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A Single-Nucleotide Polymorphism of *PARK2* Affects the Phenotype in Sporadic Parkinson Disease

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Objective: Sporadic Parkinson's disease (PD) is thought to be a complex multifactorial, age-related neurodegenerative disease caused by the interaction between genetic and environmental factors. Whether *PARK2*, a major responsible gene causing familial PD, affects to the disease susceptibility or the phenotypic variability in sporadic PD remains controversial. In this study, we perform the sequence analysis of *PARK2* and assess the correlation between clinical features of sporadic PD patients and the detected variants. **Materials and Methods:** A total of 92 sporadic PD patients were sequenced and underwent the clinical examinations. MIBG scintigraphy was performed in 61 patients and the cardiac uptake was measured as the heart/mediastinum (H/M) ratio. **Results:** We only detected two novel variants (R51R, L272I) in 3 patients and three common polymorphisms, S167N, V380L, and R366W, which had the allele frequencies of 38.6%, 7% and 0.5%, respectively. There were no significant difference of the allele frequencies between patients and controls. On the evaluation of clinical features, the patients with S167N had the younger onsets of age and the tendency of preserved cardiac uptake of MIBG in the early Hoehn and Yahr (HY) stage compared to the patients without S167N. **Conclusions:** These results suggest the common polymorphisms of *PARK2* might affect the phenotype of sporadic PD without altered susceptibility to PD.

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Introduction

Sporadic Parkinson's disease (PD) is thought to be a complex multifactorial, age-related neurodegenerative disease caused by the interaction between genetic and environmental factors.[1] The overlapping of clinical and pathological features between sporadic PD and familial PD suggests common underlying molecular mechanisms in both types of PD. Therefore, the responsible genes causing familial PD might have an important role as genetic factors, which affect to the disease susceptibility or the phenotypic variability in sporadic PD.

The mutations of *PARK2*, encoding E3 ubiquitin-protein ligase; parkin, cause a juvenile-autosomal recessive parkinsonism which is the most common form in familial PD.[2] However, the role of its genetic variation in sporadic PD still remains controversial. Some reports showed the common coding polymorphisms, S167N or V380L were associated to PD,[3-5] whereas others concluded neither polymorphisms were associated to PD.[6-10] Likewise, heterozygous mutations were showed to be risk factors in sporadic PD,[11-13] whereas the frequency of heterozygous mutations in sporadic PD patients was found to be as well as in control subjects.[6,9,14] Even if any associations between heterozygous mutations and PD exist, the attributable risk might be negligible for most of sporadic PD due to the rarity of heterozygous mutations.[14]

In addition to motor symptoms, autonomic abnormalities have been reported in PD. Decreased cardiac uptake of ^{123}I-MIBG scintigraphy in the early stage of PD has the diagnostic potential,[15] which reflects the degeneration of post-ganglionic sympathetic nerve terminals in the myocardium.[16] Typically, the involvement of the cardiac sympathetic nerve precedes the impairment that of the dopaminergic nigrostrial neurons causing motor symptoms.[17] Meanwhile, cardiac uptake of ^{123}I-MIBG in genetic PD associated with *PARK2* is predisposed to be preserved, suggesting that cardiac sympathetic denervation occurs less frequently in genetic PD than in idiopathic PD.[18] Genetic PD associated with *PARK2*, which generally lacks the formation of

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Lewy bodies, has younger onset, but good response to L-dopa for a long time.[19]

Here, we performed the sequence analysis of *PARK2* in sporadic PD patients residing in Nagasaki prefecture on the west edge of Japan and assessed the correlation to clinical features including the cardiac uptake of ^{123}I-MIBG to examine the influence of genetic variants on the phenotype.

Materials and Methods

Subjects

The subjects included 92 (32 men and 60 women) unrelated idiopathic PD patients who were ethnically Japanese resided in Nagasaki prefecture. Patients with apparent positive family history were excluded. The diagnosis of PD was based on international criteria. The mean age at onset was 57.6 ± 11.2 years with range from 28 to 94. The mean disease duration was 9.9 ± 6.74 years with range from 1 to 34. One hundred Japanese without neurodegenerative disorders were used as control subjects for sequence analysis and 32 healthy controls were recruited for MIBG Scintigraphy. All PD patients underwent clinical examination, including the Hoehn and Yahr (HY) staging scale. Informed consent was obtained from all PD patients. This study was approved by ethical committee of Nagasaki University Hospital.

