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The identified clinical features of Parkinson's disease in homo-, heterozygous and digenic variants of *PINK1*

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ABSTRACT

To investigate the prevalence and genotype-phenotype correlations of *phosphatase and tensin homolog induced putative kinase 1* (*PINK1*) variants in Parkinson's disease (PD) patients, we analyzed 1700 patients (842 familial PD and 858 sporadic PD patients from Japanese origin). We screened the entire exon and exon-intron boundaries of *PINK1* using Sanger sequencing and target sequencing by Ion torrent system. We identified 30 patients with heterozygous variants, 3 with homozygous variants, and 3 with digenic variants of *PINK1-PRKN*. Patients with homozygous variants presented a significantly younger age at onset than those with heterozygous variants. The allele frequency of heterozygous variants in patients with age at onset at 50 years and younger with familial PD and sporadic PD showed no differences. [^{123}I] meta-iodobenzylguanidine (MIBG) myocardial scintigraphy indicated that half of patients harboring *PINK1* heterozygous variants showed a decreased heart to mediastinum ratio (12/23). Our findings emphasize the importance of *PINK1* variants for the onset of PD in patients with age at onset at 50 years and younger and the broad spectrum of clinical symptoms in patients with *PINK1* variants.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, following Alzheimer's disease. The initial symptoms of PD are tremor, rigidity, akinesia, and gait disturbance (Postuma et al., 2015). Approximately, 10%–20% of PD cases are familial. Recent advances in genetic analyses revealed pathogenic variants related to familial PD such as *synuclein alpha* (*SNCA*), *leucine-rich repeat kinase 2* (*LRRK2*), *parkin RBR E3 ubiquitin-protein ligase* (*PRKN*), *phosphatase and tensin homolog* (*PTEN*) *induced putative kinase 1* (*PINK1*), and *vacuolar protein sorting 13 homolog C* (*VPS13 C*) (Deng et al., 2018). Each gene variant yields distinctive symptoms and disease courses. In families with autosomal recessive (AR) inheritance, *PRKN* and *PINK1* have been revealed as causative genes in young-onset or juvenile PD. These forms of PD are characterized by symptoms not only seen in parkinsonism but also by foot dystonia, sleep benefit, and an excellent response to levodopa (Matsumine et al., 1997; Valente et al., 2004). *PRKN* variants were originally identified from Japanese pedigrees with AR inheritance and *PINK1* variants were identified from a large Italian pedigree (Matsumine et al., 1997, Valente et al., 2001). Parkin, encoded by *PRKN*, is an E3 ubiquitin ligase with an amino-terminal ubiquitin-like (Ubl) domain and a carboxyl-terminal ubiquitin ligase domain. *PRKN* has various types of mutations or abnormal gene structures including duplication, triplication, deletions, frameshift, splicing, and missense variants (Corti et al., 2011). In contrast, *PINK1* variants are commonly identified as missense, truncating, or intronic variants close to exon-intron boundaries (Bonifati et al., 2005).

The proteins of parkin and PINK1 cooperatively regulate mitochondrial quality control (Pickrell and Youle, 2015). Physiologically, parkin is recruited from the cytosol to depolarized mitochondria to remove the damaged mitochondria. PINK1 accumulates on dysfunctional mitochondria and facilitates mitophagy along with parkin (Matsuda et al., 2010). Impaired mitochondrial maintenance results in dopaminergic cell death and a decrease in dopamine levels in the striatum, causing the symptoms of PD. There were differences in the penetrance ratio between homozygous or compound heterozygous variants of *PRKN* or *PINK1* and their heterozygous variants (approximately 100% and 1%–25%, respectively) (Klein et al., 2007). The age at onset of PD also differs between homozygous or compound heterozygous variants (younger) and heterozygous variants (older) of *PINK1* (Bonifati et al., 2005). The clinical character might also depend on the number of affected alleles. *PINK1* heterozygous variants could be relatively a high-risk factor for the onset of PD in older patients via dominant-negative effects (Puschmann et al., 2017).

The heterozygous *PINK1* variant, p.G411S, significantly increases the risk of PD by causing a reduction in kinase activity, dysfunction in mitochondrial quality control, and interfering with ubiquitin phosphorylation, via a partial dominant-negative effect (Puschmann et al., 2017). The presence of heterozygous *PINK1* variants could be a genetic risk factor for both familial and sporadic PD. However, a recent report analyzing a large cohort of patients did not support the association of p.G411S and PD; 13,708 patients with PD and 362,850 controls of European ancestry, whose age at examination or age at onset was broadly distributed, from young to elderly. The report also revealed no association of heterozygous pathogenic variants in *PINK1* with PD in 6712 patients and 45,113 controls (Krohn et al., 2020). One variant, p.G411S, showed population differences. Most reports of p.G411S were based on European ancestry and the current literature is lacking Asain ancestry reports (Krohn et al., 2020, Lin et al., 2019, Puschmann et al., 2017, Tan et al., 2006, Weng et al., 2007). In light of these previous studies, we aim to scrutinize the prevalence and association among the patients from a single cohort of Japanese, focusing in particular on the differences in age at onset among the patients.

Herein, we aimed to examine the prevalence and clinical findings in patients with heterozygous variants of *PINK1*, using samples from 842 familial PD and 858 sporadic PD patients. All patients were newly enrolled and the patients from our previous studies were excluded (Funayama et al., 2008; Hatano et al., 2004; Kumazawa et al., 2008). We identified 36 patients with probably pathogenic *PINK1* variants and aimed to depict the genotype-phenotype correlations of these patients.

2. Methods

2.1. Material and methods

2.1.1. Protocol of participants

This study was approved by the ethics review committee of Juntendo University School of Medicine. All subjects gave informed and written consent before participation. We clinically defined autosomal dominant (AD) inheritance as having family members with PD in at least 2 consecutive generations, and AR inheritance as having at least one sibling with PD in the same generation. The occurrence of PD was defined as sporadic when there was no family history of PD. Consanguinity was determined by clinical information. The study included 1700 patients: 842 familial PD patients (620 AD and 222 AR; age at onset, 54.9 ± 13.9 years; and age at sampling, 61.8 ± 13.1 years) and 858 sporadic PD patients (age at onset, 41.8 ± 11.5 years; age at sampling, 49.2 ± 12.3 years) (Table 1). Additionally, based on sporadic PD, we divided the study

Table 1
Demographic data of our enrolled patients with Parkinson's disease for the screening of *PINK1*

