: a study of 230 European cases. European Consortium on Genetic Susceptibility in Parkinson's Disease. Ann Neurol 1998;44:270–273.
19. Farrer M, Kachergus J, Forno L, et al. Comparison of kindreds with parkinsonism and α-synuclein genomic multiplications. Ann Neurol 2004;55:174–179.
20. Muenter MD, Forno LS, Hornykiewicz O, et al. Hereditary form of parkinsonism-dementia. Ann Neurol 1998;43:768–781
21. Hughes AJ, Daniel SE, Kilford L, et al. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinicopathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992;55: 181–184.
22. Johnson J, Hague SM, Hanson M, et al. SNCA multiplication is not a common cause of Parkinson disease or dementia with Lewy bodies. Neurology 2004;63:554–556.
23. Ariyama T, Inazawa J, Uemura Y, et al. Clonal origin of Philadelphia chromosome negative cells with trisomy 8 appearing during the course of alpha-interferon therapy for Ph positive chronic myelocytic leukemia. Cancer Genet Cytogenet 1995; 81:2023.
24. Saito-Ohara F, Fukuda Y, Ito M, et al. The Xq22 inversion breakpoint interrupted a novel ras-like GTPase gene in a patient with Duchenne muscular dystrophy and profound mental retardation. Am J Hum Genet 2002;71:637–645.
25. Inazawa J, Inoue J, Imoto I. Comparative genomic hybridization (CGH)-arrays pave the way for identification of novel cancer-related genes. Cancer Sci 2004;95:559–563.
26. Sonoda I, Imoto I, Inoue J, et al. Frequent silencing of low density lipoprotein receptor-related protein 1B (LRP1B) expression by genetic and epigenetic mechanisms in esophageal squamous cell carcinoma. Cancer Res 2004;64:3741–3747.
27. Takada H, Imoto I, Tsuda H, et al. Screening of DNA copy-number aberrations in gastric cancer cell lines by array-based comparative genomic hybridization. Cancer Sci 2005;96: 100–110.
28. Gispert S, Trenkwalder C, Mota-Vieira L, et al. Failure to find alpha-synuclein gene dosage changes in 190 patients with familial Parkinson disease. Arch Neurol 2005;62:96–98.
29. Lockhart PJ, Kachergus J, Lincoln S, et al. Multiplication of the alpha-synuclein gene is not a common disease mechanism in Lewy body disease. J Mol Neurosci 2004;24:337–342.
30. Hayward CP, Hassell JA, Denomme GA, et al. The cDNA sequence of human endothelial cell multimerin. A unique protein with RGDS, coiled-coil, and epidermal growth factor-like domains and a carboxyl terminus similar to the globular domain of complement C1q and collagens type VIII and X. J Biol Chem 1995;270:18246–18251.
31. Singleton A, Gwinn-Hardy K, Sharabi Y, et al. Association between cardiac denervation and parkinsonism caused by alpha-synuclein gene triplication. Brain 2004;127:768–772.
32. Nagayama H, Hamamoto M, Ueda M, et al. Reliability of MIBG myocardial scintigraphy in the diagnosis of Parkinson's disease. J Neurol Neurosurg Psychiatry 2005;76:249–251.
33. Orimo S, Ozawa E, Nakade S, et al. [^{123}I] metaiodobenzylguanidine myocardial scintigraphy differentiates corticobasal degeneration from Parkinson's disease. Intern Med 2003;42:127–128.
34. Suzuki M, Hattori N, Orimo S, et al. Preserved myocardial [^{123}I]metaiodobenzylguanidine uptake in autosomal recessive juvenile parkinsonism: first case report. Mov Disord 2005;20: 634–636.
35. Mueller JC, Fuchs J, Hofer A, et al. Multiple regions of alpha-synuclein are associated with Parkinson's disease. Ann Neurol 2005;57:535–541.
36. Pals P, Lincoln S, Manning J, et al. α-Synuclein promoter confers susceptibility to Parkinson's disease. Ann Neurol 2004;56: 591–595.

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Clinicogenetic Study of Mutations in *LRRK2* Exon 41 in Parkinson's Disease Patients From 18 Countries

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Abstract: We screened *LRRK2* mutations in exon 41 in 904 *parkin*-negative Parkinson's disease (PD) patients (868 probands) from 18 countries across 5 continents. We found three heterozygous missense (novel I2012T, G2019S, and I2020T) mutations in *LRRK2* exon 41. We identified 11 (1.3%) among 868 PD probands, including 2 sporadic cases and 8 (6.2%) of 130 autosomal dominant PD families. The *LRRK2* mutations in exon 41 exhibited relatively common and worldwide distribution. Among the three mutations in exon 41, it has been reported that Caucasian patients with G2019S mutation have a single-founder effect. In the present study, Japanese patients with G2019S were unlikely to have a single founder from the Caucasian patients. In contrast, I2020T mutation has a single-founder effect in Japanese patients. Clinically, patients with *LRRK2* mutations had typical idiopathic PD. Notably, several patients developed dementia and psychosis, and one with I2020T had low cardiac ^{123}I-metaiodobenzylguanidine (MIBG) heart/mediastinum ratio, although the ratio was not low in other patients with I2020T or G2019S. Clinical phenotypes including psychosis, dementia, and MIBG ratios are also heterogeneous, similar to neuropathology, in PD associated with *LRRK2* mutations. © 2006 Movement Disorder Society