Mutational analysis

Genomic DNA was extracted from peripheral blood using standard methods. All 12 exons of parkin and surrounding intronic sequence were amplified using previously reported primers[20] and sequenced using BigDye 3.1 terminator (Applied Biosystems, Foster City, CA) on an ABI 3100 Genetic Analyzer. In 50 control subjects, the codon 167 Ser/Asn (167S/N) polymorphism in exon 4 was analyzed by digestion with AlwN I (New England Biolabs, Beverly, MA).

MIBG Scintigraphy

Single-photon emission tomography was performed with using intravenous injection of 111 MBq of ^{123}I-MIBG (Diichi Radioisotope Laboratory, Tokyo, Japan). Data were collected at 30 min (early image) and 240 min (delayed image) after the isotope injection. Static planar imaging and regional MIBG uptake were obtained with 128x128 matrix. Regions of interest (ROI) were manually drawn around the heart and mediastinum of the anterior image, and tracer uptake was measured within each ROI to calculate the heart/mediastinum (H/M) ratio. Values of the H/M ratio were considered abnormal if they were more than two standard deviations below the control mean.

Statistical Analysis

Statistical analyses were performed using StatView 5.0 (Abacas Concept, SAS Inc, San Francisco, CA). The x^2 test and Fisher's exact test was used to test the allele and the genotype frequencies and Mann-Whitney U test was used to compare mean age at onset and H/M ratio between groups. Values were expressed as mean ± SD and $p < 0.05$ was considered significant.

Results

Mutational analysis

In sequence analysis of 92 PD patients, we detected two novel variants R51R (c.254G>A), L272I (c.915C>A) and three known polymorphisms, S167N (c.601G>A), V380L (c.1239G>C), R366W (c.1197C>T) (Figure 1), but we did not detect any common mutations including missense, nonsense, frameshift, and splice-site mu-

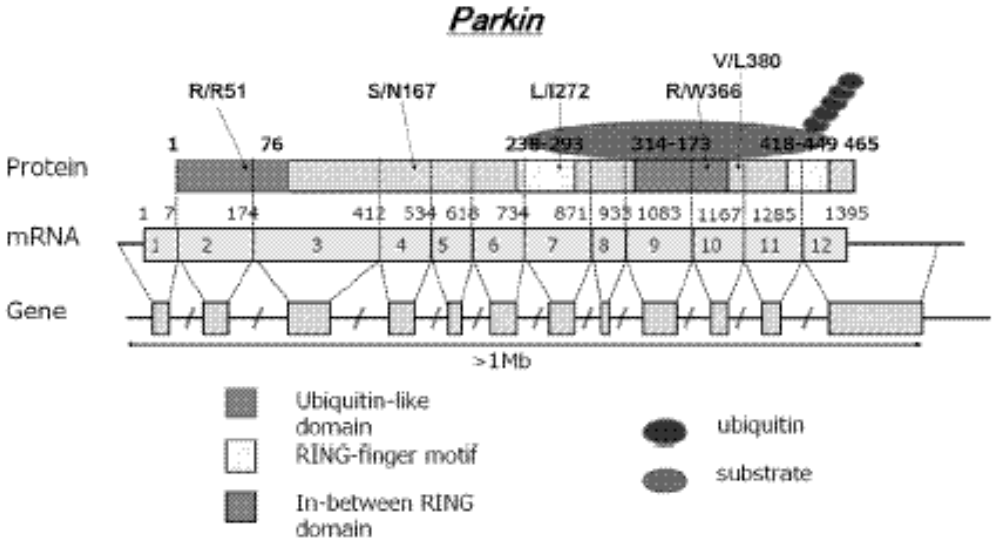

Figure 1. Identified variants in parkin in this study.