	Number of patients (n = 1700) (Male: Female)	Age at sampling [years ±SD (range)]	Age at onset [years ±SD (range)]	Disease duration [years ±SD (range)]
Familial Parkinson's disease (n = 842)				
Total number of patients	842 (794 probands) (400:442)	61.8 ± 13.1 (17–91)	54.9 ± 13.9 (12–88)	7.0 ± 6.9 (0–46)
Autosomal dominant	620 (594 probands) (303:317)	59.1 ± 12.7 (22–91)	52.3 ± 13.2 (13–88)	6.8 ± 6.8 (0–46)
Autosomal recessive	222 (200 probands) (97:125)	69.5 ± 11.0 (17–87)	62.0 ± 13.5 (12–84)	7.7 ± 7.3 (0–37)
Sporadic Parkinson's disease (n = 858)				
Age at onset of 50 y and younger				
Total number of patients	858 (448:410)	49.2 ± 12.3 (12–83)	41.8 ± 11.5 (7–80)	7.4 ± 7.5 (0–72)
Total number of patients	704 (394:310)	45.8 ± 10.3 (12–83)	37.9 ± 8.0 (7–50)	7.8 ± 8.0 (0–72)
Consanguinity (+)	24 (13:11)	51.7 ± 10.3 (24–66)	39.2 ± 10.6 (7–49)	12.5 ± 12.2 (2–59)
Consanguinity (−)	680 (381:299)	45.5 ± 10.2 (12–83)	37.9 ± 7.9 (7–50)	7.7 ± 7.8 (0–72)
Age at onset of over 51 y				
Total number of patients	154 (54:100)	64.9 ± 8.0 (51–83)	59.6 ± 7.1 (51–80)	5.4 ± 4.2 (0–20)
Consanguinity (+)	19 (6:13)	61.8 ± 7.7 (51–78)	57.5 ± 6.2 (51–70)	4.7 ± 4.2 (0–16)
Consanguinity (−)	135 (48:87)	65.4 ± 8.0 (53–83)	59.9 ± 7.2 (51–80)	5.5 ± 4.2 (0–20)

Autosomal dominant and autosomal recessive were clinically defined.
Key: SD, standard deviation.

population into 2 groups—(i) disease onset at ≤50 years of age (704 patients [male:female = 394:310]; 37.9 ± 8.0 years of age at onset; range, 7–50 years) and (ii) disease onset at >50 years of age (154 patients [male:female = 54:100]; 59.6 ± 7.1 years of age at onset; range, 51–80 years). We also set up an in-house healthy control group with a male: female ratio of 79:137 and average age at the time of examination of 68.2 ± 12.1 years. All cases were of Japanese origin, and we did not include patients that we reported previously (Funayama et al., 2008, Hatano et al., 2004, Kumazawa et al., 2008). The diagnosis of PD, Parkinson's disease with dementia, and dementia with Lewy bodies were confirmed by the clinical criteria (Emre et al., 2007; Gibb and Lees, 1988; McKeith et al., 2017). The Unified Parkinson's Disease Rating Scale (UPDRS) part from I to IV was examined by the standard protocol (Fahn, 1987). For [^{123}I] meta-iodobenzylguanidine (MIBG) myocardial scintigraphy, a previously described method was used (Yoshii et al., 2017). The sensitivity and specificity of the heart-to-mediastinum ratio for diagnosing PD as 82% and 89%, respectively, in the early phase, and 89% and 82%, respectively, in the delayed phase (Orimo et al., 2012). All controls showed normal values of heart-to-mediastinum ratio. Each institute may use different radiological equipment and a different cutoff. We collected the data based on the decrease or lack of change in the heart-to-mediastinum ratio, as reported by the attending doctors. All examined patients did not take monoamine oxidase inhibitors such as selegiline.

2.2. Procedures of genetic screenings

Genomic DNA was extracted from peripheral blood samples using a standard protocol. We used primer sets to amplify the coding region of *PINK1* and its exon-intron boundaries. Sanger sequencing of polymerase chain reaction (PCR) products was performed using a BigDye Terminators v3.1 Cycle Sequencing Kit with a 3130 Genetic Analyzer (Life Technologies, Foster City, CA, USA). PCR and sequence primers were the same as described previously (Valente et al., 2004). Additionally, we screened genes related to familial PD or dementia using target sequencing by Ion Torrent system (Thermo Fisher Scientific, Waltham, MA, US); the panel (IAD103177_182) was set up to screen *SNCA*, *PARK2*, *UCHL1*, *PINK1*, *DJ-1*, *LRRK2*, *ATP13A2*, *GIGYF2*, *HTRA2*, *PLA2G6*, *FBXO7*, *VPS35*, *EIF4G1*, *DNAJC6*, *SYNJ1*, *DNAJC13*, *CHCHD2*, *GCH1*, *NR3A2*, *VPS13 C*, *RAB7L1*, *BST1*, *c19orf12*, *RAB39 B*, *MAPT*, *PSEN1*, *GRN*, *APP*, and *APOE*. The identified variants were confirmed by Sanger sequencing. The panel for sequencing was designed with Ion AmpliSeq Designer (https://www.ampliseq.com). For library preparation, we used an Ion AmpliSeq Kit for Chef DL8 (Thermo Fisher Scientific) and Ion Chef System (Thermo Fisher Scientific). Emulsion PCR was performed using an Ion 530 Kit-Chef. The sequencing was performed on an Ion S5 Plus Sequencer using an Ion 530 Chip. For sequence alignment, the Torrent Mapping Alignment Program aligner implemented in v5.10 of the Torrent Suite software (Thermo Fisher Scientific) was used. The sequencing mean depth was >500-fold (>95% target regions; >100-fold depth). The result of sequencing depths was 100%, while that of coverage was 95%. The primer sequence of *LRRK2* and PCR conditions are available on request. We have selected the rare variants matching the criteria of allele frequency under 0.001 of autosomal dominant inheritance and under 0.005 of autosomal recessive inheritance, with reference to the public gene database, including the genome aggregation database (gnomAD) (Lek et al., 2016), Human Genetic Variation Database (HGVD) (Higasa et al., 2016), and Tohoku Medical Megabank Organization (Kuriyama et al., 2016). Further methodological details have been described previously (Shin et al., 2017). Among the enrolled patients, we screened 179 patients with AD PD and 52 patients with AR PD, and 226 patients with sporadic PD using the Ion Torrent System. Copy number variations in *PINK1* were analyzed using the multiplex ligation-dependent probe amplification (MLPA) methods with the SALSA MLPA P051/P052 Parkinson probe mix (MRC-Holland, Amsterdam, Netherlands).

We excluded any patients with *SNCA* multiplication or missense mutations in any part of exons 31, 41, or 48 of *LRRK2*. We excluded patients harboring rare variants in *PRKN* including deletion, multiplication, heterozygous, homozygous, and compound heterozygous variants. The methods were described in the supplementary material and methods. If patients harbored digenic variants of *PRKN* and *PINK1*, they were included in the cohort. Regarding the patients analyzed by Ion Torrent System, we excluded those patients having rare variants in other genes in the panel IAD103177_182.