Key words: genetics; Parkinson's disease; *PARK8*; *leucin-rich repeat kinase 2* (*LRRK2*); cardiac ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy

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Parkinson's disease (PD) is the second most common neurodegenerative disorder with a prevalence of 3% in individuals older than 65 years.[1] Although the majority of PD cases are sporadic, it is now clear that genetic factors contribute to the pathogenesis of PD. Indeed, *alpha-synuclein*,[2] *UCH-L1*,[3] and *LRRK2*[4,5] have been reported as causative genes of autosomal dominant PD (ADPD); and *parkin*,[6] *DJ-1*,[7] and *PINK1*[8] as causative genes of autosomal recessive PD (ARPD). Recently, *LRRK2* was identified as the causative gene for *PARK8*,[4,5] and a common single *LRRK2* mutation in exon 41 (G2019S) has been found in mainly Caucasian patients with familial PD.[9] In the present study, we performed direct sequencing of *LRRK2* exon 41 in a large number of patients with familial and sporadic PD of different ethnic origins. We also analyzed the haplotype to determine whether a single haplotype was associated with *LRRK2* mutations. In addition, we investigated the clinicogenetic features, including cardiac ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy, of patients with *LRRK2* mutations.

PATIENTS AND METHODS

All blood samples and clinical information were obtained by the neurologists working in the respective countries after obtaining informed consent from their patients. The study was approved by the ethics review committee of Juntendo University. Diagnosis of PD was made by the participating neurologists based on the presence of Parkinsonism, good response to anti-PD treatment, and radiological findings. In all countries, the clinical evaluation was based on the Unified Parkinson's Disease Rating Scale. Controls were evaluated by neurologists to ensure none of them had PD. For control subjects, the mean age of the Japanese was 56.1 years (range, 23–98) and that of the Taiwanese was 34.4 years (range, 21–86). DNA was prepared using standard methods. The mutation screening was performed as described previously.[6,10] Dideoxy cycle sequencing was performed with Big Dye Terminater Chemistry (Applied Biosystems, Foster City, CA). This was followed by exon sequencing on ABI377 and 310 automated DNA sequence analyzers (Applied Biosystems). None of the participants had *parkin* mutations (including exonic deletions and multiplications), as confirmed by polymerase chain reaction (PCR), direct sequencing, and quantitative assays based on real-time PCR with Taq-Man probes (Applied Biosystems) of all exons.

Direct sequencing of *LRRK2* exon 41 was performed in 904 PD patients (male, 413; female, 437; not reported, 54; age, 7–100 years; mean, 47.5 years), including 868 probands from 18 countries (Japan, Korea, Taiwan, China, Philippines, Australia, Israel, Tunisia, Morocco, Turkey, Greece, Poland, Bulgaria, Yugoslavia, United Kingdom, United States, Canada, and Brazil). Asian probands (Japanese, Koreans, Taiwanese, and Chinese) formed the majority (763/868; 87.9%). The remainder included 72 Caucasian probands (Israelis, Australians, Turkish, Greek, Polish, Bulgarians, Yugoslavs, British, Americans, and Canadians) and 15 North African probands (Tunisians and Moroccans). The other 18 probands were Filipinos (n = 3), Brazilians (n = 1), or could not be specified (n = 14). In this study, families with more than two affected members or two members with a mutation at least in two generations were classified as ADPD, and families with at least two affected siblings in only one generation as (potential or pseudo-) ARPD. Among 904 PD patients, we considered 130 families as ADPD, 80 families as ARPD, and 417 patients as sporadic PD.

Haplotype analysis was performed as described previously.[10,11] Genotyping of patients with *LRRK2* mutations was performed using the microsatellite markers (D12S2514, D12S2515, D12S2516, D12S2518, D12S2519, D12S2520, D12S2521, D12S2522, and D12S2523) and the single nucleotide polymorphisms (rs1427263, rs11176013, and rs11564148).[10] Alleles were sized by GENESCAN (Applied Biosystems).

Cardiac MIBG scintigraphy was performed 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 cardiac MIBG uptake was measured on a planar image as the heart/mediastinum (H/M) activity ratio.

RESULTS

Genetic Studies

We found two heterozygous missense mutations (6059T>C, I2020T, and 6055G>A, G2019S), which had been reported previously,[5,9,12–14] and one novel heterozygous missense mutation (6035T>C; I2012T; Fig. 1). The novel I2012T mutation is also highly conserved across various species, similar to the other previously reported mutation sites of I2020T and G2019S.[5,12–14] We did not find any of these mutations in 200 chromosomes from normal Taiwanese and 200 chromosomes from normal Japanese populations. We found 3 Japanese patients (two probands) with the I2020T. Patient A1 with the novel I2012T mutation was of Taiwanese origin.

