Published in final edited form as:

Neurobiol Aging. 2014 November ; 35(11): 2656.e17–2656.e23. doi:10.1016/j.neurobiolaging.2014.05.025.

Identification of a Japanese family with LRRK2 p.R1441G-related Parkinson's disease

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Abstract

Leucine-rich repeat kinase 2 (*LRRK2*) is a causative gene of autosomal dominant familial Parkinson's disease (PD). We screened for *LRRK2* mutations in 3 frequently reported exons (31, 41, and 48) in our cohort of 871 Japanese patients with PD (430 with sporadic PD and 441 probands with familial PD). Direct sequencing analysis of *LRRK2* revealed 1 proband (0.11%) with a p.R1441G mutation, identified for the first time in Asian countries, besides frequently reported substitutions including, the p.G2019S mutation (0.11%) and p.G2385R variant (11.37%). Several studies have suggested that the *LRRK2* p.R1441G mutation, which is highly prevalent in the Basque country, is extremely rare outside of northern Spain. Further analysis of family members of the proband with the p.R1441G mutation revealed that her mother and first cousin shared the same mutation and parkinsonism. Haplotype analysis revealed a different haplotype from that of the original Spanish families. Our patients demonstrated levodopa-responsive parkinsonism with intrafamilial clinical heterogeneity. This is the first report of familial PD because of the *LRRK2* p.R1441G mutation in Asia.

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Disclosure statement: The authors report no conflicts of interest and have no financial interest related to the material described in the manuscript.

Keywords

Parkinson's disease; *LRRK2*; p.R1441G; Asia; Intrafamilial clinical heterogeneity

1. INTRODUCTION

Leucine-rich repeat kinase 2 (*LRRK2*) is one of the causative genes of autosomal dominant Parkinson's disease (PD) (Paisan-Ruiz et al., 2004; Zimprich et al., 2004). To date, 7 mutations in *LRRK2* p.G2019S, p.I2020T, p.N1437H, p.R1441G/C/H, and p.Y1699C have been proven as pathogenic mutations. Although p.G2019S and p.R1441G/C/H are frequent mutations in *LRRK2* and the prevalence of the p.G2019S mutation, the most common *LRRK2* substitution in populations of European origin, is estimated at 5%-13% of individuals with familial PD (Haugarvoll and Wszolek, 2009), the p.R1441C, p.R1441H, and p.G2019S mutations have been found to be very rare in Asia (Haugarvoll et al., 2008; Ross et al., 2009). The p.R1441G mutation is frequent in Spain, and especially in the Basque country, where it accounts for 16% of familial and 4% of sporadic cases (Haugarvoll and Wszolek, 2009). However, p.R1441G carriers are extremely rare outside of Spain (Haugarvoll and Wszolek, 2009; Mata et al., 2005, 2009b; Simon-Sanchez et al., 2006). To date, only 4 probands with p.R1441G outside of northern Spain have been reported (Cornejo-Olivas et al., 2013; Deng et al., 2006; Mata et al., 2009a; Yescas et al., 2010). Interestingly, most of the reported p.R1441G PD patients share a common founder, and this mutation is regarded as a rare haplotype (Haugarvoll and Wszolek, 2009; Mata et al., 2005, 2009b; Simon-Sanchez et al., 2006). It remains unclear whether there are patients carrying the p.R1441G mutation in Asia. In this study, we performed a mutation screening of exons 31, 41, and 48 of *LRRK2* in a Japanese cohort of PD patients and found a proband with the p.R1441G mutation. Here, we report the results of a clinicoradiological and haplotype analysis of the first familial PD patients linked to p.R1441G mutation in Asian countries.

