


# A Clinical and Genetic Study of Early-Onset and Familial Parkinsonism in Taiwan: An Integrated Approach Combining Gene Dosage Analysis and Next-Generation Sequencing

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**ABSTRACT: Background:** Recent genetic progress has allowed for the molecular diagnosis of Parkinson's disease. However, genetic causes of PD vary widely in different ethnicities. Mutational frequencies and clinical phenotypes of genes associated with PD in Asian populations are largely unknown. The objective of this study was to identify the mutational frequencies and clinical spectrums of multiple PD-causative genes in a Taiwanese PD cohort.

**Methods:** A total of 571 participants including 324 patients with early-onset parkinsonism (onset age, <50 years) and 247 parkinsonism pedigrees were recruited at a tertiary referral center in Taiwan from 2002 to 2017. Genetic causes were identified by an integrated approach including gene dosage analysis, a targeted next-generation sequencing panel containing 40 known PD-causative genes, repeat-primed polymerase chain reaction, and whole-exome sequencing analysis.

**Results:** Thirty of the 324 patients with early-onset parkinsonism (9.3%) were found to carry mutations in *Parkin*, *PINK1*, or *PLA2G6* or had increased trinucleotide

repeats in *SCA8*. Twenty-nine of 109 probands with autosomal-recessive inheritance of parkinsonism (26.6%) were found to carry mutations in *Parkin*, *PINK1*, *GBA*, or *HTRA2*. The genetic causes for the 138 probands with an autosomal-dominant inheritance pattern of parkinsonism were more heterogeneous. Seventeen probands (12.3%) carried pathogenic mutations in *LRRK2*, *VPS35*, *MAPT*, *GBA*, *DNAJC13*, *C9orf72*, *SCA3*, or *SCA17*. A novel missense mutation in the *UQCRC1* gene was found in a family with autosomal-dominant inheritance parkinsonism via whole-exome sequencing analysis.

**Conclusions:** Our findings provide a better understanding of the genetic architecture of PD in eastern Asia and broaden the clinical spectrum of PD-causing mutations. © 2019 The Authors. *Movement Disorders* published by Wiley Periodicals, Inc. on behalf of International Parkinson and Movement Disorder Society.

**Key Words:** early onset; genetics; next-generation sequencing; Parkinson's disease; parkinsonism

Parkinson's disease (PD) is a progressive neurodegenerative disorder caused by a combination of genetic and environmental risk factors.<sup>1</sup> Approximately 5%-10%

of patients have monogenic forms of the disease with either an autosomal-dominant (AD) or autosomal-recessive (AR) pattern of inheritance.<sup>2</sup> Mutations in a

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**Relevant conflicts of interests/financial disclosures:** No author reports any conflicts of interest.

**Funding agencies:** This work was supported by grants from National Taiwan University Hospital (NTUH 107C101-82), National Taiwan University (106R8805C1), the Ministry of Science and Technology (MOST 106-2314-B-002-072-MY3), and the T.H. Wu Foundation.

**Received:** 17 July 2018; **Revised:** 10 January 2019; **Accepted:** 14 January 2019

Published online 20 February 2019 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.27633

number of genes implicated in both familial and sporadic PD have been identified.<sup>3</sup> Identification of these PD-causative genes provides biological insights into underlying disease processes.<sup>3</sup> Neuromodification therapy by targeting these pathways provides an avenue for future drug discovery to mitigate disease progression.<sup>4</sup>

Evidence has shown that genetic causes can vary depending on the geographic and ethnic backgrounds of the studied populations.<sup>5</sup> For example, the major genetic cause for AD inheritance of parkinsonism is a mutation in leucine-rich repeat kinase 2 (*LRRK2*)<sup>6,7</sup> with the *LRRK2* p.G2019S mutation having the highest frequency among North African Arab-Berbers and Ashkenazi Jews.<sup>8,9</sup> However, the *LRRK2* p.G2019S mutation is rare in Asian patients.<sup>10</sup> As mutations in *LRRK2* result in hyperactivation of *LRRK2* kinase activity, *LRRK2* inhibitors have entered clinical trials that offers the prospect of elaborating disease-modifying treatments for PD.<sup>11</sup> These observations indicate a pressing need to expand the knowledge of ethnically appropriate genetics in diverse populations.

We have previously described the clinical features of Taiwanese patients with early-onset parkinsonism.<sup>12</sup> Here we take an integrative approach, including gene dosage analysis, a targeted next-generation sequencing (NGS) panel, repeat-primed polymerase chain reaction (PCR), and whole-exome sequencing (WES) to elucidate genetic causes and the relationships between genotypes and clinical phenotypes in patients with early-onset parkinsonism and familial parkinsonism in a Taiwanese population.