Including all ethnic groups, we found 11 (1.3%) of 868 PD probands and 8 (6.2%) of 130 ADPD families (1 Taiwanese, 2 Japanese, 2 Israelis, and 3 Tunisians) with

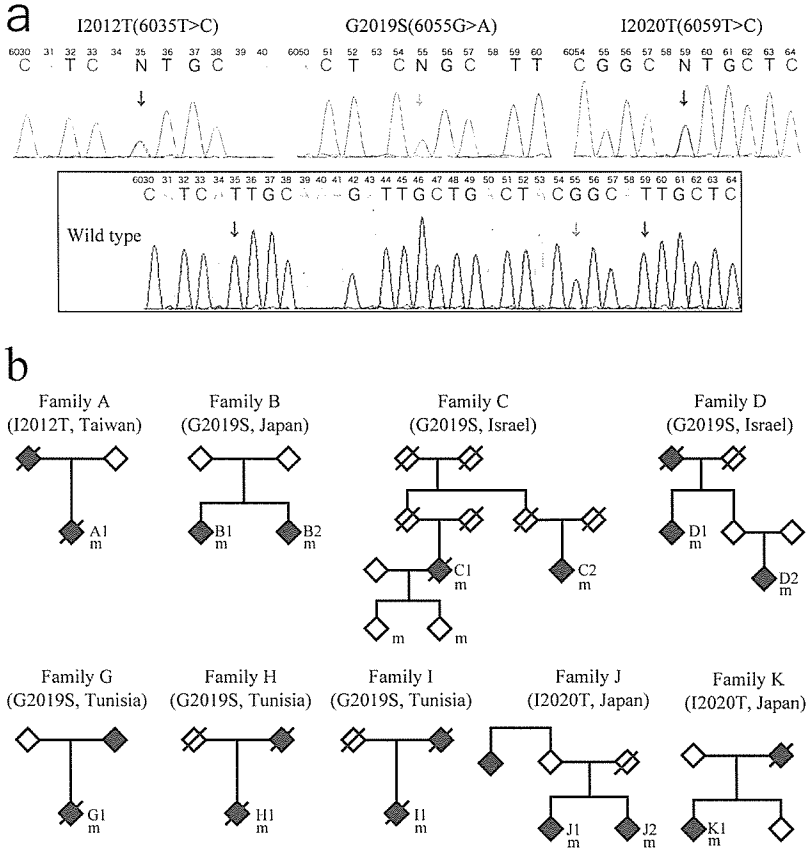

FIG. 1. a: Chromatogram showing 3 mutations in *LRRK2* exon 41. A novel 6035T>C (I2012T) mutation was found in Family A. The 6055G>A (G2019S) mutation was found in Families B-I. The 6059T>C (I2020T) mutation was found in Families J and K. **b:** Pedigree structures of Families A-D and G-K. Solid symbols denote affected members with PD and "m" denotes members with mutation. To provide confidentiality for participants, some unaffected members and some affected members in whom haplotype analysis was not performed are not shown on the trees.

3 different mutations in *LRRK2* exon 41. The G2019S mutation was detected in 8 probands (1 Japanese, 3 Israelis, 1 Moroccan, and 3 Tunisians). Considering the mode of inheritance, 2 (0.5%) of 417 sporadic PD patients had the G2019S with the age at onset of 41 and 42 years, respectively. Including all ethnic groups, 5 ADPD families (2 Israelis and 3 Tunisians) had the G2019S (5 of 130 = 3.8%). Interestingly, 1 (1.3%) of 80 potential or pseudo-ARPD families had the G2019S.

Considering each ethnic group, the frequency of PD probands with the G2019S was as follows: 0.1% (1 of 763) in Asian people, 4.2% (3 of 72) in Caucasian people, and 26.7% (4 of 15) in North African people. The frequency of PD probands with the I2020T was 0.3% (2 of 763) in Asian people. In addition to the pathogenic mutations, we found a novel single polymorphism (6054C>T) that was synonymous (T2018T) in 2 Korean families.

The parent and the parent's sibling of Japanese Patients J1 and J2 with the I2020T were born in the Sagamihara area, and the Japanese Patient K1 with the I2020T was also born near the Sagamihara area. The haplotype with 5 microsatellite markers in this region was exactly the same as that of the Sagamihara family.[11,12] In contrast, the haplotype of the other Japanese Family B (Patients B1 and B2) with the G2019S was quite different from that of the Caucasian families reported previously (Fig. 2).[10]

Clinical Features

Most patients with *LRRK2* mutations in exon 41 showed cardinal signs of idiopathic PD and good responses to levodopa (Table 1). Other clinical features noted in some patients included dementia, hallucination, delusion, and depression. Patients C1 and C2 with the G2019S mutation developed visual hallucinations, paranoidal thoughts, and dementia. Patient H1 with the G2019S mutation developed hallucinations and delusion. Patients B1 and C2 with the G2019S mutation also suffered from depression. Patient J2 with the I2020T mutation developed severe auditory and visual hallucinations and severe delusion. Psychoses were not induced by medication (levodopa/dopamine agonists) and did not relent when *off* medication. The doses of anti-PD drugs for Patient J2 were selegiline 2.5 mg/day, cabergoline 3 mg/day, amantadine 150 mg/day, and talipexole 1.2 mg/