2. METHODS

2.1. Subjects

We studied 871 Japanese PD patients (430 patients with sporadic PD and 441 probands with familial PD; age, 56.4 ± 14.5 years; age at onset, 49.8 ± 14.6 years; disease duration; 6.67 ± 6.89 years). A clinical diagnosis of PD was determined by the presence of at least 2 of 3 cardinal signs (rest tremor, bradykinesia, and rigidity) and improvement following adequate dopaminergic therapy (when available). In this study, families with 2 or more affected members or 2 members with a mutation in at least 2 generations were classified as autosomal dominant PD families, and families with at least 2 affected siblings in only 1 generation were classified as (potential or pseudo-) autosomal recessive PD families. Written informed consent was obtained from all participants, and the local ethics authorities approved the project. A 2-generation pedigree was established for the Japanese family with the *LRRK2* p.R1441G mutation (Fig. 1A). Among the 12 subjects in the family, 4 (II:5, II:4, I:6, and II:2) were willing to participate in this clinicogenetic study, whereas the remainder, including 2 reportedly parkinsonian subjects (I:2 and I:3), refused to participate. Participating individuals were examined by neurologists specializing in movement

disorders. A full history was collected and a neurologic examination was performed for each patient.

2.2. Genetic analysis

Genomic DNA from each subject was extracted from peripheral blood using the QIA amp DNA Blood Maxi Kit (QIAGEN, Valencia, CA, USA). Three exons of *LRRK2* that have been frequently reported to contain PD-associated mutations (exons 31, 41, and 48) were analyzed by polymerase chain reaction-direct sequencing using the Big Dye Terminator v. 1.1 Cycle Sequencing Kit (Life Technologies, Foster City, CA, USA) as previously described (Zimprich et al., 2004). Haplotype analysis of *LRRK2* flanking region was performed using previously described methods (Mata et al., 2005, 2009a, 2009b).

3. RESULTS

3.1 Genetic Findings

In this cohort, we identified 1 proband (0.11%) with a p.R1441G mutation, 1 with a p.G2019S mutation (0.11%), and 103 with a p.G2385R variant (11.37%). We failed to detect p.R1441C, p.R1441H, or p.I2020T mutations in this cohort.

The pedigree of the family with the p.R1441G mutation is shown in Fig. 1A. Five members of this family presented with parkinsonism (I:2, I:3, I:6, II:2, and II:5). Direct sequencing analysis of *LRRK2* revealed a heterozygous p.R1441G (*LRRK2* 4321C>G) mutation in II:5 (proband), I:6, and II:2. A healthy brother (II:4) of the proband did not share the mutation (Fig. 1B). Two individuals (II:2: a first cousin of the proband with the p.R1441G mutation, 50-year-old female; and II:4: a healthy brother of the proband without the p.R1441G mutation, 35-year-old male) were heterozygous for the p.G2385R (*LRRK2* 7153G>A) variant, which was observed on a different haplotype to that in individuals with the p.R1441G mutation. These results show co-segregation of parkinsonism and the mutation in the family, although the remaining members were unable to be tested.

Genotypes for 15 markers from our patients (II:2 and II:5) and the corresponding haplotype shared among 29 p.R1441G carriers from Spanish families are presented in Table 1. Allele sharing was evident at only 3 of the 15 markers and was not observed at c.4937T>C (p.M1646T), a rare polymorphism immediately flanking c.4321C>G (p.R1441G). Thus, our Japanese family members do not share a common founder with the Spanish families.

3.2. Case reports

The clinicoradiological findings in these patients are summarized in Table 2.

3.2.1. p.R1441G-Patient II:5—This 36-year-old female developed clumsiness in the right hand and leg at the age of 34 years. Subsequently, she developed right leg tremor and gait disturbance. She did not have any problems with cognition, sleep, or bowel movements. Neurologic examination revealed hemiparkinsonism including resting tremor, muscular rigidity, and akinesia in her right upper and lower limbs along with hyperreflexia in all extremities. Her motor score on the unified PD rating scale (UPDRS part III) was 17 points