## Materials and Methods

### Subjects

A total of 571 participants including 324 patients with early-onset sporadic parkinsonism (onset age, <50 years) and 247 probands with familial parkinsonism (at least 1 of the family members in 3 generations affected with parkinsonism) were recruited from the Centre for Parkinson and Movement Disorders at a tertiary referral center in Taiwan from 2002 to 2017. Among the 247 probands with familial parkinsonism, 57 probands had an age at onset younger than 50 years. Of all participants, 522 patients fulfilled the United Kingdom PD Society Brain Bank diagnostic criteria of PD,<sup>13</sup> and 49 patients also presented with mixed neurodegenerative features, including cognitive decline (n = 18), ataxia (n = 28), and motor neuron disorders (n = 3). All participants received regular evaluations of motor and cognitive functions. Motor symptom severity was evaluated using the Unified Parkinson's Disease Rating Scale (UPDRS) motor subscale<sup>14</sup> and Hoehn-and-Yahr staging.<sup>15</sup> Cognition was evaluated with the Mini-Mental State Examination,<sup>16</sup> and some patients received complete neuropsychological

tests.<sup>17</sup> All participants provided informed consent, and the institutional ethics review board of National Taiwan University Hospital approved this study.

Of the 247 probands with familial parkinsonism, 138 were compatible with an AD inheritance pattern, and 109 were compatible with AR inheritance or had at least 1 other affected first- and/or second-degree relative with parkinsonism. Of the 324 patients with early-onset parkinsonism, 72 had previously been reported to screen for *Parkin*, *PINK1*, and *DJ-1* mutations.<sup>12</sup> In the current study, we enrolled additional patients with early-onset parkinsonism and applied an integrated genetic approach.

### Genetic Analysis

The flowchart of the genetic analysis is presented in Figure 1.

#### Multiplex Ligation-Dependent Probe Amplification

DNA was extracted from venous blood using standard protocols.<sup>12</sup> Large deletions or duplications of common PD-causative genes including *SNCA*, *Parkin*, *PINK1*, *DJ-1*, *ATP13A2*, *PLA2G6*, *FBXO7*, *DNAJC6*, and *LRRK2* were detected using the salsa multiplex ligation-dependent probe amplification (MLPA) kit P051-c1/P52-c1 (MRC-Holland, Amsterdam, The Netherlands). Patients with deletions or duplications then received Sanger sequencing of the target gene to identify missense mutations on the other allele in a compound heterozygous state, and relative quantification of implicated exons was performed to confirm a homozygous deletion state.

#### Repeat-Primed PCR

Repeat-primed PCR was carried out as previously described to screen for GGGGCC hexanucleotide expansions in *C9orf72*<sup>18</sup> and CAG, CTG, and ATTCT

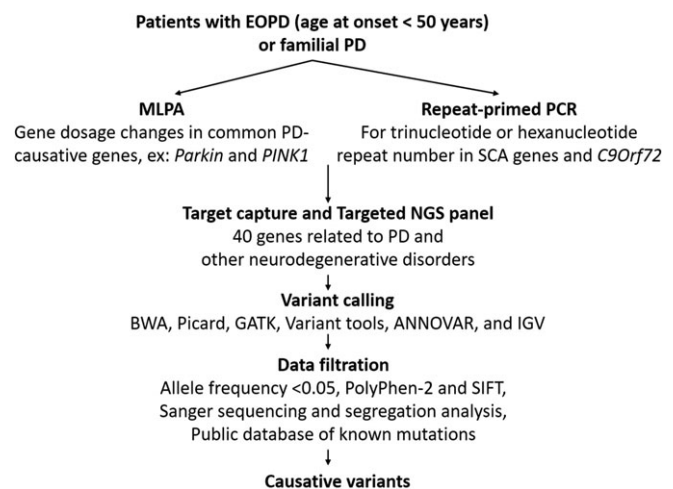


FIG. 1. Pipeline for the identification of causative variants in patients with early-onset parkinsonism or familial parkinsonism.

repeats in *SCA1*, *SCA2*, *SCA3*, *SCA6*, *SCA8*, and *SCA17*.<sup>19,20</sup>

### Targeted NGS Panel

A custom-designed NGS panel, including 40 genes associated with parkinsonism (Supplementary Table 1), was performed as previously described.<sup>21</sup> Figure 1 depicts the criteria for identifying causative variants in the affected families, including target enrichment, variant calling, and data filtering. The details are described in the Supplementary Methods.