a

Patient		Family (country)																			
		B (Japan)				C (Israel)				D (Israel)				E (Israel)		F (Morocco)		G (Tunisia)		H (Tunisia)		I (Tunisia)	
		B1		B2		C1		C2		D1		D2		E1		F1		G1		H1		I1	
Marker	D12S345	210	214	210	232	214	214	214	210	214	218	214	212	214	231	212	224	211	217	214	223	226	234
	D12S2514	295	295	295	295	**291**	291	**291**	291	**291**	295	**291**	295	**291**	291	**291**	291	293	285	**291**	291	291	291
	D12S2515	216	228	216	228	**224**	224	**224**	216	**224**	216	**224**	224	**224**	216	**224**	220	228	228	**224**	212	228	232
(*LRRK2* IVS 30)	**D12S2516**	252	252	252	252	**254**	254	**254**	252	**254**	252	**254**	252	**254**	254	**254**	254	**254**	254	**254**	254	**254**	254
(*LRRK2* Exon 34)	**rs1427263**	C	C	C	C	A	A	A	C	A	C	A	C	A	A	A	A	A	C	A	A	A	A
(*LRRK2* Exon 34)	**rs11176013**	A	A	A	A	G	G	G	A	G	A	G	A	G	G	G	G	G	A	G	G	G	G
(*LRRK2* Exon 34)	**rs11564148**	T	T	T	T	A	A	A	T	A	T	A	T	A	A	A	T	A	T	A	A	A	A
(*LRRK2* IVS 45)	**D12S2518**	154	168	154	168	**154**	154	**154**	168	**154**	154	**154**	154	**154**	154	**154**	154	**154**	168	**154**	154	**154**	154
	D12S2519	140	140	140	132	**132**	132	**132**	132	**132**	132	**132**	138	**132**	132	**132**	140	**132**	138	**132**	132	**132**	132
	D12S2520	257	257	257	254	**260**	254	**260**	257	**260**	260	**260**	260	**260**	257	**260**	257	**260**	248	**260**	257	**260**	257
	D12S2521	320	339	320	364	**359**	363	**359**	357	**359**	367	**359**	323	**359**	359	**359**	320	**359**	324	**359**	363	**359**	363
	D12S2522	285	281	285	297	**297**	297	**297**	297	**297**	297	**297**	297	**297**	297	**297**	283	**297**	281	**297**	297	**297**	297
	D12S2523	314	317	314	320	**320**	320	**320**	320	**320**	320	**320**	320	**320**	320	**320**	314	**320**	318	**320**	320	**320**	320
	D12S1653	202	206	202	206	210	218	210	206	210	220	210	220	210	220	210	215	217	206	217	217	217	219

b

Patient		Family (country)						
		J (Japan)				K (Japan)		Sagamihara (Japan)
		J1		J2		K1		
Marker	D12S1631	**185**	181	**185**	177	**185**	177	**185**
	D12S87	**155**	155	**155**	157	**155**	155	**155**
	D12S345	**224**	214	**224**	212	**224**	230	**224**
	D12S1653	**222**	210	**222**	210	**222**	216	**222**
	D12S85	**121**	123	**121**	103	**121**	103	**121**

FIG. 2. Haplotype analysis. **a:** The haplotype of Families C-I with the G2019S from around Europe was similar to the previously reported Caucasian family.[10] Genotypes shared are highlighted in boldface. The haplotype of Japanese Family B with the G2019S was different from that of Families C-I and the previously reported Caucasian family.[10] The size of these markers are corrected using CEPH 1331-01 and -02 as controls. **b:** The haplotype of Families J and K with the I2020T was similar to that of the Japanese Sagamihara family. Shared genotypes are highlighted in boldface. The haplotype with 5 microsatellite markers in this region spans 10.4 cM.

day. Patient B1 developed upward-gaze palsy. None of the patients showed severe autonomic disturbances or amyotrophy. The age at onset of the patients with these three mutations varied widely from 37 to 73 years (average, 55.0 years). In addition to these patients, two grown-up children (aged 36 and 38 years) with mutations were asymptomatic (Family C, Fig. 1b). Pathological studies could not be conducted in all patients with *LRRK2* mutations in this study.

Cardiac MIBG scintigraphy was performed in Patients B1 and B2 with G2019S mutation and Patients J1, J2, and K1 with I2020T mutation (Table 1). For Patient B1, B2, and K1, the H/M ratio was normal. For Patient J1, the H/M ratio was 1.56 in early image and 1.39 in delayed image. For Patient J2, the H/M ratio was 1.45 in early image and 1.30 in delayed image. The cutoff value of delayed H/M ratio is 2.09 in Patients B1 and B2 and 1.45 in Patients J1, J2, and K1 (each cutoff value is different because the MIBG scintigraphy was performed in different hospitals). Patient J2 had no ischemic heart disease, chronic heart failure, diabetes mellitus, or medication history of antidepressants that may influence MIBG uptake. Notably, Patient J2, who developed severe psychosis, had a low H/M ratio in delayed MIBG images.

DISCUSSION

The present study revealed that the *LRRK2* G2019S mutation spreads worldwide across different ethnic groups with variable frequencies. Whereas the G2019S mutation is quite rare in Asia, this mutation is more frequent around Europe[9,13,14] and especially in North Africa. Demographically, it is possible that the distribution and frequencies of this mutation is associated with human migration history. In a previous report, European and North American patients with the G2019S shared a common ancestral haplotype indicative of a single-founder effect.[10] Indeed, our patients from around Europe and North Africa also had the same haplotype. On the other hand, the haplotype of Japanese Family B was quite different from that of Caucasian patients, suggesting they do not share a common founder and they are of independent origin. The possibility that Japanese patients have a common haplotype on the very narrow region could not be excluded. However, it is unlikely that the Japanese G2019S mutation originated from a common and extremely old founder, because the G2019S mutation is rare in Asian people compared with North African and Caucasian peoples.