(Supplementary Video 1). Neurocognitive assessments, such as the Mini Mental State Examination, Frontal Assessment Battery (FAB), and the Japanese version of the Montreal cognitive assessment (MoCA-J) were normal. The odor stick identification test for Japanese revealed mild hyposmia. The administration of levodopa/benserazide (200 mg/50 mg), pramipexole (4 mg), and selegiline (5 mg) resulted in a marked amelioration of her parkinsonism without psychiatric problems. After treatment, her UPDRS part III score was 4 points. Although she did not have any cerebrovascular risk factors, fluid level attenuated inversion recovery (FLAIR) magnetic resonance imaging (MRI) revealed bilateral scattered deep white matter lesions with no structural abnormality (Fig. 2C-F). The results of iodine-123 metaiodobenzylguanidine (^{123}I -MIBG) myocardial scintigraphy were normal (Fig. 2B). Iodine-123 iodoamphetamine single photon emission computed tomography (^{123}I -IMP SPECT) revealed normal regional cerebral blood flow (rCBF) (Fig. 2A).

3.2.2. p.R1441G-Patient I:6—This 65-year-old female is the mother of Patient II:5. Her sister (I:2) and brother (I:3) were also diagnosed as having PD in middle age with no detailed history obtained. She had a history of depression, insomnia over the past 12 years, and right frontoparietal traumatic injury at the age of 55 years. At the age of 60 years, sulpiride (150 mg/d) was administered for depression, resulting in the development of parkinsonism. Cessation of sulpiride ameliorated her parkinsonism and brotizolam (0.25 mg/d), paroxetine (10 mg/d), zolpidem (5 mg/d), flunitrazepam (2 mg/d), etizolam (1.5 mg/d), and trazodone (50 mg/d) were given for the treatment of her depression and insomnia. At the age of 65 years, she impulsively attempted suicide. The administration of perospirone (12 mg/d) induced a marked deterioration of her motor and swallowing functions. After cessation of perospirone, quetiapine (37.5 mg/d) was used to treat her psychiatric symptoms without aggravation of motor function. Three weeks after the suicide attempt, neurologic examinations revealed minimal left-dominant parkinsonism (Supplementary Video 2) and mild hyposmia (odor stick identification test for Japanese; 6 points).

Although she did not have any problems with orientation, memory, and calculation, the FAB and MoCA-J revealed dysfunctions of verbal fluency and conceptualization (FAB and MoCA-J scores of 14/18 and 24/30, respectively). Although she did not have any cerebrovascular risk factors, T2-weighted, and FLAIR MRI revealed frontotemporal atrophy and small abnormal high intensity lesions in the bilateral frontal white matter (Fig. 2I-L). The results of ^{123}I -MIBG myocardial scintigraphy were normal (Fig. 2H). ^{123}I IMP SPECT showed decreased rCBF in the bilateral frontal lobes, corresponding to the cranial MRI (Fig. 2G).

3.2.3. p.R1441G-Patient II:2—This 50-year-old female is a first cousin of Patient II:5. She carries both p.R1441G and p.G2385R mutations in *LRRK2*. She had panic syndrome at the age of 18 years. She developed bradykinesia and tremor in the left lower limb at the age of 28 years. On neurologic examination, 2 years after disease onset, she showed left hemiparkinsonism and hyperreflexia in all extremities without any cognitive dysfunction. The administration of levodopa/carbidopa (200 mg/20 mg), trihexyphenidyl (3 mg), talipexole (2.4 mg), and pergolide (0.3 mg) resulted in a marked amelioration of her

parkinsonism. Cranial FLAIR MRI was normal and ^{123}I -IMP SPECT showed decreased rCBF in the right basal ganglia (Fig. 2M-O). After the third year of her illness, she developed motor fluctuations with severe ballistic dyskinesia in the left lower limb as a result of high doses of anti-parkinsonian medications. After the 11th year of her illness, bilateral deep brain stimulation of the subthalamic nucleus (STN-DBS) was performed (Fig. 2P). Although STN-DBS was able to temporarily ameliorate her motor complications, she developed dystonic motor complications owing to right-sided STN-DBS stimulation 3 months after the commencement of DBS. In addition, she showed schizophrenic psychosis with severe delusions associated with STN-DBS at 1 year after the operation. After the 13th year of illness, she showed severe wearing off and dyskinesia with off-time pain and dopamine dysregulation syndrome. The left-sided STNDBS partially ameliorated her off-phase painful dystonia, but caused severe dyskinesia in the on-phase. Eventually, she was put on levodopa/benserazide (700 mg/175 mg/d) and quetiapine (25 mg/d) using left-side STN-DBS in the off-phase only, enabling her to enjoy bowling and ride a bicycle in the on-phase. However, in the off-phase, she showed severe akinesia and panic attacks with delusional ideation.