### Whole-Exome Sequencing

Affected individuals from 1 multiplex family with AD-inheritance parkinsonism and without mutations in known PD genes received WES analyses using the Ion Torrent Next-Generation Sequencing Exon v2 kit and platform (Life Technologies) as previously described.<sup>22</sup>

### Generation and Quantitative Analysis of Neurites of UQCRC1 Knock-In SH-SY5Y Cells

We generated mutant *UQCRC1* knock-in (KI) SH-SY5Y cell lines with clustered, regularly interspaced short palindromic repeats-associated nuclease 9 (CRISPR-Cas9) technology, as described in the Supplementary Methods.<sup>23</sup> Neurite length for each genotype of SH-SY5Y cells was quantified manually with Image J software (National Institutes of Health, Bethesda, MD), which is described in the Supplementary Methods.<sup>24</sup>

### Measurement of Mitochondrial Respiratory Chain Activity

We measured mitochondrial respiratory chain activity in wild-type and mutant *UQCRC1* SH-SY5Y cells with a Seahorse XFe24 extracellular flux analyzer (Seahorse Bioscience, North Billerica, MA), as previously described.<sup>25</sup>

## Results

### Genetic Analyses

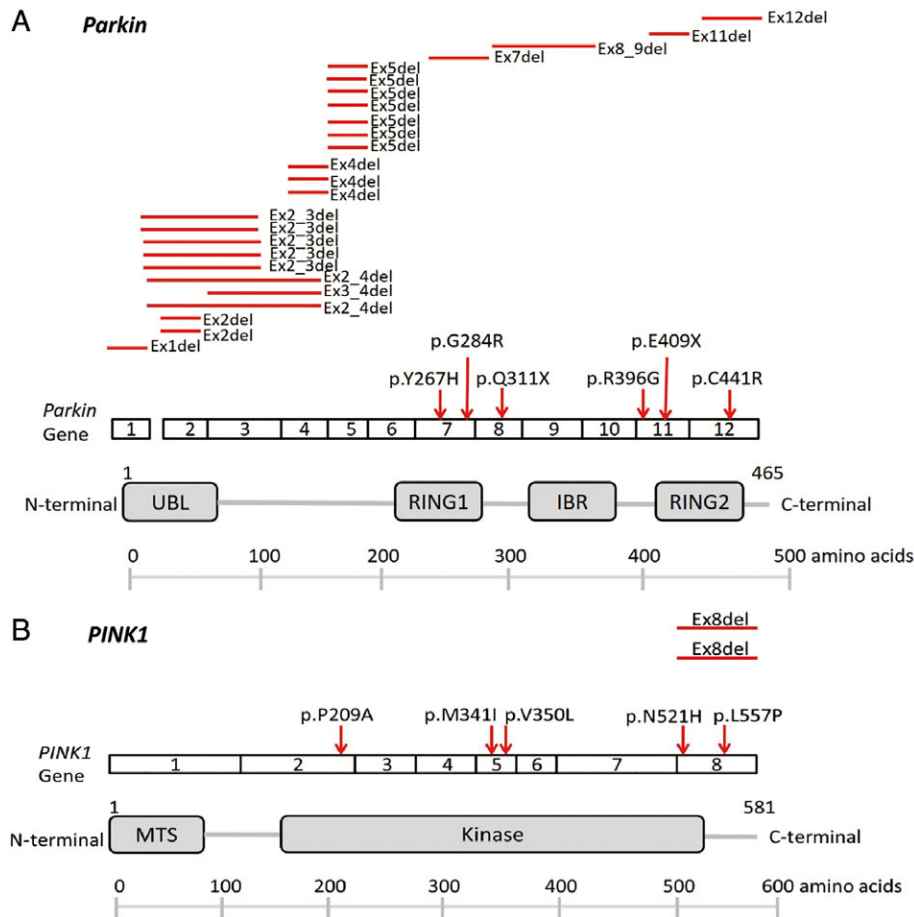
The mean age at onset of patients with early-onset parkinsonism was  $41.6 \pm 6.4$  years, and 50.1% were men, whereas the mean age at onset of probands with familial parkinsonism was  $54.4 \pm 13.7$  years, and 53.3% were men. Using target gene capture sequencing, we covered 656 exons in 40 genes representing a total coding region of 158,073 bp. The average coverage was 143-fold, with 92.3% of sequences having coverage greater than 30-fold and 89.1% greater than 50-fold.