2.3. Bioinformatics analysis for the identified variants

The potential pathogenicity of variants of *PINK1* was assessed using Polyphen-2 (Adzhubei et al., 2010), Mutation Taster (Schwarz et al., 2010), Protein Variation Effect Analyzer (PROVEAN) (Choi and Chan, 2015), sorting intolerant from tolerant (SIFT) (Kumar et al., 2009), and human splicing finder (Desmet et al., 2009). The frequency of any variants was assessed using gnomAD (Lek et al., 2016), HGVD (Higasa et al., 2016), Japanese Multi Omics Reference Panel (jMorp) (Tadaka et al., 2018), and the BioBank Japan Whole-

Genome Sequencing (BBJWGS) (Okada et al., 2018). Evolutionary conservation of any mutated amino acids was evaluated using NCBI Homologene (http://www.ncbi.nlm.nih.gov/homologene/). To estimate *p*-values between clinical data from our cases and data from previous studies, we used unpaired Student's *t*-test (GraphPad Prism®6, Graph-Pad Software, Inc., San Diego, CA, USA).

2.4. Comparative study between one-allele variants and two-allele variants

We defined one-allele variants as heterozygous variants and two-allele variants as homozygous or compound heterozygous variants. To estimate the precise values, we then summarized the clinical data from 8 previous studies, including 53 patients with one-allele variants and 82 patients with two-allele variants (Abou-Sleiman et al., 2006, Bonifati et al., 2005; Eggers et al., 2010, Hatano et al., 2004; Hedrich et al., 2006; Ishihara-Paul et al., 2008, Kumazawa et al., 2008; Marongiu et al., 2008; Ricciardi et al., 2014). We then performed a statistical analysis using Student's *t*-test or the Chi-square test after Bonferroni correction, to compare the following 3 groups: (i) one-allele versus two-allele variants, (ii) PD with a one-allele variant versus sporadic PD, and (iii) PD with two-allele variants versus sporadic PD. These cases were combined with the cases in our study which included: 30 patients with one allele variants and 3 patients with two-allele variants.

3. Results

3.1. Genetic analysis

We identified 36 patients harboring *PINK1* variants, including 3 homozygous, 30 heterozygous, and 3 with digenic variants in *PRKN-PINK1* (Table 2). The allele frequency of heterozygous variants among the patients with familial PD and sporadic PD with young onset was 0.01 of familial 19/1684, and 0.009 of sporadic 13/1408 ($p = 0.578$). We identified 19 families harboring heterozygous variants; named as Family A to S, of which 11 families showed an AD and 8 families showed an AR pattern (Fig. 1). Three patients harboring homozygous variants were sporadic. Eight variants were located in the protein kinase domain: p.H271Q, p.R279 C, p.V285 M, p.R312Q, p.M341I, p.C388 R, p.R407Q, and p.V482 M. Four variants were located in the mitochondrial targeting sequence domain: p.Y29Rfs*70, p.G30 V, p.G43 R, and p.R58_V59insGR. The frequency of digenic variants of *PRKN-PINK1* was 0.2 % (3/1700). The types of digenic variants in *PRKN-PINK1* in 3 patients were, (a) compound-heterozygous deletion (exon2–4del and exon3del) and heterozygous p.V482 M, (b) homozygous p.T175pfsX2 and heterozygous p.R58_V59insGR, and (c) heterozygous p.M434 K and heterozygous p.V122I (*PRKN* variant and *PINK1* variant). There were no exon rearrangements or deletions in *PINK1*.

3.2. Clinical findings of the patients with PINK1 variants

We divided our enrolled patients into 3 groups: (i) heterozygous variants (n = 30), (ii) homozygous variants (n = 3), and (iii) digenic variants of *PRKN-PINK1* (n = 3) (Table 3). The age at onset was 48.8 ± 15.0 years, 31.3 ± 10.1 years, and 38.3 ± 3.79 years, respectively. The patients with homozygous variants showed the youngest age at onset. The ratio of consanguinity was 4/30, 2/3, and 1/3, respectively. Most patients with sporadic PD were young-onset (≤50 years of age at onset) (11/12). In the comparison of the data of familial PD and sporadic PD with heterozygote variants, sporadic PD group indicated significantly unique symptoms, such as young age at onset, high prevalence of dystonia at onset, and hyperreflexia, and low prevalence of constipation. The prevalence of other symptoms related to PD were largely the same among the groups of heterozygous, homozygous, and digenic variants groups. In contrast, dystonia, sleep benefit, ataxia, and dysautonomia, such as urinary incontinence, orthostatic hypotension, and psychosis (such as hallucination, delusion, depression, and cognitive decline), were less often reported. Rapid eye movement sleep behavior disorder, restless legs syndrome, and olfactory disturbance were also rare. Family pedigrees indicated a complex form of AD (11) and AR (8) types. All of them harbored heterozygous variants, indicating the incomplete penetrance of the patients with a heterozygous variant in *PINK1* (Fig. 1). None of the variants existed or were extremely rare in the public gnomAD, HGVD, jMorp, and BBJWGS gene databases (Table 4). Furthermore, all variants identified in our cohort were not seen in our 216 in-house controls. The prediction tool mostly indicated “probably or possibly damaging” or “disease-causing” estimated by Mutation taster and Polyphen-2. All variants had evolutionarily conserved amino acids apart from 2 variants, p.M341I and p.V482 M.

In the neuro-radiological analysis, brain magnetic resonance imaging (MRI) indicated that the patients usually had no morphological abnormalities. [^{123}I] MIBG myocardial scintigraphy revealed that half of the patients had a decreased heart to mediastinum ratio (51.9 %, 14/27) in all patients of PD, and (52.2%, 12/23) in the patient with the heterozygous variant. Four patients showed a cognitive decline. Among them, 3 patients were clinically diagnosed as PD with dementia or dementia with Lewy bodies.

Additionally, we found a family (Family H) harboring digenic variant of *PINK1* and *PRKN*, complicated with typical parkinsonism,

Table 2
Results of our genetic screening of *PINK1*

	PINK1			Digenic variants of *PRKN-PINK1*		
	Homozygote	Heterozygote	Total	*PRKN homo./PINK1 hetero.*	*PRKN hetero./PINK1 hetero.*	Total
Familial Parkinson's disease (n = 842)	0	18	18 (2.1%)	1	0	1 (0.1%)
Sporadic Parkinson's disease (n = 858)						
Age at onset of 50 y and younger (n = 704)						
Consang. (+) (n = 24)	1	0	1 (4.2%)	0	0	0
Consang. (−) (n = 680)	2	11	13 (1.9%)	1	1	2 (0.3%)
Total	3	11	14 (2.0%)	1	1	2 (0.3%)
Age at onset over 51 y (n = 154)						
Consang. (+) (n = 19)	0	0	0	0	0	0
Consang. (−) (n = 135)	0	1	1 (0.7%)	0	0	0
Total	0	1	1 (0.6%)	0	0	0
Total	3	12	15 (1.7%)	1	1	2 (0.2%)
Total number of screened patients (n = 1700)	3	30	33 (1.9%)	2	1	3 (0.2%)

Key: AD, autosomal dominant; AR, autosomal recessive; PD, Parkinson's disease.