PARK8 was originally mapped from Japanese Sagamihara family showing late-onset ADPD.[11] The coincidence of haplotype in Japanese Family J, Family K, and Sagamihara family indicates a single-founder effect. The same mutation I2020T was found in a Caucasian patient of European origin (personal communication, Dr. Thomas Gasser),[5] suggesting this mutation also has a worldwide distribution, although the haplotype is unknown at present. Interestingly, G2019S and I2020T mutations affect adjacent codons in the amino acid sequence. In addition to the two mutations, I2012T is also located in the very narrow region within exon 41. This narrow region could be a hot spot and mutations in exon 41 could be relatively frequent. Since *LRRK2* consists of 51 exons, it is important to decide which exon(s) of this gene should be first screened for mutation analysis. In this regard, worldwide screening of exon 41 prior to all other exons is a reasonable strategy. Analysis of other putatively pathogenic coding substitutions and exon 31 is now also recommended.[15]

TABLE 1. *Clinical features of patients (Families A-K) with heterozygous LRRK2* mutations in exon 41

	LRRK2 mutation														
	I2012T	G2019S											I2020T		
Country	Taiwan	Japan		Israel		Israel		Israel	Morocco	Tunisia	Tunisia	Tunisia	Japan		Japan
Mode of inheritance	AD	AR		AD		AD		SP	SP	AD	AD	AD	AD		AD
Consanguinity	−	−		−		−		−	−	−	−	+	−		−
Patient's number	A1	B1	B2	C1	C2	D1	D2	E1	F1	G1	H1	I1	J1	J2	K1
Age at onset (yr)	47	59	73	72	66	64	60	41	42	58	46	37	53	64	43
Disease duration (yr)	14	8	2	13	8	21	18	9	7	2	6	16	8	6	3
Sex	M	F	M	M	F	M	M	F	M	F	F	F	F	F	M
Resting tremor	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−
Rigidity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bradykinesia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Postural instability	−	+	+	+	+	+	+	+	−	−	−	−	−	+	+
Gait disturbance	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+
Clinical response to L-dopa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	NT
Wearing *off*	−	+	+	+	+	+	+	+	+	−	−	−	+	−	−
Asymmetry at onset	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Incontinence	−	−	−	−	−	−	−	−	NA	−	−	−	−	−	−
Urinary urgency	−	−	−	+	−	−	−	−	NA	+	−	−	−	−	−
L-dopa–induced dyskinesia	+	−	−	+	+	+	+	+	+	−	−	+	−	−	−
Sleep benefit	−	+	−	−	−	−	−	−	NA	+	−	−	−	NA	+
Dystonia at onset	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Hyperreflexia	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Psychosis	−	DEP	−	HA, DEL	HA, DEL, DEP	−	−	−	−	−	HA, DEL	−	−	HA, DEL	−
Dementia	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−
Other special comment		Upward gaze palsy													
UPDRS III (*on/off*)	34/57	18/23	29/NA	43/56	38/52	25/33	25/31	16/32	NA	NA	7/37	19/72	7/NA	6/−	15/−
MIBG H/M ratio (early/delay)	ND	2.95/3.08	2.35/2.89	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.56/1.39	1.45/1.30	1.99/1.91

AD, autosomal dominant; AR, autosomal recessive; SP, sporadic; M, male; F, female; MIBG H/M ratio, ^{123}I-metaiodobenzylguanidine heart/mediastinum ratio; ND, not determined; NA, not available; NT, not tried; HA, hallucination; DEP, depression; DEL, delusion; UPDRS, Unified Parkinson's Disease Rating Scale.

With regard to the mode of inheritance, our study showed that not only patients with ADPD but also those with potential ARPD and sporadic PD had *LRRK2* mutations. Moreover, we detected the mutations in late- and early-onset PD. A shared *LRRK2* G2019S haplotype in the vast majority of carriers argues against de novo occurrence.[13] Based on the widely variable age at onset and various modes of inheritance, the phenotype of PD patients with *LRRK2* mutations must be influenced by stochastic, environmental, and other genetic factors.[10]

The clinical features of most patients with *LRRK2* mutations resemble those of patients with typical idiopathic PD.[10] Previously reported patients with *LRRK2* mutations, especially those with G2019S and I2020T, showed no evidence of psychiatric or cognitive dysfunction apart from mild symptoms, despite the long disease duration.[5,11,12,16] However, some of our patients with the *LRRK2* mutations developed severe hallucination, moderate dementia, delusion, and depression, even during the early stages of the disease. Both Patients C1 and C2 with G2019S of the same family had psychosis and dementia. On the other hand, although of the same family, Patient J2 with I2020T showed psychosis and Patient J1 did not show psychosis. Notably, only Patient J2 developed severe psychosis and had low H/M ratio in delayed MIBG images. In contrast, markedly low H/M ratio was not noted in other patients without psychosis.