3.2.4. Other mutations—A 58-year-old male with PD due to p.G2019S, whose father and 2 of 5 siblings had PD developed levodopa-responsive parkinsonism, including resting tremor, rigidity, postural instability, and right lower-limb akinesia without cognition and autonomic dysfunctions. The results of ^{123}I -MIBG myocardial scintigraphy were normal. ^{123}I -IMP SPECT showed decreased rCBF in the frontal lobe, basal ganglia, and bilateral thalamus (Table 2). Approximately 80% of patients with the p.G2385R variant showed typical parkinsonism with levodopa effectiveness. Autonomic dysfunction is present in <20% of them. In 60% of them, the results of ^{123}I -MIBG myocardial scintigraphy were normal (Table 2).

4. DISCUSSION

Here, we report for the first time an *LRRK2* p.R1441G mutation in Asia. Whereas the p.R1441G mutation is the most prevalent mutation in the Basque country, it is extremely rare outside of northern Spain (Haugarvoll and Wszolek, 2009; Marti-Masso et al., 2009; Mata et al., 2005, 2009b; Simon-Sanchez et al., 2006). In our family, the lack of sharing at p.M1646T (Table 1), located 9.7 kb downstream from the mutation, is particularly informative because the “*LRRK2* c.4937 T<C” allele occurs at a frequency of <2% in controls from northern Spain but has been observed in all mutation carriers described in the literature (Mata et al., 2005). Therefore, we concluded that our family has a different haplotype from that of all the Spanish families reported to date. Recently, a cohort study revealed a Uruguayan patient carrying a p.R1441G mutation, who did not share the haplotype of the original Spanish families (Mata et al., 2009a). Our patients did not appear to have an ancestor from outside Japan based on historical records. Thus, they are only the second family with this mutation outside of Spain, with a different haplotype, reinforcing the hypothesis that p.R1441 is a mutational hot spot. It is therefore possible that patients with a mutation at *LRRK2* p.R1441 are distributed worldwide.

Previous reports have described that the clinical features of the original Basque family members (Paisan-Ruiz et al., 2005) and the Uruguayan patient (Mata et al., 2009a) with *LRRK2* p.R1441G were similar to those of typical idiopathic PD (iPD), including good response to levodopa therapy, rigidity, and akinesia (Table 2). Although most of the members of the Basque families showed late onset PD (Gonzalez-Fernandez et al., 2007; Mata et al., 2005; Simon-Sanchez et al., 2006), Gonzalez-Fernandez et al. (2007) reported that 5 of 15 patients showed an early age at onset (<50 years). These findings indicate that some of the Basque families might exhibit intrafamilial clinical heterogeneity. In our Japanese family, Patients II:2 and II:5 also showed younger age at onset compared with iPD patients. In contrast to Patients I:2, I:3, II:2, and II:5, Patient I:6, albeit in old age, showed trivial hemiparkinsonism only detected by detailed neurologic examination with no functional disabilities, whereas the patient showed frontal dysfunction with impaired verbal fluency and conceptualization. Severe psychosis appeared to be unique to Patient II:2 in our family. Therefore, our family also exhibited intrafamilial clinical heterogeneity. The presence of such heterogeneity suggests that additional factors, that is, genetic and/or environmental factors, contribute to the development of the disease in association with the p.R1441G mutation.