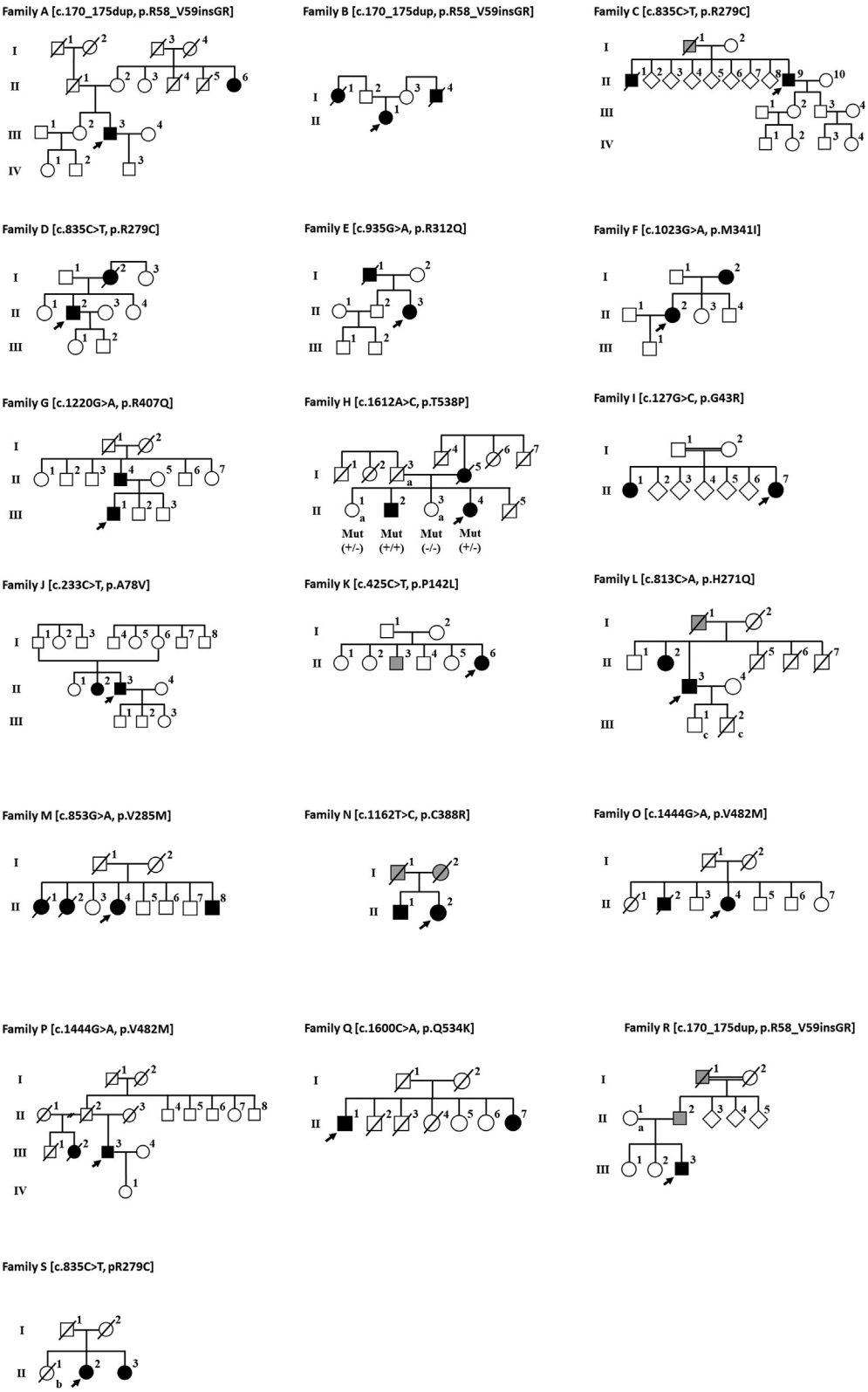

Fig. 1. Family pedigree harboring *PINK1* variants (Family A to S). The figure indicated the family pedigree harboring PINK1 variants we identified. The families include 11 of autosomal dominant inheritance and 8 of autosomal recessive inheritance. The symbols are black filled square and circle, Parkinson's disease; gray filled square and circle, parkinsonism; 'a', essential tremor; 'b', Multiple System Atrophy; 'c', psychiatric symptoms; black arrow, proband.