MIBG scintigraphy is a clinically useful diagnostic method and has high sensitivity. MIBG uptake shows remarkable decrease in Lewy body–positive PD patients from the early stage, except for some of the patients with stage I. Indeed, in a previous study, a more severely reduced H/M ratio was seen in dementia with Lewy bodies compared with PD, and the strong negative correlation between H/M ratio and Hoehn–Yahr stage in PD was observed, suggesting that Lewy body pathology itself may cause low MIBG uptake.[17] Histopathological studies showed a high incidence of Lewy bodies in the sympathetic ganglia and visceral autonomic nervous system[18] and the presence of Lewy bodies and Lewy neurites in the cardiac plexus in PD.[19] These findings may account for low cardiac uptake of MIBG in Lewy body disease, reflecting actual cardiac sympathetic denervation, which precedes the neuronal loss in the sympathetic ganglia.[19,20] In this regard, the reduced cardiac uptake of MIBG in Patient J2 is compatible with Lewy body disease, and Patient J2 with severe psychosis may have cardiac sympathetic denervation and spread of Lewy bodies. On the other hand, Patients B1 and B2 with G2019S and Patients J1 and K1 with I2020T may have no Lewy bodies, similar to most patients of the Sagamihara family.[11,12] The normal MIBG uptake in autosomal recessive juvenile parkinsonism (AR-JP), which is caused by the *parkin* gene,[21,22] generally without Lewy bodies, psychosis, and dementia,[23] supports our findings concerning MIBG scintigraphy and clinical features.

In the present study, the clinical features and cardiac uptake of MIBG varied even in patients with the same I2020T mutation, and also in members of the same family. Indeed, one PD patient with the I2020T mutation from the Sagamihara family had Lewy bodies, although most of the other family members were negative for Lewy bodies (personal communication, Dr. Ogino). Thus, variable pathologies such as positive and negative Lewy body and variable clinical phenotypes were observed in patients with *LRRK2* mutations.

In the previously reported German–Canadian family and western Nebraska family, the phenotypes of *PARK8*-linked parkinsonism with signs of dementia, diffuse Lewy body disease (DLBD), progressive supranuclear palsy (PSP), and motor neuron degeneration showed pathological features of synucleinopathies and tauopathies.[24–27] In the present study, one patient with G2019S showed upward-gaze palsy like PSP, although downward-gaze palsy was not seen. Interestingly, some of the patients with *LRRK2* R1441C mutation had Lewy bodies, while patients with Y1699C mutation in the German–Canadian family and most patients with I2020T of the Sagamihara family had no Lewy bodies.[5,24–27] Thus, clarification of the function of *LRRK2* product and its interaction with other proteins is important for our understanding of the pathogenesis of dopaminergic neuronal death with or without Lewy bodies.

The domain structure of *LRRK2* includes five conserved major functional domains, including leucine-rich repeat (LRR), a Roc (Ras in complex proteins) domain, a COR domain (C-terminal of Roc), a tyrosine kinase catalytic domain (TyrKc), and a WD40 domain.[5] The I2012T, G2019S, and I2020T mutations are located in the kinase domain. The G2019S and I2020T mutations may be involved in the alteration of kinase activities of the *LRRK2* product.[28] Furthermore, this protein may be potentially associated with phosphorylation of both alpha-synuclein and tau.[4,5,29] Indeed, concurrent pathology of alpha-synuclein and tau accumulation within the same aggregates was reported previously.[29] Therefore, the kinase activity of the *LRRK2* product could be a key event in the accumulation and aggregation of these unfolded proteins within the degenerating neurons[4,5] and could be an upstream event.

In our study, we showed that PD caused by *LRRK2* mutations is common and widespread, showing variable pathology and variable clinical phenotypes, including psychosis and dementia. Our study could have major

implications on the clinical understanding of phenotype–genotype correlations and could help in genetic counseling for PD. Insights into the pathogenesis of the disease may lead to new treatment of PD and neurodegenerative disorders.