tations. S167N was more frequent polymorphism (allele frequency, 38.6%) as compared to the other polymorphisms, V380L and R366W, which had the allele frequencies in the PD patients, 7% and 0.5%, respectively. The frequencies of homozygous (G/G or A/A) and heterozygous (G/A) S167N did not differ significantly between the control group and the PD patients (Table 1). There was no significant difference between observed frequency and expected genotype frequency from Hardy-Weinberg equilibrium in the PD patients and control subjects. Two novel variants, heterozygous R51R and L272I were not found in 50 control subjects. In spite of substitution in the branched chain amino acids, L272I might have some effect to the normal function of *parkin*, because leucine at codon 272 is located in RING-finger motif of the functional domains and evolutionarily conserved among species.

Comparison of clinical features

The mean age at onset of PD was significantly earlier in PD patients with genotype A/A (50.8 ± 13.5) as compared to PD patients with genotype G/G (59.1 ± 11.6) (p = 0.046). The mean age at onset of PD patients with genotype G/A (58.4 ± 9.9) was not significantly differentas compared to PD with genotype G/G (p = 0.44). There was no difference in the other clinical features between PD patients with and without allele A or G (Table 2).

Comparison of cardiac uptake of MIBG scintigraphy

MIBG scintigraphy was performed on 61 of 92 PD patients. The H/M ratio was significantly lower in the PD patients (early; 1.64 ± 0.26, delayed; 1.49 ± 0.39) as compared to 32 healthy controls (early; 2.31 ± 0.21, delayed; 2.37 ± 0.22) (early; p < 0.0001, delay; p < 0.0001). The H/M ratio was significantly higher in 33 PD patients with S167N (early; 1.74 ± 0.3, delayed; 1.63 ± 0.48) as compared to 28 PD patients without S167N (early; 1.57 ± 0.24, delayed; 1.36 ± 0.24) (early; p = 0.021, delay; p = 0.014). According to the HY staging scale, 61 PD patients were separated into two stages: the early stage comprising of HY stage II or less, and the advanced stage comprising of HY stage III or above. In the early stage, the early H/M ratio was significantly higher in 20 PD patients with S167N (1.89 ± 0.28) as compared to 12 PD patients without S167N (1.59 ± 0.18) (p = 0.013). Similarly, the delayed H/M ratio also showed significant difference (1.73 ± 0.42 vs 1.40 ± 0.23, p = 0.020). The H/M ratio of PD patients was not differed between both PD patients in the advanced stage. There was no association of the endpoint with sex and age.

Table 1. Genotype and Allele Frequencies of the polymorphism S167N.
No statistical significant differences in genotype frequencies between PD patients and controls.

Group	Genotype frequency			Allele frequency	
	G/G (%)	G/A (%)	A/A (%)	G allele (%)	A allele (%)
PD	36 (39.1)	41 (44.6)	15 (16.3)	113 (61.4)	71 (38.6)
Control	40 (40.0)	48 (48.0)	12 (12.0)	128 (64.0)	72 (36.0)

Table 2. The difference of clinical features in PD patients with S167N.
The mean age at onset of PD was significantly earlier in PD patients with S167N (G/A + A/A) than without S167N (G/G). Data are presented as number (percentage) of corresponding patients except for the mean age at onset. There were no significant differences between (G/G + G/A) and A/A. p-values were estimated by comparisons to genotype G/G.
† indicates statistically significant differences (P = 0.046).

Genotype	G/G	G/A	A/A	G/A + A/A
n	34	39	12	51
Mean age ± SD at onset, yr	59.1 ± 11.6	58.4 ± 9.9	50.8 ± 13.5†	56.58 ± 11.2
Male/female ratio	0.5	0.44	0.55	0.46
Resting tremor	31 (91.1)	32 (82.1)	10 (83.3)	42 (82.4)
Rigidity	34 (100)	39 (100)	12 (100)	51 (100)
Dyskinesia	10 (29.4)	9 (23.1)	4 (33.3)	13 (25.5)
Levodopa, mg/day	292.2 ± 100.1	277.8 ± 115.5	304.1 ± 140.1	284.4 ± 121.2

Table 3. The Heart/Mediastinum (H/M) of ^{123}I-MIBG uptake in PD patients with S167N.
The H/M ratio was significantly greater in PD patients with S167N (G/A + A/A) than PD patients without S167N (A/A) in the early stage comprising of HY stage II or less. Data represent mean ± SD. There were no significant differences between (G/G + G/A) and A/A. p-values were estimated by comparisons to genotype G/G.
† indicates statistically significant differences ($P < 0.05$).