Based on the results of 2 studies, patients with the *LRRK2* p.R1441G mutation seem to display fewer of the non-motor features characteristic of iPD, such as hyposmia and cardiac sympathetic denervation (Table 2) (Ruiz-Martinez et al., 2011; Tijero et al., 2013). Consistent with these observations, our patients showed no autonomic dysfunctions, including bowel and urinary dysfunctions and orthostatic hypotension, REM-sleep behavior disorders, or reduced cardiac ¹²³I-MIBG uptake (Table 2). Hyposmia detected in our patients (II:5 and I:6) appeared less severe than that in iPD, requiring further studies to determine whether the p.R1441G mutation is associated with hyposmia. In terms of neuroimaging, we also observed differences between patients with the p.R1441G mutation and iPD patients. Patient I:6 showed frontal atrophy on MRI and frontal hypoperfusion on ¹²³I-IMP SPECT. Furthermore, ¹²³I-IMP SPECT of Patient II:2 revealed hypoperfusion in the basal ganglia. These findings contrast with those in iPD patients, which typically show a decrement of blood flow in the occipital lobes (Abe et al., 2003). On the basis of these results, the phenotypes of our patients seemed to be different from those of iPD patients with Lewy pathology. Pathologic findings in PD patients with *LRRK2* mutations are very variable despite them exhibiting a relatively uniform clinical phenotype of parkinsonism (Cookson et al., 2008; Hasegawa et al., 2009; Ross et al., 2006; Ujiie et al., 2012; Zimplich et al., 2004). A clinicopathological study of patients with the p.G2019S mutation revealed no correlation between Lewy pathology and clinical phenotypes, including dementia and psychiatric symptoms (Ross et al., 2006). These observations suggest that additional factors besides *LRRK2* mutations could play roles in the development of dementia and/or psychiatric problems as well as Lewy pathology. It therefore remains to be clarified whether our cases show Lewy pathology, although a previously reported patient with a p.R1441G mutation did not have Lewy pathology (Marti-Masso et al., 2009).

It has been described that the phenotype of PD patients with the p.G2019S mutation cannot be distinguished from iPD (Haugarvoll and Wszolek, 2009). However, the patient with the

p.G2019S mutation in our cohort showed no autonomic dysfunction, no psychosis, no dementia, and normal cardiac ^{123}I -MIBG uptake, resembling the phenotype of p.R1441G-patient II:5 without early age of onset. The prevalence of autonomic dysfunctions of PD patients with p.G2385R was also seemed to be less than that in iPD patients (Khoo et al., 2013). Two members (II:2 and II:4) of our family also had the p.G2385R variant. Although combining p.R1441G with p.G2385R in II:2 might cause severe psychosis, it remains unclear whether the p.G2385R variant combined with an *LRRK2* pathogenic mutation might be associated with a deterioration of clinical phenotypes. Therefore, we could not determine the contribution of this variant to the phenotypic modifications in our family.

In Patient II:2, STN-DBS exacerbated psychiatric problems without any improvement of motor function. Gomez-Esteban et al. (2008) also described a poor response to STN-DBS therapy in patients with the p.R1441G mutation. These results might be attributable to the p.R1441G mutation.

We found 1 patient with the p.G2019S mutation (0.11%) in this cohort. Parkinsonism because of p.G2019S is common in Caucasian populations (Haugarvoll and Wszolek, 2009), and the frequencies in the Ashkenazi Jewish and North African Arab cohorts are 18.3% and 39%, respectively (Lesage et al., 2006; Ozeliuss et al., 2006). However, this mutation has been reported to be very rare in Asian populations (Tan et al., 2005; Tomiyama et al., 2006; Zabetian et al., 2006), consistent with our cohort. The finding of a relatively high prevalence of the *LRRK2* p.G2385R variant in our cohort is consistent with the findings of previous reports (Funayama et al., 2007; Ross et al., 2011). Although Ross et al. (2011) described that the prevalence of PD with p.G2385R in large Asian populations, including Japanese was about 3%, the prevalence of p.G2385R in the previously reported Japanese cohort was 11.6% (Funayama et al., 2007), similar to that in the present cohort. On the other hand, the association of p.G2385R with PD in the Caucasian population was not supported by the results of a previous large population study (Ross et al., 2011).

