



Clinicogenetic study of mutations in LRRK2 exon 41 in Parkinson's disease patients from 18 countries

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**Clinicogenetic study of mutations in *LRRK2* exon 41 in Parkinson's disease patients
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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 3 heterozygous missense (novel I2012T, G2019S, and I2020T) mutations in *LRRK2* exon 41. We identified 11 among 868 PD probands (1.3%) including 2 sporadic cases and 8 of 130 (6.2%) autosomal dominant PD families. The *LRRK2* mutations in exon 41 exhibited relatively common and worldwide distribution. Among the 3 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 ¹²³I-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.

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

Parkinson's disease (PD) is the second most common neurodegenerative disorder with a prevalence of 3% in individuals older than 65 years of age.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 ¹²³I-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 (range, 23-98) years and that of the Taiwanese was 34.4 (range, 21-86) years. DNA was prepared using standard methods. The mutation screening was performed as

described previously.^{6, 10} Dideoxy cycle sequencing was performed with Big Dye Terminator
85 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.

90 Direct sequencing of *LRRK2* exon 41 was performed in 904 PD patients (males 413,
females 437, not reported 54; aged 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, USA, Canada,
and Brazil). Asian probands (Japanese, Koreans, Taiwanese, and Chinese) formed the
95 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
100 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,
105 D12S2515, D12S2516, D12S2518, D12S2519, D12S2520, D12S2521, D12S2522, and
D12S2523) and the SNPs (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 ¹²³I-MIBG (Daiichi Radioisotope Laboratories Co., Tokyo, Japan). Early images were
110 obtained 15 minutes and delayed images were obtained 3-4 hours after injection. Whole cardiac MIBG uptake was measured on a planar image as the heart/mediastinum (H/M) activity ratio.

Results

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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. 1a, b). 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 three Japanese patients (two probands) with the I2020T. Patient A1 with the novel I2012T mutation was of Taiwanese origin.

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Including all ethnic groups, we found 11 of 868 (1.3%) PD probands and 8 of 130 (6.2%) ADPD families (one Taiwanese, two Japanese, two Israelis, and three Tunisians) with three different mutations in *LRRK2* exon 41. The G2019S mutation was detected in 8 probands (one Japanese, three Israelis, one Moroccan, and three Tunisians). Considering the mode of inheritance, two of 417 sporadic PD patients ($2/417 = 0.5\%$) had the G2019S with the age at onset of 41 and 42 years. Including all ethnic groups, five ADPD families (two Israelis and three Tunisians) had the G2019S ($5/130 = 3.8\%$), and interestingly 1 of 80 potential or pseudo ARPD families had the G2019S ($1/80 = 1.3\%$).

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Considering each ethnic group, the frequency of PD probands with the G2019S was as follows: 0.1% (1/763) in Asian people; 4.2% (3/72) in Caucasian people; and 26.7% (4/15) in North African people. The frequency of PD probands with the I2020T was 0.3% (2/763) in Asian people. In addition to the pathogenic mutations, we found a novel single polymorphism (6054C>T) that was synonymous (Y2018Y) in two Korean families.

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The parent and the parent's sibling of Japanese Patients J1 and J2 with the I2020T were born in the Sagami-hara area, and the Japanese Patient K1 with the I2020T was also born near the Sagami-hara area. The haplotype with five microsatellite markers in this region was exactly the same as that of the Sagami-hara 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. 2a, b).¹⁰

145 *Clinical features*

Most patients with *LRRK2* mutations in exon 41 showed cardinal signs of idiopathic PD and good responses to levodopa (Table). Other clinical features noted in some patients included dementia, hallucination, delusion, and depression. Patients C1 and C2 with the G2019S mutation developed visual hallucinations, paranoid 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/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). Pathologic 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). 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 cut off value of delayed H/M ratio is 2.09 in Patients B1 and B2, and is 1.45 in
165 Patients J1, J2, and K1 (each cut off value is different because the MIBG scintigraphy was performed in different hospitals). Patient J2 had no ischemic heart disease, chronic heart failure, diabetes mellitus, and no medication history of anti-depressants that may influence MIBG uptake. Notably, Patient J2, who developed severe psychosis, had a low H/M ratio in
170 delayed MIBG images.

Discussion

The present study revealed that the *LRRK2* G2019S mutation spreads worldwide across
175 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
180 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
185 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 firstly 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

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
215 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
220 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
225 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
230 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.