*Parkin* was the most prevalent mutated gene in 324 patients with early-onset parkinsonism. Of the 14 *Parkin* mutation carriers (4.3% of patients with

early-onset parkinsonism), 4 had compound heterozygous mutations, and 10 had single heterozygous mutations. Exon deletions were the most common type of mutations, especially exon 5 and between exons 2 and 3 (Fig. 2A). Six patients had missense mutations: 2 had p. R396G, 1 had p.Y267H, and 3 had p.C441R mutations (Fig. 2A).<sup>26,27</sup> We also identified 7 patients with heterozygous *PINK1* mutations (2.2% of patients with early-onset parkinsonism), including 5 missense mutations (2 with p.M341I,<sup>28</sup> 1 with p.P209A, 1 with p.V350 L, 1 with p.L557P), and 2 heterozygous exon 8 deletions; see Figure 2B. The novel *PINK1* p.V350 L and p.L557P mutations were not found in the 1517-exome database from Taiwan Biobank and have not been described in public databases. In addition, 2 patients from consanguineous families had a common *PLA2G6* homozygous mutation, p.D331Y (0.6% of early-onset parkinsonism; Supplementary Fig. 2A), one of which was previously reported.<sup>29</sup> One patient had increased expansion of CTA/CTG trinucleotide repeats in *SCA8* (86 repeats). Several novel heterozygous mutations were identified, including 2 with *FBXO7* substitutions, p.I87T and p.D328R,<sup>30</sup> 1 with p.A551V in *SYNJ1*, and another is p.G394 V in the *DNAJC13* gene. An additional 2 patients had heterozygous *GBA* p.L444P mutations.<sup>31</sup>

Of 109 probands with AR inheritance of parkinsonism, *Parkin* was the most prevalent mutated gene. Of the 16 probands carrying *Parkin* mutations (14.7%), 11 had compound heterozygous or homozygous mutations, and 5 had single heterozygous mutations. All biallelic *Parkin* mutations were found to segregate with disease status within the affected families (Supplementary Fig. 1). Exon deletions were the most common mutation in the *Parkin* gene and were found in 11 probands. Three of the 4 p. G284R substitution carriers and all the p.C441R mutation carriers had a compound heterozygous mutation with *Parkin* exon deletions (Fig. 2A). We also identified 3 probands carrying heterozygous *PINK1* mutations, 1 with a p.N521H mutation, 1 with an exon 8 deletion, and another with a p.V350 L mutation. However, the affected family member associated with the proband carrying the heterozygous *PINK1* exon 8 deletion was deceased; therefore, we were unable to check the segregation of this substitution in this family. We also identified a proband having a heterozygous *HTRA2* p.P143A mutation.<sup>32</sup> Nine probands (8.3%) had heterozygous *GBA* p.L444P mutations.<sup>31</sup>

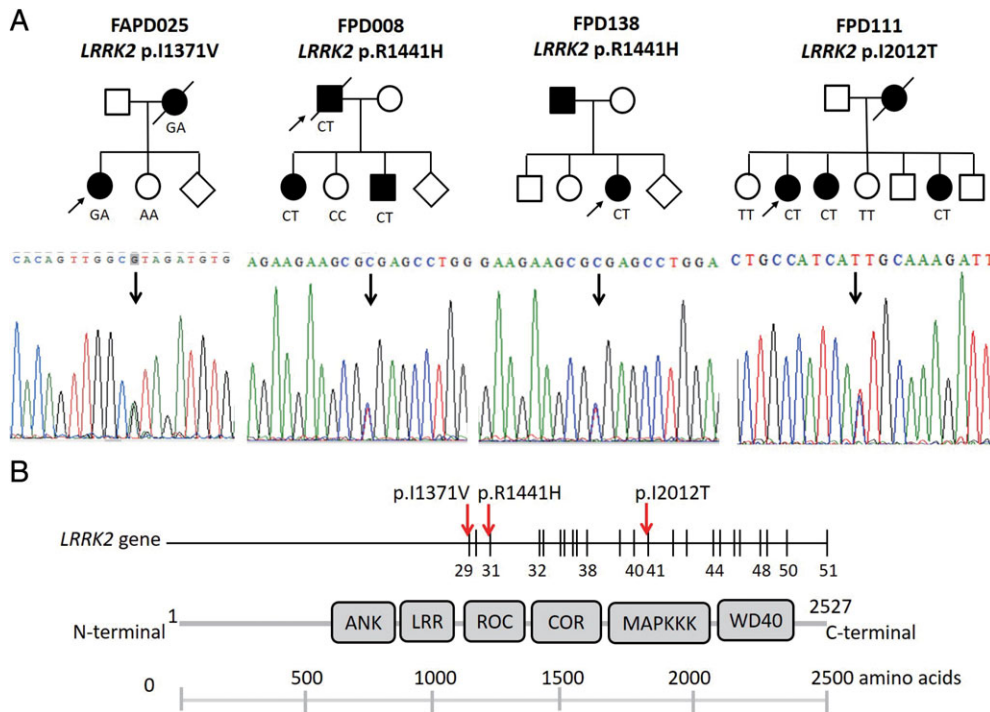
Seventeen of the 138 probands with an AD inheritance pattern of parkinsonism (12.3%) were found to carry pathogenic mutations in known parkinsonism-causative genes. We did not find a *LRRK2* p.G2019S mutation, which is the most prevalent mutation in Western populations, but we identified 4 probands with other *LRRK2* mutations (2.9%): 1 had a p.I1371V mutation,<sup>33,34</sup> 2 had p.R1441H mutations,<sup>35,36</sup> and