To the best of our knowledge, this is the first report of an *LRRK2* p.R1441G mutation in Asia and only the third outside of northern Spain (Mata et al., 2009a). Further mutation screening of *LRRK2* p.R1441G in Asian populations may confirm the characteristic features of patients with this mutation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Dr. Hideo Mori (Juntendo Koshigaya Hospital) and all the participants in this study. This work was supported by a Strategic Research Foundation Grant-in-Aid Project for Private Universities, Grants-in-Aid for Scientific Research (KAKENHI) (to Nobutaka Hattori, 24390224 and to Taku Hatano, 25461290) and a Grant-in-Aid for Scientific Research on Innovative Areas (to Nobutaka Hattori, 23111003 and to Manabu Funayama, 25129707) from the Japanese Ministry of Education, Culture, Sports, Science and Technology. This work was also supported by grants from the Parkinson's Disease Foundation, United States and the National Institutes of Health, United States (R01 NS065070 and P50 NS062684).

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Figure. 1. Pedigree and direct sequencing analysis of the Japanese family with the *LRRK2* p.R1441G mutation

(A) Pedigree of the Japanese family with the *LRRK2* p.R1441G mutation. The arrow indicates the proband. (B) Direct sequencing analysis of *LRRK2* in this family. Arrow heads indicate sites of heterozygous substitution of *LRRK2* 4321C>G.

Figure 2. Cranial MRI, CT, ^{123}I -IMP SPECT, and ^{123}I -MIBG myocardial scintigraphy of Patients II:5, I:6, and II:2

In Patient II:5, ^{123}I -IMP SPECT and ^{123}I -MIBG myocardial scintigraphy show no abnormality (A, B). Cranial T2-WI and FLAIR MRI depict several abnormal high-intensity lesions without any atrophy (C–F). In Patient I:6, ^{123}I -IMP SPECT shows hypoperfusion of bilateral frontal area (G). Cranial T2-WI and FLAIR MRI show several abnormal high-intensity lesions with frontal lobe atrophy (I–L). The results of ^{123}I -MIBG myocardial scintigraphy were normal (E). In Patient II:2, ^{123}I -IMP SPECT shows hypoperfusion of the right basal ganglia (M). Cranial T2-WI MRI showing no abnormality (O, P). Cranial CT

showed that electrodes for deep brain stimulation of the subthalamic nucleus were implanted into the bilateral subthalamic nucleus (N). Abbreviations: CT, computerized tomography; FLAIR, fluid level attenuated inversion recovery; ^{123}I -IMP SPECT, Iodine-123 iodoamphetamine single photon emission computed tomography; ^{123}I -MIBG, iodine-123 metaiodobenzylguanidine; MRI, magnetic resonance imaging.

Table 1

Comparison of R1441G-containing haplotypes across the *LRRK2* region. “Spanish-Basque” indicates the haplotype shared among patients with the *LRRK2* p.R1441G mutation from northern Spain. In the members of the Japanese family, allele sharing was observed at only 5 markers, indicated in bold.

Location	Marker	Spanish-Basque	Uruguay	II:2	II:5
33305760	D12S2080	188	188 /192	192	192
34142287	rs1511547	T	C	C	C
34142413	rs55917927	C	T	T	T
34142467	rs56260627	A	G	G	G
37708307	rs10876410	A	A	A	A
38738007	D12S2194	249	253/257	253	253
<i>LRRK2</i>	D12S2516	252	254	252 /254	252 /254
	R1441G	G	G /C	G /C	G /C
	M1646 T	C	T	T	T
	D12S2518	154	154	154	154
39116885	D12S2519	138	132/140	132/140	132/140
39120098	D12S2520	248	257	257	257
39128754	D12S2521	323	319/359	315	315
39132380	D12S2522	281	283/297	283/297	283/297
39176380	D12S2517	184	188/198	202	202
39312730	D12S1048	211	211 /214	220	220