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REFERENCES

1. Lang AE, Lozano AM. Parkinson's disease: first of two parts. N Engl J Med 1998;339:1044–1053.
2. Polymeropoulos MH, Lavedan C, Leroy E, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 1997;276:2045–2047.
3. Leroy E, Boyer R, Auburger G, et al. The ubiquitin pathway in Parkinson's disease. Nature 1998;395:451–452.
4. Paisán-Ruíz C, Jain S, Evans EW, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 2004;44:595–600.
5. Zimprich A, Biskup S, Leitner P, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 2004;44:601–607.
6. Kitada T, Asakawa S, Hattori N, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 1998; 392:605–608.
7. Bonifati V, Rizzu P, van Baren MJ, et al. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 2003;299:256–259.
8. Valente EM, Abou-Sleiman PM, Caputo V, et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 2004;304:1158–1160.
9. Nichols WC, Pankratz N, Hernandez D, et al. Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease. Lancet 2005;365:410–412.
10. Kachergus J, Mata IF, Hulihan M, et al. Identification of a novel LRRK2 mutation linked to autosomal dominant parkinsonism: evidence of a common founder across European populations. Am J Hum Genet 2005;76:672–680.
11. Funayama M, Hasegawa K, Kowa H, et al. A new locus for Parkinson's disease (PARK8) maps to chromosome 12p11.2-q13.1. Ann Neurol 2002;51:296–301.
12. Funayama M, Hasegawa K, Ohta E, et al. An LRRK2 mutation as a cause for the parkinsonism in the original PARK8 family. Ann Neurol 2005;57:918–921.
13. Gilks WP, Abou-Sleiman PM, Gandhi S, et al. A common LRRK2 mutation in idiopathic Parkinson's disease. Lancet 2005;365:415–416.
14. Di Fonzo A, Rohe CF, Ferreira J, et al. A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease. Lancet 2005;365:412–415.
15. Mata IF, Kachergus JM, Taylor JP, et al. Lrrk2 pathogenic substitutions in Parkinson's disease. Neurogenetics 2005;17:1–7.
16. Aasly JO, Toft M, Fernandez-Mata I, et al. Clinical features of LRRK2-associated Parkinson's disease in central Norway. Ann Neurol 2005;57:762–765.
17. Nagayama H, Hashimoto M, Ueda M, et al. Reliability of MIBG myocardial scintigraphy in the diagnosis of Parkinson's disease. J Neurol Neurosurg Psychiatry 2005;76:249–251.
18. Wakabayashi K, Takahashi H, Ohama E, et al. Lewy bodies in the visceral autonomic nervous system in Parkinson's disease. In: Narabayashi H, Nagatsu N, Yanagisawa N, Mizuno Y, editors. Advances in neurology. New York: Lippincott-Raven; 1993. p 609–612.
19. Iwanaga K, Wakabayashi K, Yoshimoto M, et al. Lewy body–type degeneration in cardiac plexus in Parkinson's and incidental Lewy body diseases. Neurology 1999;52:1269–1271.
20. Orimo S, Amino T, Itoh Y, et al. Cardiac sympathetic denervation precedes neuronal loss in the sympathetic ganglia in Lewy body disease. Acta Neuropathol (Berl) 2005;109:583–588.
21. Suzuki M, Hattori N, Orimo S, et al. Preserved myocardial [123I]metaiodobenzylguanidine uptake in autosomal recessive juvenile parkinsonism: first case report. Mov Disord 2005;20:634–636.
22. Kitami T, Nomoto T, Nagao T, et al. ^{123}I-MIBG myocardial scintigraphy in juvenile parkinsonism. Mov Disord 1998;13(Suppl. 2):247.
23. Lücking CB, Dürr A, Bonifati V, et al. Association between early-onset Parkinson's disease and mutations in the *parkin* gene. N Engl J Med 2000;342:1560–1567.
24. Wszolek ZK, Vieregge P, Uitti RJ, et al. German–Canadian family (family A) with parkinsonism, amyotrophy, and dementia: longitudinal observations. Parkinsonism Relat Disord 1997;3:125–139.
25. Wszolek ZK, Pfeiffer B, Fulgham JR, et al. Western Nebraska family (family D) with autosomal dominant parkinsonism. Neurology 1995;45:502–505.
26. Zimprich A, Müller-Myhsok B, Farrer M, et al. The PARK8 locus in autosomal dominant parkinsonism: Confirmation of linkage and further delineation of the disease-containing interval. Am J Hum Genet 2004;74:11–19.
27. Wszolek ZK, Pfeiffer RF, Tsuboi Y, et al. Autosomal dominant parkinsonism associated with variable synuclein and tau pathology. Neurology 2004;62:1619–1622.
28. Hernandez DG, Paisán-Ruíz C, Mclnerney-Leo A, et al. Clinical and positron emission tomography of Parkinson's disease caused by LRRK2. Ann Neurol 2005;57:453–456.
29. Duda JE, Giasson BI, Mabon ME, et al. Concurrence of alpha-synuclein and tau brain pathology in the Contursi kindred. Acta Neuropathol (Berl) 2002;104:7–11.

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Research Review

Recessive Parkinson's Disease

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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disease caused by loss of dopaminergic neurons in the substantia nigra pars compacta. Although the etiology of PD remains unclear, it is now clear that genetic factors contribute to the pathogenesis of the disease. Recently, several causative genes have been identified in monogenic forms of PD. Accumulating evidence indicates that their gene products play important roles in mitochondrial function, oxidative stress response, and the ubiquitin–proteasome system, which are also implicated in sporadic PD, suggesting that these gene products share a common pathway to nigral degeneration in both familial and sporadic PD. Here, we review recent advances in knowledge about genes associated with recessive PD, including *parkin*, *PINK1*, and *DJ-1*. © 2006 Movement Disorder Society

Key words: *parkin*; *PINK1*; *DJ-1*; recessive Parkinson's disease; mitochondria; oxidative stress; ubiquitin–proteasome system

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 discovery of levodopa, a precursor of dopamine, has dramatically improved the functional prognosis of PD. Levodopa, however, is a symptomatic drug and long-term treatment with levodopa is associated with adverse effects, such as motor fluctuations (wearing-off and on–off phenomenon) and dyskinesias. New therapies are therefore required to improve the long-term prognosis. To develop a new remedy for PD, it will be essential to elucidate the pathogenic mechanisms underlying the degeneration of dopaminergic neurons.

Mitochondrial dysfunction and oxidative stress are critical components of most current theories of nigral degeneration in PD.[2–5] However, the mechanisms responsible for the cell death are largely unknown. Recently, there has been increasing evidence that genetic factors play an important role in PD. Although most PD is sporadic, a small proportion of cases shows a Mendelian inheritance. The identification of responsible genes for rare familial forms of PD has provided vital clues to understanding the molecular pathogenesis of the more common sporadic forms of this disease.