	G/G	G/A	A/A	G/A + A/A
n	28	21	12	33
H/M early	1.57 ± 0.24	1.75 ± 0.29†	1.73 ± 0.31	1.74 ± 0.30†
H/M delayed	1.36 ± 0.24	1.61 ± 0.44†	1.66 ± 0.56	1.63 ± 0.48†
Hoehn-Yahr ≦ 2				
n	12	13	7	20
H/M early	1.59 ± 0.18	1.89 ± 0.28†	1.73 ± 0.31	1.81 ± 0.28†
H/M delayed	1.40 ± 0.23	1.81 ± 0.45†	1.71 ± 0.54	1.73 ± 0.42†
Hoehn-Yahr ≧ 3				
n	16	8	5	13
H/M early	1.55 ± 0.27	1.51 ± 0.10	1.72 ± 0.36	1.59 ± 0.26
H/M delayed	1.34 ± 0.34	1.29 ± 0.12	1.57 ± 0.65	1.39 ± 0.43

Discussion

We demonstrated that a common polymorphism, S167N in PD patients was associated with the younger age at onset and the tendency of preserved cardiac uptake of MIBG, but has no increase in the susceptibility to PD. In the other words, PD patients with S167N have the predisposition to develop motor symptoms rather than the sympathetic autonomic dysfunction of the heart in the early stage of disease.

Several reports studied the association between common polymorphisms and PD. Although S167N is almost thought to be not associated to PD, Sato et al. revealed the association to PD as a genetic risk factor,[5] which was a study in the prefecture neighboring to our prefecture, Nagasaki. On the other hand, Lincoln et al. in USA[6] and Lucking et al. in Germany[4] revealed the association between a common polymorphism, V380L and PD. S167N is apparently lower allele frequency in non-Asian population (less than 10%) than Asian population (around 40%). V380L is vice versa. Even if such polymorphisms have the potential to substantially contribute to disease susceptibility or disease modification for sporadic PD, the ethnic differences in the allele frequencies might obscure the genetic effects.

Although the diagnosis of typical PD is based on the presence of a bradykinetic rigid syndrome with asymmetric onset, a resting tremor, and a good and sustained response to levodopa,[21] the non-motor symptoms including autonomic dysfunctions, cognitive and neurobehavioural abnormalities, sleep disorders, and sensory abnormalities are a common and underappreciated feature of PD.[22] The non-motor symptoms are thought to attribute to the dysfunction of non-dopaminergic neurons, but molecular details of the pathophysiology still remains elucidated. Braak and colleagues proposed that the concept of six-stage pathological process classified by topographic distribution pattern of the Lewy pathology (the alpha-synuclein inclusion body pathology) in the autopsied brain of sporadic PD.[23] The Lewy pathology begins from the olfactory structures and the dorsal motor nucleus of the vagus nerve followed by the locus coeruleus in the lower brain stem, which may explain early non-motor symptoms as olfactory dysfunction and various sleep disorders. Subsequently, it expands to the substantia nigra pars compacta and then to higher parts of the neuraxis, which may explain cognitive and neurobehavioural abnormalities. Additionally, Lewy pathology is found in the peripheral autonomic nervous system, including neurons of the enteric plexus of the gastrointestinal tract, paravertebral autonomic ganglia and sympathetic nerve fibers in the adrenal gland and heart, and in cutaneous nerves,[23] which may explain autonomic dysfunction and sensory abnormalities. The parkin, encoded by the *PARK2* gene, might affect the distribution of Lewy pathology.