Table 2

Clinical features of patients with *LRK2* p.R1441G mutation. Key: Bil., bilateral; H/M, heart/mediastinum ratio; FAB, frontal assessment battery; 123I-MIBG, iodine-123 metaiodobenzylguanidine; iPD, idiopathic Parkinson's disease; MMSE, mini mental state examination; MoCA-J, Japanese version of the Montreal cognitive assessment; MRI, magnetic resonance imaging; N.A., not applicable; OSIT-J, odor stick identification test for Japanese; PD, Parkinson's disease; rCBF, regional cerebral blood flow; S-like, schizophrenic-like; Slt., slightly.

	p.R1441G our cohort			p-G2019S our cohort	p-G2385R our cohort	p.R1441G northern Spain	p.R1441G Uruguay	iPD
	Patient II:5	Patient I:6	Patient II:2					
Age at onset (y)	34	55	28	54	52 (21-83)	55 (29-80)	50	Around 60
Disease duration (y)	2	10	16	4	7		4	
Resting tremor	+	-	+	+	59%	+	+	+
Bradykinesia	+	+	+	+	86.7%	+	+	+
Rigidity	+	+	+	+	82.9%	+	+	+
Gait disturbance	+	-	+	+	80%	+	N.A.	+
Postural instability	-	-	+	+	62.9%	+	+	+
Clinical response to levodopa	+	N.A.	+	+	69.5%	+	+	+
Wearing off	-	-	+	N.A.	28.6%	+	N.A.	+
Levodopa-induced dyskinesia	-	-	+	N.A.	24.8%	+	N.A.	+
Asymmetry at onset	+	+	+	+	73.3%	+	+	+
Hyposmia (OSIT-J)	Mild impairment (9, cutoff; 11.09 ^d)	Mild impairment (6, cutoff; 9.78 ^d)	N.A.	N.A.	N.A.	Mild impairment	N.A.	Severe impairment
Orthostatic hypotension	-	-	-	-	12.4%	-	N.A.	+
Incontinence	-	-	-	-	18.1%	-	N.A.	+
Sleep benefit	+	-	Unknown	-	16.2%	-	N.A.	-
Dystonia at onset	-	-	-	N.A.	14.3%	-	N.A.	-
Hyperreflexia	+	-	+	N.A.	17.1%	-	N.A.	-
Hallucination	-	-	+	-	16.2%	-	N.A.	-
Dementia (FAB/MMSE/MoCA-J)	-(18/30/29)	Slt. frontal dysfunction (14/N.A./24)	-	-	12.4%	-	-	-

	p.R1441G our cohort			p-G2019S our cohort	p-G2385R our cohort	p.R1441G northern Spain	p.R1441G Uruguay	iPD
	Patient II:5	Patient I:6	Patient II:2					
Other psychiatric problems	–	Depression, executive dysfunction	S-like, delusion	–	±	±	N.A.	–
Brain MRI	Abnormal small spots scatter in white matter	Abnormal small spots scatter in white matter, bilateral frontal lobe atrophy, post traumatic hemorrhage in right frontoparietal cortex	No abnormality	N.A.	N.A.	No abnormality	N.A.	No abnormality
¹²³ I-imp spect	Normal record	Decreased rCBF in bil. frontal lobes	Decreased rCBF in the right basal ganglia	Decreased rCBF in frontal, basal ganglia, and right thalamus,	N.A.	N.A.	N.A.	Decreased rCBF in bil. occipital lobes
¹²³ I-MIBG myocardiac scintigraphy	H/M ratio (E/L) = 2.38/2.68; cutoff, 1.4 ^a ; wash out ratio=4.15%	H/M ratio (E/L) = 3.18/3.63; cutoff, 2.0 ^b ; wash out ratio = 20.1%	Not performed	Normal	Decreased 40%, normal 60%	Less decreased than PD	N.A.	Decreased H/M ratio

^a Cutoff points in OSIT-J vary according to age and sex.

^b Cutoff points in ¹²³I-MIBG myocardiac scintigraphy were different in every institution.