Table 3
Clinical overview of all patients harboring *PINK1* variants

	Heterozygote			Homozygote	Digenic variants of *PRKN-PINK1*			*p* values
	Total	Familial PD[(i)]	Sporadic PD[(ii)]	Total	Total	ADPD	SPD with young onset[#]	(i) vs (ii)
Number of patients	30	18	12	3	3	1	2	
Gender (M:F)	15:15	8:10	7:5	1:2	2:1	Male	1:1	
Age at onset	48.8 ± 15.0	55.7 ± 13.6	38.4 ± 10.8	31.3 ± 10.1	38.3 ± 3.8	34	40.5 ± 0.7	0.0006
Age at exam	56.7 ± 13.7	63.7 ± 11.4	46.2 ± 9.5	47.0 ± 19.5	48.0 ± 3.6	49	47.5 ± 4.9	0.0001
Consanguinity	13.3 (4/26)	16.7 (3/15)	8.3 (1/11)	2/1	1/2	+	0/2	0.51
Resting tremor	56.7 (17/13)	44.4 (8/10)	75.0 (9/3)	1/2	3/0	+	2/0	0.1
Akinesia	86.7 (26/4)	94.4 (17/1)	75.0 (9/3)	2/1	3/0	+	2/0	0.12
Rigidity	93.3 (28/2)	94.4 (17/1)	91.7 (11/1)	2/1	3/0	+	2/0	0.77
Loss of postural reflex	60.0 (18/12)	66.7 (12/6)	50.0 (6/6)	2/1	3/0	+	2/0	0.36
Gait disturbance	83.3 (25/5)	88.9 (16/2)	75.0 (9/3)	3/0	3/0	+	2/0	0.32
Response to levodopa	78.6 (22/6)	83.3 (15/3)	70.0 (7/3)	NA	1/2	+	0/2	0.41
Dystonia at onset	20.0 (6/24)	5.56 (1/17)	41.7 (5/7)	0/3	0/3	–	0/2	0.02
Hyperreflexia	31.0 (9/20)	16.7 (3/15)	54.5 (6/5)	1/2	0/3	–	0/2	0.03
Sleep benefit	15.4 (4/22)	13.3 (2/13)	18.2 (2/9)	2/1	2/1	+	1/1	0.73
Ataxia	0 (0/25)	0 (0/15)	0 (0/10)	0/3	0/3	–	0/2	NA
Constipation	48.0 (12/13)	66.7 (10/5)	20.0 (2/8)	0/3	1/2	+	0/2	0.02
Urinary incontinent	26.7 (8/22)	38.9 (7/11)	8.3 (1/11)	1/1	1/2	+	0/2	0.06
Orthostatic hypotension	20.7 (6/23)	29.4 (5/12)	8.3 (1/11)	0/2	1/2	+	0/2	0.17
Hallucination	13.3 (4/26)	16.7 (3/15)	8.3 (1/11)	0/3	2/1	+	1/1	0.51
Delusion	3.3 (1/29)	0 (0/18)	8.3 (1/11)	0/3	1/2	-	1/1	0.21
Depression	13.8 (4/25)	5.9 (1/16)	25.0 (3/9)	0/3	1/2	+	0/2	0.14
Cognitive dysfunction	13.3 (4/26)	16.7 (3/15)	8.3 (1/11)	0/3	0/3	–	0/2	0.51
REM behavior disorder	26.1 (6/17)	40.0 (6/9)	0 (0/8)	0/3	1/1	+	0/1	0.04
Restless legs syndrome	19.0 (4/17)	28.6 (4/10)	0 (0/7)	0/3	0/2	–	0/1	0.12
Smell disturbance	31.8 (7/15)	23.1 (3/10)	44.4 (4/5)	1/2	0/2	–	0/1	0.29
Brain MRI (atrophic changes: normal)	7.1 (2/26)	6.3 (1/15)	8.3 (1/11)	1:2	0:3	normal	0:2	0.83
^{123}I-MIBG myocardial scintigraphy (decreasing of heart to mediastinum ratio: normal)	52.2 (12/11)	53.8 (7/6)	50.0 (5/5)	1:1	1:1	normal	1:0	0.85

Each column showed (average, ± standard deviation), [percentage (positive/negative)]. # SPD with young onset means the PD group with under 50 year old of age at onset.
Key: REM; rapid eye movement, MRI; magnetic resonance imaging, MIBG; metaiodobenzylguanidine; SPD; sporadic Parkinson's disease.

essential tremors, and epilepsy. Further information is provided in Fig. 1 and Supplementary data.

3.3. Comparative study between our patients and the patients reported previously

When we combined the data of our patients with those of previously-reported patients, the statistical analysis clearly showed differences in the category of the age at onset, which was 35.3 ± 10.6 years with two-allele variants, 45.8 ± 13.6 years with one-allele variants and 49.6 ± 8.2 years with sporadic PD (Table 5). The age at onset seems to depend on the number of affected alleles. Additionally, gait disturbance has a relatively low frequency in patients with two-allele variants. However, most of the symptoms did not show any differences between one-allele variants, two-allele variants, and sporadic patients in the categories of tremor, rigidity, bradykinesia, postural instability, cognitive dysfunction, psychosis, and response to levodopa. These results indicated that the number of variants in *PINK1* affects the age at onset but not the clinical symptoms and course of the disease. With the digenic mutation of *PRKN-PINK1*, the age at onset was 38.3 ± 3.79 years. These patients showed common symptoms of PD and a good response to levodopa without cognitive decline. These findings were quite similar with those of patients with *PINK1* variants.

4. Discussion

We reported the genotype-phenotype correlations of 36 patients harboring *PINK1* variants. The sample included 30 patients with heterozygous variants, 3 patients with homozygous, and 3 patients with digenic variants of *PRKN-PINK1*, who were identified from a large cohort of 1700 PD patients, comprising 842 familial PD and 858 sporadic PD patients. The prevalence of patient and allele frequency with heterozygous variants was 2.3 % (19/842) and 0.01 (19/1684) in familial PD and 1.8 % (13/704) and 0.009 (13/1408) in sporadic PD with young onset. There was no significant difference with the prevalence of heterozygous *PINK1* variants between familial and sporadic-young onset PD. None of the identified variants occurred in our control subjects and they were also extremely rare in genetic databases. Thus, our results emphasize that heterozygous variants relate to both familial and sporadic PD with young-onset. The frequency of heterozygous variants in *PINK1* was reported as 1.2 % (9/768) and 0.006 (9/1536) among sporadic PD patients and 2.6 % (4/116) and 0.02 (4/232) among sporadic PD patients with young-onset (Abou-Sleimanet al., 2006; Bonifatiet al., 2005). Our results are consistent with these previous reports. However, a recent report did not provide evidence of heterozygous pathogenic variants in *PINK1* and PD, including p.G411S. The data were obtained from a large cohort: 13,708 PD patients and 362,850 controls of European ancestry (Krohn et al., 2020). There are some differences between our study and the previous study. The previous study enrolled PD patients across all ages, from young to elderly. While our study also included both young-onset and late-onset PD cases, the participants were separated into 2 groups by age at onset: 50 years and younger or over 51 years. Additionally, rare variants in *PINK1* seem to have population differences, especially, p.G411S. The variant is frequently found in European ancestry but no study has reported the presence of p.G411S in Asians, including ours (Linet al., 2019; Puschmannet al., 2017; Tanet al., 2006; Wenget al., 2007). The differences in study design or cohort, or reduced penetrance of *PINK1* variants may lead to the conflicting conclusions between the previous report and our study.