References

1. Schapira AH. Etiology of Parkinson's disease. *Neurology* 66: S10-23, 2006
2. Lücking CB, Durr A, Bonifati V, et al. Association between early-onset Parkinson's disease and mutations in the parkin gene. *N Engl J Med* 342: 1560-7, 2000
3. Biswas A, Maulik M, Das SK, Ray K, Ray J. Parkin polymorphisms: risk for Parkinson's disease in Indian population. *Clin Genet* 72: 484-486, 2007
4. Lucking CB, Chesneau V, Lohmann E, et al. Coding polymorphisms in the parkin gene and susceptibility to Parkinson disease. *Arch Neurol* 60: 1253-1256, 2003
5. Satoh J, Kuroda Y. Association of codon 167 Ser/Asn heterozygosity in the parkin gene with sporadic Parkinson's disease. *Neuroreport* 10: 2735-2739, 1999
6. Lincoln SJ, Maraganore DM, Lesnick TG, et al. Parkin variants in North American Parkinson's disease: cases and controls. *Mov Disord* 18: 1306-1311, 2003
7. Hu CJ, Sung SM, Liu HC, Lee CC, Tsai CH, Chang JG. (2000). Polymorphisms of the parkin gene in sporadic Parkinson's disease among Chinese in Taiwan. *Eur Neurol* 44: 90-93, 2000
8. Oliveira SA, Scott WK, Nance MA, et al. Association study of Parkin gene polymorphisms with idiopathic Parkinson disease. *Arch Neurol* 60: 975-980, 2003
9. Sinha R, Racette B, Perlmutter JS, Parsian A. Prevalence of parkin gene mutations and variations in idiopathic Parkinson's disease. *Parkinsonism Relat Disord* 11: 341-347, 2005
10. Wang M, Hattori N, Matsumine H, et al. Polymorphism in the parkin gene in sporadic Parkinson's disease. *Ann Neurol* 45: 655-658, 1999

11. Foroud T, Uniacke SK, Liu L, et al. Heterozygosity for a mutation in the parkin gene leads to later onset Parkinson disease. *Neurology* 60: 796-801, 2003
12. Oliveira SA, Scott WK, Martin ER, et al. Parkin mutations and susceptibility alleles in late-onset Parkinson's disease. *Ann Neurol* 53: 624-629, 2003
13. Lesage S, Lohmann E, Tison F, Durif F, Durr A, Brice A. Rare heterozygous parkin variants in French early-onset Parkinson disease patients and controls. *J Med Genet* 45: 43-46, 2008
14. Kay DM, Moran D, Moses L, et al. Heterozygous parkin point mutations are as common in control subjects as in Parkinson's patients. *Ann Neurol* 61: 47-54, 2007
15. Sawada H, Oeda T, Yamamoto K, et al. Diagnostic accuracy of cardiac metaiodobenzylguanidine scintigraphy in Parkinson disease. *Eur J Neurol* 16: 174-182, 2009
16. Orimo S, Takahashi A, Uchihara T, et al. Degeneration of cardiac sympathetic nerve begins in the early disease process of Parkinson's disease. *Brain Pathol* 17: 24-30, 2007
17. Goldstein DS, Sharabi Y, Karp BI, et al. Cardiac sympathetic denervation preceding motor signs in Parkinson disease. *Cleve Clin J Med* 76: S47-50, 2009
18. Orimo S, Amino T, Yokochi M, et al. Preserved cardiac sympathetic nerve accounts for normal cardiac uptake of MIBG in PARK2. *Mov Disord* 20: 1350-1353, 2005
19. Lucking CB, Durr A, Bonifati V, et al. Association between early-onset Parkinson's disease and mutations in the parkin gene. *N Engl J Med* 342: 1560-1567, 2000
20. Kitada T, Asakawa S, Hattori N, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392: 605-608, 1998
21. Hughes AJ, Daniel SE, Ben-Shlomo Y, Lees AJ. The accuracy of diagnosis of parkinsonian syndromes in a specialist movement disorder service. *Brain* 125: 861-870, 2002
22. Jankovic J. Parkinson's disease: clinical features and diagnosis. *J Neurol Neurosurg Psychiatry* 79: 368-376, 2008
23. Braak H, Del Tredici K. Neuroanatomy and pathology of sporadic Parkinson's disease. *Adv Anat Embryol Cell Biol.* 201:1-119, 2009