235 In the present study, the clinical features and cardiac uptake of MIBG varied even in patients with the same I2020T mutation, and also even 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
240 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,
245 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
250 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
255 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
260 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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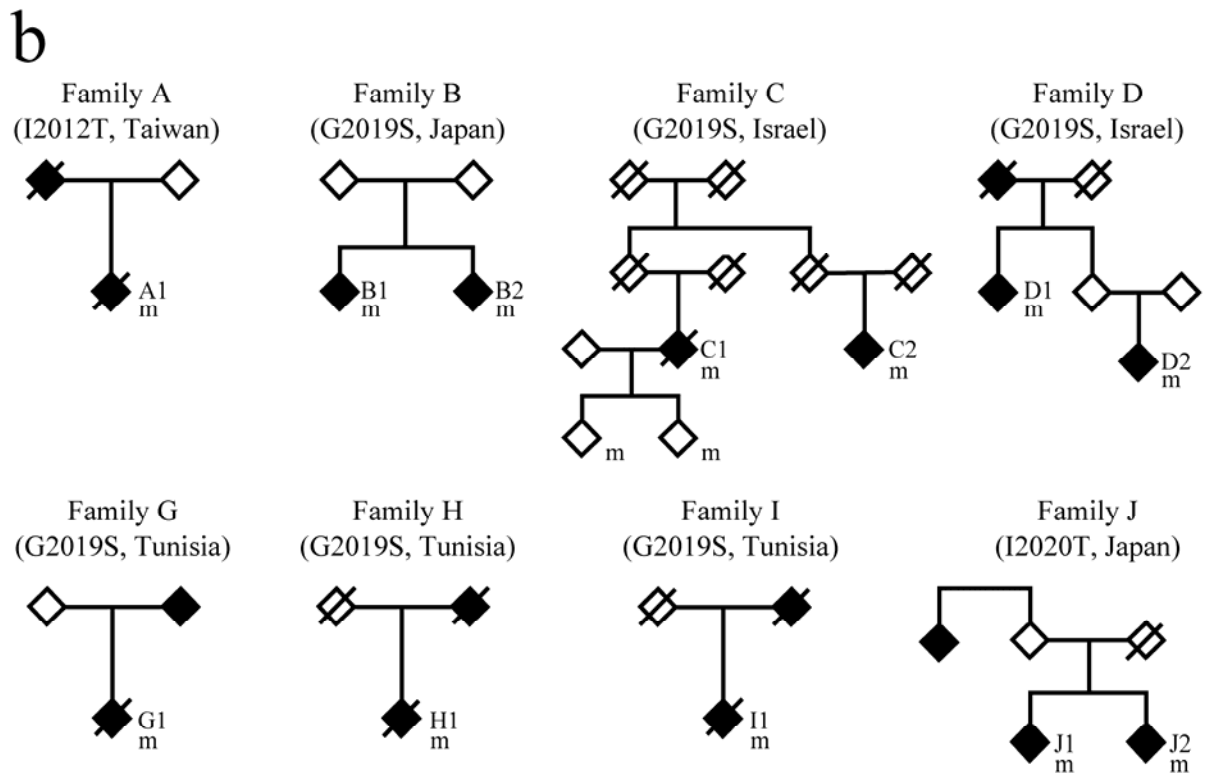
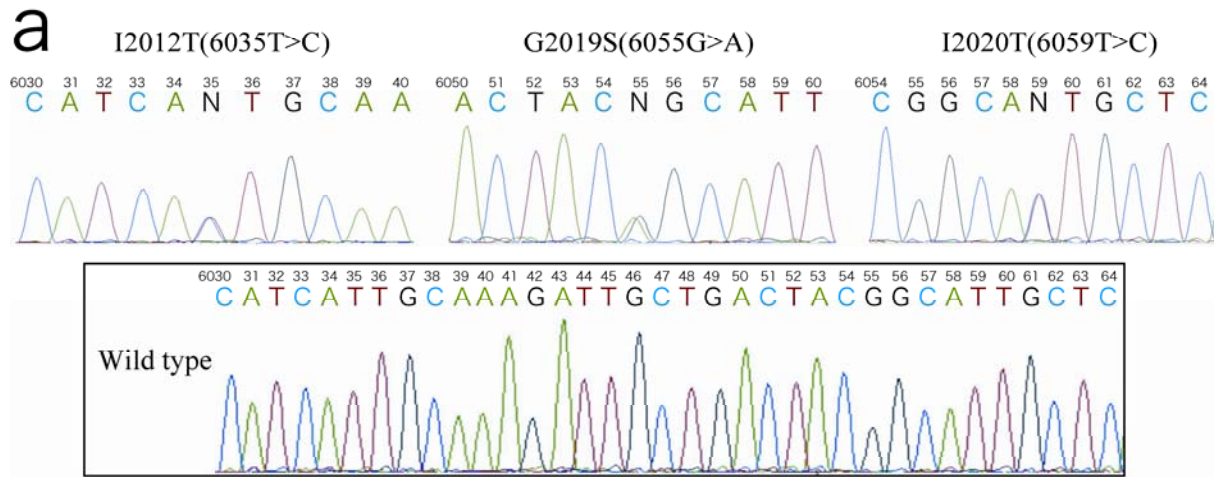
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Legends for figures



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Figure 1. (a) Chromatogram showing three 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 symbols of “m” denote 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.

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a

Patient	Marker	Family (country)																					
		B (Japan)				C (Israel)				D (Israel)				E (Israel)		F (Morocco)		G (Tunisia)		H (Tunisia)		I (Tunisia)	
		B1	B2	B3	B4	C1	C2	C3	C4	D1	D2	D3	D4	E1	E2	F1	F2	G1	G2	H1	H2	I1	I2
	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
(<i>LRRK2</i> 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
(<i>LRRK2</i> 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
(<i>LRRK2</i> 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
(<i>LRRK2</i> 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
(<i>LRRK2</i> 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	Marker	Family (country)						
		J (Japan)		K (Japan)		Sagamihara (Japan)		
		J1	J2	K1	K2			
	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

360 Figure 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 red. 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 365 Families J and K with the I2020T was similar to that of the Japanese Sagamihara family. Shared genotypes are highlighted in blue. The haplotype with five microsatellite markers in this region spans 10.4 cM.