Regarding the symptoms, there are no large differences between PD patients with heterozygous (one-allele) variants, homozygous or compound heterozygous (two-allele) variants, and sporadic PD, except for the age at onset, although we collected the data by cross-sectional study without long-term observation. Age at onset is the most prominent factor to differentiate patients with two-allele

Table 4
Bioinformatics data of the identified *PINK1* variants

Patients	Nucleotide	Amino acid	Position	Zygosity	Exon	rs number	Allele frequency					In Silico prediction				
							gnomAD (Total)	gnomAD (East Asia)	HGVD	jMorp (ToMMo3.5JPN)	BBJWGS	Polyphen2*	PROVEAN	SIFT	Mutation taster	Human splicing finder
Family L	c.813 C>A	p.H271Q	1:20971019	Hetero	4		no data	no data	no data	no data	no data	Pb Dam/Pb Dam (0.999/0.983)	Deleterious	Damaging	Disease causing	
Family N	c.1162 T>C	p.C388 R	1:20975036	Hetero	6		no data	no data	no data	no data	no data	Pb Dam/Pb Dam (1.000/1.000)	Deleterious	Damaging	Disease causing	
Family G	c.1220 G>A	p.R407Q	1:20975094	Hetero	6	rs556540177	0.0001035 (26/251126)	0.0009788 (18/18,390	0.0009	0.0006 (4/6898)	no data	Pb Dam/Pb Dam (0.999/0.936)	Neutral	Damaging	Disease causing	
Family H	c.1612 A>C	p.T538P	1:20977050	Hetero	8		no data	no data	no data	no data	no data	Ps Dam/Ps Dam (0.901/0.470)	Neutral	Damaging	Polymorphism	
Family I	c.127 G>C	p.G43 R	1:20960168	Hetero	1	rs1443552897	no data	no data	0.001	no data	no data	Benign/Benign (0.013/0.002)	Neutral	Damaging	Polymorphism	
Family J	c.233 C>T	p.A78 V	1:20960274	Hetero	1	rs1409111496	no data	no data	0.0004	no data	no data	Pd Dam/Ps Dam (0.996/0.627)	Neutral	Damaging	Disease causing	
Family K	c.425 C>T	p.P142 L	1:20964772	Hetero	2		no data	no data	no data	no data	no data	Ps Dam/Benign (0.855/0.285)	Deleterious	Damaging	Disease causing	
Family M	c.853 G>A	p.V285 M	1:20971059	Hetero	4	rs557503577	0.00002121 (6/282884)	0 (0/19,950)	no data	0.0001 (1/7042)	no data	Pb Dam/Ps Dam (0.997/0.859)	Deleterious	Damaging	Disease causing	
Family Q	c.1600 C>A	p.Q534 K	1:20977038	Hetero	8		no data	no data	no data	0.0003 (2/7076)	no data	Ps Dam/Ps Dam (0.928/0.491)	Neutral	Tolarated	Disease causing	
Case 14	c.85_10622bpdel	p.Y29Rfs*70	1:20960126_20960148	Homo	1		no data	no data	no data	no data	no data					Potential alteration of splicing
Case 15	c.336delG	p.E113 fs	1:20960377	Homo	1		no data	no data	no data	no data	no data				Disease causing	probably no impact on splicing
Case 16	c.342_343insA	p.Q115Tfs*27	1:20960383_20960384	Homo	1		no data	no data	no data	no data	no data				Disease causing	Potential alteration of splicing
Case 1	c.89 G>T	p.G30 V	1:20960130	Hetero	1		no data	no data	no data	0.0003 (2/6952)	0.00975 (2/2052)	Benign/Benign (0.190/0.076)	Neutral	Damaging	Disease causing	

Case 3	c.286 T>C	p.C96 R	1:20960327	Hetero	1		no data	no data	no data	no data	no data	Benign/ Benign (0.008/ 0.005)	Neutral	Tolarated	Disease causing	
Case 4	c.448 G>A	p.G150S	1:20964395	Hetero	2		no data	no data	no data	no data	no data	Pb Dam/Pb Dam (1.000/ 0.999)	Neutral	Tolarated	Disease causing	
Case 17	c.364 G>A	p.V122I	1:20960405	Hetero	1		no data	no data	no data	no data	no data	Benign/ Benign (0.001/ 0.002)	Neutral	Tolarated	Polymorphism	
Family A, B, R Case 2	c.169_170dup	p.R58_V59insGR	1:20960210 _20960211	Hetero	1	rs751456355	0.00003205 (4/124812)	0.0004023 (4/9942)	no data	no data	no data				Polymorphism	Potential alteration of splicing
Family C, D, S Case 5,	c.835 C>T	p.R279 C	1:20971041	Hetero	4	rs61735932	0.00006363 (18/ 282886)	0.0004511 (9/19,952)	0.0008	0.0008 (6/7060)	0.001462 (3/2052)	Pb Dam/Pb Dam (1.000/ 0.987)	Neutral	Tolarated	Disease causing	
Family E Case 7	c.935 G>A	p.R312Q	1:20971141	Hetero	4	rs202128685	0.00002476 (7/282698)	0.00005015 (1/19,940)	no data	0.0006 (4/7012)	no data	Pb Dam/Ps Dam (0.998/ 0.876)	Neutral	Tolarated	Disease causing	
Family F Case 8, 9	c.1023 G>A	p.M341I	1:20972116	Hetero	5	rs35813094	0.0001839 (52/ 282810)	0.002606 (52/19,952)	no data	0.0004 (3/6940)	0.001462 (3/2052)	Benign/ Benign (0.045/ 0.033)	Neutral	Tolarated	Disease causing	
Family O, P Case 10, 11, 12, 13, 18	c.1444 G>A	p.V482 M	1:20975680	Hetero	7	rs773843241	0.00003188 (9/282344)	0.0001504 (3/19,946)	0.0017	0.0021 (15/7026)	0.00341 (7/2052)	Ps Dam/ Benign (0.867/ 0.222)	Neutral	Tolarated	Polymorphism	

The reference number of *PINK1* sequence is NM_032409. *Pb Dam, Probably damaging; Ps Dam, Possibly damaging. Hetero means heterozygote. Homo means homozygote.
Key: HGVD, the Human Genetic Variation Database; BBJWGS, the BioBank Japan Whole-Genome Sequencing; PROVEAN, Protein Variation Effect Analyzer; SIFT, sorting intolerant from tolerant.

Table 5
Summarizing data of the patients harboring *PINK1* mutations reported previously, comparing between heterozygous (one allele) and homozygous or compound-heterozygous (2 alleles) variants