To date, at least nine distinct genetic loci have been recognized to be linked to PD (PARK1–3, 5–8, 10, and 11).[6–14] Recently, the family that was originally mapped to PARK4 has been mapped to PARK1.[15] PARK9 has been found not to be a genetic locus for PD because of the atypical associated phenotypes such as spasticity resulting from corticospinal tract degeneration, a supranuclear upward-gaze paresis, and the development of dementia in all affected subjects.[16] Among the PD-associated loci, mutations have been identified in five genes (*α-synuclein*,[17] *parkin*,[18] *PINK1*,[19] *DJ-1*,[20] and *LRRK2*[21,22]) that definitely cause familial forms of PD. *UCH-L1* is another gene that may cause PARK5. So far, only one mutation in the *UCH-L1* gene that is potentially linked to PD was identified in a small German family.[9] Among these genes, mutations in *parkin* (PARK2), *PINK1* (PARK6), and *DJ-1* (PARK7) are associated with

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TABLE 1. *Mutations linked to recessive PD*

	PARK2	PARK6	PARK7
Locus	6q25-27	1p35-36	1p36
Gene	*Parkin*	*PINK1*	*DJ-1*
Inheritance	AR	AR	AR
Number of mutations	> 95	> 17	> 9
Type of mutations	Exonic deletion and duplication, insertion, nonsense, missense	Insertion, deletion, nonsense, missense	Exonic deletion, missense
Lewy body	(−) except for two cases	?	?

autosomal recessive PD, in which loss of function of a single gene product can lead to the degeneration of dopaminergic neurons and the clinical manifestations of parkinsonism (Table 1). This suggests that these gene products are essential for the survival of nigral neurons. Therefore, identification of the physiological functions of these proteins is expected to lead to elucidation of the pathogenic mechanisms underlying the disease. Here, we review the clinical findings in recessive forms of PD and the molecular biology of *parkin*, *PINK1*, and *DJ-1*.

PARKIN (PARK2)

Clinical manifestations of *parkin*-associated PD were originally characterized by parkinsonism associated with early onset before the age of 40, wearing-off phenomenon, and slow disease progression.[23] More recent studies have suggested a broader phenotypic spectrum of *parkin*-associated PD that includes later age at onset and tremor-dominant manifestations without foot dystonia, hyperreflexia, diurnal fluctuations, sleep benefit, and early susceptibility to levodopa-induced dyskinesias.[24–26] Many *parkin*-associated PD patients, therefore, appear to be clinically indistinguishable from sporadic PD. Remarkably, a wide variation of age at onset has been reported, even within single families with mutations in the *parkin* gene,[26] suggesting that additional factors, either genetic or environmental, contribute to the phenotype. However, Lohmann and colleagues[27] have reported that *parkin*-associated PD patients tend to have earlier and more symmetrical onset, slow progression of the disease, and greater response to levodopa despite low doses compared with those with early-onset PD without *parkin* mutations. Clinical features including atypical phenotypes are summarized in Table 2.[27–31] At present, there appears to be no genotype–phenotype correlation. Histopathological characteristics of *parkin*-associated PD are neuronal loss and gliosis limited to the substantia nigra and locus coeruleus. Although the entire knowledge on the pathology is based on limited cases, Lewy bodies, which are cytoplasmic proteinaceous inclusion bodies that are considered the hallmark of PD, are absent[28,29,32–37] except in two reported cases with compound heterozygous mutations.[38,39] Atypical pathological findings have been reported in *parkin*-associated PD, such as tau pathology in the cerebral cortex and brainstem nuclei,[29,32] degeneration of the spinocerebellar system,[28] and α-synuclein–positive inclusions in the neuropils of the pedunclopontine nucleus.[36] Presence of α-synuclein–positive inclusions including Lewy bodies, although detected in only a small number of patients, suggests a possible relationship between *parkin*-associated PD and idiopathic PD.

The gene responsible for *parkin*-associated PD, *parkin*,[18] contains 12 exons spanning over 1.4 megabases and encodes a 465 amino acid (aa) protein with moderate homology to ubiquitin at the amino terminus (ubiquitin-like domain, Ubl) and two RING finger motifs at the carboxy terminus. Various *parkin* mutations have been reported worldwide, including exonic deletions, exonic duplications, insertions, and many different point mutations.[26,30,31,40] Mutations in the *parkin* gene are a relatively frequent cause of early-onset PD, especially in cases with a positive family history and an autosomal recessive mode of transmission. To date, more than 95 different mutations in approximately 400 patients have been reported worldwide.[41] Frequency of the mutations in early-onset PD has been estimated at 40% to 50% in

TABLE 2. *Clinical features of* parkin*-associated PD*

Age of onset usually < 40 years
Typical presenting phenotype: young-onset PD
Normal cognition
Frequent foot dystonia
Early instability, freezing, festination, or retropulsion in some cases
Dramatic response to levodopa, and dose-sensitive motor and psychiatric complications of medication
Excellent response to anticholinergics in some cases
Usually benign and slow disease course
Atypical presenting phenotypes include:
Later onset, mimicking idiopathic PD
Psychosis, panic attacks, depression, hypersexuality, obsessive–compulsive behavior
Exercise-induced dystonia
Atremulous bilateral akinetic rigid syndrome
Focal dystonia (writer's cramp, cervical)
Autonomic or peripheral neuropathy
Cerebellar and pyramidal tract dysfunction