Author/year/country	Number of samples	Mean age at onset	Tremor	Rigidity	Bradykinesia	Postural instability	Gait disturbance	Cognitive dysfunction	Psychosis/hallucination	Response for levodopa
One allele of variant										
Eggers et al.,/2010/Germany	1	51	0/1	1/0	1/0	0/1	0/0	0/1	0/1	NA
Kumazawa et al.,/2008/Japan	5	47.4 ± 3.78	5/0	5/0	5/0	4/1	5/0	0/5	0/5	NA
Maroungiu et al.,/2008/Itarly	20	52.2 ± 10.2	NA	NA	NA	NA	NA	NA	NA	NA
Ishihara-Paul et al.,/2008/Tunisia	3	69.0 ± 8.0	43,893	NA	NA	NA	0/3	NA	NA	NA
Abou-Sieman et al.,/2006/United Kingdom	9	53.7 ± 9.19	44,014	9/0	9/0	NA	NA	NA	NA	9/0
Hedrich et al.,/2006/Germany	11	NA	0/11	5/6	6/5	2/9	NA	0/11	NA	NA
Bonifati et al.,/2005/Italy	4	39.0 ± 4.97	2/2	4/0	4/0	2/2	NA	0/4	1/3	4/0
Our cases	30	48.8 ± 15.0	18/12	28/2	26/4	19/11	25/5	4/26	4/26	22/6
Total	83	45.8 ± 13.6	35/31 (53.0)	52/8 (86.7)	51/9 (85.0)	27/24 (52/9)	30/8 (78.9)	4/47 (7.84)	5/35 (12.5)	35/6 (85.4)
Two alleles of variant										
Ricciardi et al.,/2014/Italy	5	36.8 ± 2.90	NA	NA	NA	NA	NA	0/5	0/5	5/5
Eggers et al.,/2010/Germany	4	35.0 ± 23.4	0/4	4/0	4/0	4/0	0/4	0/4	0/4	4/0
Kumazawa et al.,/2008/Japan	16	32.6 ± 8.5	11/5	13/3	12/4	9/7	15/1	2/14	2/14	NA
Ishihara-Paul et al.,/2006/Tunisia	46	36.0 ± 12.0	24/22	NA	NA	NA	17/29	NA	NA	NA
Hedrich et al.,/2006/Germany	4	50.0 ± 9.31	1/3	4/0	4/0	4/0	1/3	0/4	NA	4/0
Bonifati et al.,/2005/Italy	4	30.8 ± 2.99	3/1	4/0	4/0	4/0	NA	0/4	0/4	3/0
Hatano et al.,/2004/Japan	3	28.6 ± 5.13	2/1	3/0	3/0	3/0	0/3	0/3	43,832	NA
Our cases	3	31.3 ±10.1	1/2	2/1	2/1	2/1	3/0	0/3	0/3	1/2
Total	85	35.3 ±10.6	42/38 (55.4)	30/4 (88.2)	29/5 (85.3)	24/10 (70.6)	36/40 (47.4)	2/37 (5.13)	3/32 (8.57)	17/7 (70.8)
Sporadic Parkinson's disease										
Our case	196	49.6 ± 8.2	108/88 (55.1)	182/14 (92.9)	168/28 (85.7)	123/73 (62.8)	163/33 (83.2)	22/174 (11.5)	25/171 (12.8)	152/25 (85.9)
Statiscal analysis										
One allele vs 2 alleles		<0.0001	1	1	1	0.12	0.001	0.69	0.72	0.2
One allele vs sporadic		0.02	0.78	0.18	0.84	0.2	0.49	0.61	1	1
Two alleles vs sporadic		<0.0001	0.79	0.32	1	0.44	<0.0001	0.39	0.59	0.07

The significant level of p value was defined under 0.0167 (0.05/3) after Bonferroni correction. Each column showed (average, ± standard deviation), [positive/negative (percentage)].

variants from those with one-allele variants. Our findings are consistent with previous reports, in which the patients with two-allele variants had a younger age at onset than those with one-allele variants or sporadic cases (Abou-Sleiman et al., 2006, Bonifati et al., 2005). Another report with precise clinical findings and 12 years's follow-up reported that 5 patients with homozygous variants showed non-motor symptoms and 3 of 5 patients an additional psychosis. Three patients with heterozygous variants showed non-motor symptoms and cognitive decline, although the symptoms of the patients with homozygous variants were relatively severe (Ricciardiet al., 2014). Most of the studies reported that few patients showed cognitive dysfunction. Sleep benefit, dystonia, and hyper reflexes were not common among the patients with heterozygous variants in *PINK1* but are commonly seen in patients with homozygous variants in *PINK1* (Abou-Sleiman et al., 2006, Bonifati et al., 2005). Generally, the patients with *PINK1* variants displayed a benign clinical course with a good response to levodopa without prominent cognitive decline (Table 5).

[^{123}I] MIBG myocardial scintigraphy indicated approximately half of patients having a decrease of heart to mediastinum ratio among the patients with heterozygous variants (12/23%, 52.2%). The reduction of MIBG uptake associated with Lewy bodies pathology. Up to date, neuropathological studies of 6 patients with *PINK1* variants were reported. One was 31 of age at onset and 39 of age at death, harboring compound heterozygote in the deletion of exon 7 and splicing mutation in exon 7, whose pathology revealed Lewy body pathology in the reticular nuclei of the brainstem, substantia nigra pars compacta, and Meynert nucleus (Samaranch et al., 2010). The other was 54 of age at onset and 56 of age at death, harboring homozygous missense variants, p.C388 R, whose pathology indicated a neuronal loss in the substantia nigra pars compacta without Lewy body pathologies through the brain (Takanashi et al., 2016). Other 4 patients with heterozygous variants showed a wide degree of Braak stage, ranging from I to V, along with the appearances of brainstem Lewy bodies in the absence of cortical Lewy bodies (Gandhi et al., 2006). Lewy bodies in the substantia nigra were immuno-stained by PINK1 antibodies. Four patients commonly showed late-onset (58–64 years) and mild parkinsonism with an excellent response to levodopa, and 2 patients showed cognitive decline. Each patient presented with a different course and pathology. However, 5 of 6 patients presented with Lewy body pathology. Half of the patients with a reduction of heart-to-mediastinum ratio of MIBG myocardial scintigraphy may develop the over-expression of alpha-synuclein and Lewy body pathologies (Orimo et al., 2005), which means that heterozygous variants in *PINK1* possibly associate with synucleinopathies. In other words, heterozygous variants in *PINK1* may be a risk factor for developing PD as an alpha-synucleinopathy.

Our analysis of the family tree indicated the incomplete penetrance of heterozygous variants in *PINK1*. Incomplete penetrance could be observed in several types of genes obeying the Mendelian inheritance in familial PD. The estimated penetrance ratio of heterozygous variants in *PRKN* or *PINK1* is relatively low (1%–25%) compared to homozygous or compound heterozygous in *PRKN* or *PINK1* (nearly 100 %) (Klein et al., 2007). Heterozygous variants are less severe than homozygous or compound-heterozygous variants. In cell models, cell lines expressing 2 copies of kinase mutants are more severely affected than those expressing only one copy (Tan et al., 2009). Cell lines with 2 copies had a greater apoptosis rate and a decreased mitochondrial membrane potential. The results showed that the effect of *PINK1* variants depends on the number of affected alleles. The alteration of kinase activity could be caused by haploinsufficiency or dominant-negative effects.

In the different genes identified from a family with AR inheritance, *PRKN* is known as a pathogenic gene related to young-onset parkinsonism (Kitada et al., 1998). The patients with 2 or more-allele variants had a significantly younger age at onset than those with one-allele variants (Sun et al., 2006). *PLA2G6* is known as a pathogenic gene related to young-onset parkinsonism or adult-onset dystonia-parkinsonism (Guo et al., 2018). First, *PLA2G6* was identified as a homozygous mutation in a large family. The patients presented adult-onset dystonia-parkinsonism, pyramidal signs, and cognitive/psychiatric features (Paisan-Ruiz et al., 2009). Recently, it was reported that heterozygous variants in *PLA2G6* also induce late-onset PD (Ferese et al., 2018). The symptoms of patients with *PRKN* or *PLA2G6* variants are thought to be caused by loss-of-function mutations (Shimura et al., 2000; Sumi-Akamaru et al., 2016). *PRKN*, *PINK1*, and *PLA2G6* were first found in AR families. It was only later revealed that the heterozygous variants are also a potential risk factor. The differences between one-allele and two-allele variants may be a specific finding for those genes with haploinsufficiency mechanisms.

In contrast to *PINK1* and *PRKN*, the pathogenic mutations in *SNCA* or *LRRK2* did not show any differences in clinical symptoms between homozygous and heterozygous. The patients with homozygous variants of *SNCA* p.A53 V presented the same clinical symptoms as those with heterozygous variants of *SNCA* missense mutations, which are middle-age onset parkinsonism, cognitive decline, and psychosis (Deng et al., 2018, Yoshino et al., 2017). The patients with the p.R1441H mutation in *LRRK2* showed the same symptoms and disease course as patients with homozygous or heterozygous variants even in the same family (Takanashi et al., 2018). Their symptoms were late-onset parkinsonism with good response to levodopa and low prevalence of cognitive decline. *SNCA* and *LRRK2* are commonly thought to be caused by gain-of-function mechanisms in the manner of autosomal dominant inheritance. The influence of the number of affected alleles seems to be specific for the genes related to haploinsufficiency or dominant-negative effects, which is commonly identified in patients with AR inheritance. However, there are limited reports of homozygous variants of *SNCA* and *LRRK2*. Further studies are necessary to explore the functional differences between one-allele and two-allele variants.

Our group previously reported 3 families harboring digenic variants in *PINK1* and *PRKN* (Funayama et al., 2008). Most of their clinical symptoms occurred at a young age (teens to thirties). Prominent symptoms were rigidity and good response to levodopa with long disease duration. Other distinctive symptoms were schizophrenia, facial dyskinesia, grimacing, and severe dysarthria. Their variants were [p.T175Pfs/p.T175Pfs in *PRKN*, and p.R58_V59insGR/wt in *PINK1*] in AD PD, [p.C441 R/p.A138GfsX7 in *PRKN*, and p.R407Q/wt in *PINK1*] in AR PD, and [p.P437 L/wt in *PRKN*, and p.E476 K/wt in *PINK1*] in one sporadic PD patient, which were different variants than in our case. Till date, only a few reports described digenic variants of genes which are known to be related to familial PD, obeying the Mendelian form such as the complex form of (*PINK1* and *DJ-1*) or (*PRKN* and *LRRK2*) (Dachsel et al., 2006; Tang et al., 2006). Our patient had *PRKN* and *PINK1* variants (H-II-2) with various clinical symptoms such as parkinsonism, epilepsy, and essential tremor. In mice models, hemizygous deletion of *Mcl-1* in *PRKN*-knockout mice produced neurodegeneration, particularly in the substantia nigra, and progressive motor dysfunction (Ekholm-Reed et al., 2019). *Mcl-1* hemizygous deletion may be a modifier for neurodegeneration. Digenic variants may have the potential to enhance the distinctive phenotype. However, digenic variants remain ambiguous due to the small number of reported cases.

Our study includes some limitations: clinical study was set up as a cross-sectional study without long-term follow-up observation. It remains controversial whether *PINK1* heterozygous variants influence the symptoms or not, as shown in a previous study (Krohn et al., 2020).

To conclude, we emphasize the importance of heterozygous variants of *PINK1* among familial and sporadic PD patients, along with incomplete penetrance and heterogeneous symptoms. Haploinsufficiency or dominant-negative effects are possible pathomechanisms for the development of the disease. Some patients may relate to synucleinopathies. The *PINK1* variants may help us understand the detailed physiological mechanisms of familial and sporadic PD.

Disclosure statement

The authors report no conflicts of interest relevant to the manuscript.

CRediT authorship contribution statement

Arisa Hayashida: Formal analysis, Writing - original draft, Validation. **Yuanzhe Li:** Formal analysis, Writing - review & editing, Funding acquisition. **Hiroyo Yoshino:** Formal analysis, Data curation, Writing - review & editing, Validation, Funding acquisition. **Kensuke Daida:** Resources. **Aya Ikeda:** Resources. **Kotaro Ogaki:** Resources. **Atsuhito Fuse:** Resources. **Akio Mori:** Resources. **Masashi Takanashi:** Resources. **Toshiki Nakahara:** Resources. **Asako Yoritaka:** Resources. **Yuji Tomizawa:** Resources. **Yoshiaki Furukawa:** Resources. **Kazuaki Kanai:** Resources. **Yoshiaki Nakayama:** Resources. **Hidefumi Ito:** Resources. **Mieko Ogino:** Resources. **Yuko Hattori:** Resources. **Tatsuya Hattori:** Resources. **Yuta Ichinose:** Resources. **Yoshihisa Takiyama:** Resources. **Tsukasa Saito:** Resources. **Takashi Kimura:** Resources. **Hitoshi Aizawa:** Resources. **Hiroshi Shoji:** Resources. **Yuri Mizuno:** Resources. **Takuya Matsushita:** Resources. **Mitsuto Sato:** Resources. **Yoshiki Sekijima:** Resources. **Masayo Morita:** Resources. **Akio Iwasaki:** Resources. **Hirofumi Kusaka:** Resources. **Mikiko Tada:** Resources. **Fumiaki Tanaka:** Resources. **Yusuke Sakiyama:** Resources. **Takeshi Fujimoto:** Resources. **Yuko Nagara:** Resources. **Kenichi Kashihara:** Resources. **Hiroyuki Todo:** Resources. **Kouichi Nakao:** Resources. **Kazuhito Tsuruta:** Resources. **Masaaki Yoshikawa:** Resources. **Hideo Hara:** Resources. **Hiroaki Yokote:** Resources. **Nagako Murase:** Resources. **Kiyotaka Nakamagoe:** Resources. **Akira Tamaoka:** Resources. **Motonori Takamiya:** Resources. **Nobutoshi Morimoto:** Resources. **Kazuya Nokura:** Resources. **Tetsuharu Kako:** Resources. **Manabu Funayama:** Writing - review & editing, Supervision, Funding acquisition, Funding acquisition. **Kenya Nishioka:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Visualization, Project administration, Funding acquisition. **Nobutaka Hattori:** Funding acquisition, Supervision.

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Appendix A. Supplementary data

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