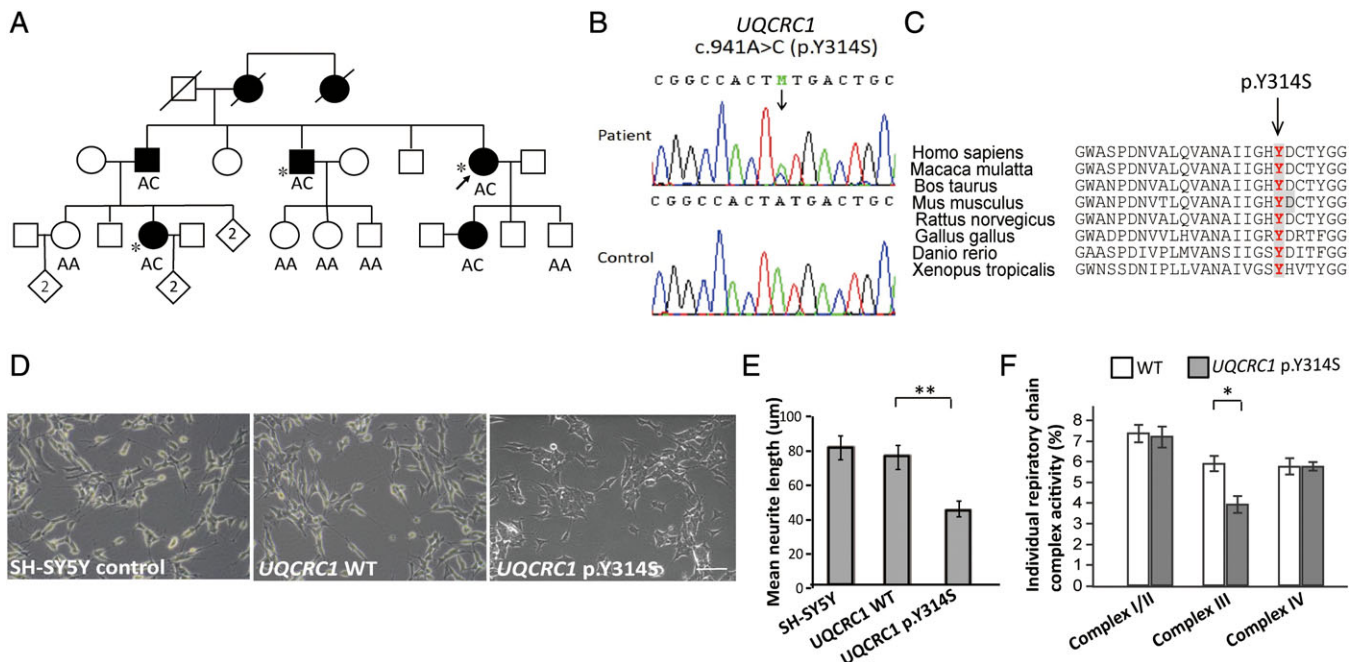
**FIG. 2.** Schematic depiction of (A) *Parkin* and (B) *PINK1* structures and associated disease-linked mutations, including missense mutations and exonic deletions. [Color figure can be viewed at wileyonlinelibrary.com]

another had a p.I2012T mutation (Fig. 3A).<sup>21</sup> These variants are all known pathogenic mutations and segregated with the parkinsonism phenotype within the families. On the LRRK2 protein, the p.I1371V and p.R1441H substitutions are both on the Ras of complex protein (ROC) domain, and p.I2012T is on the kinase domain of the LRRK2 protein (Fig. 3B). We also identified 2 probands (1.4%) carrying *VPS35* mutations, 1 with the previously reported p.D620N<sup>37,38</sup> and another with the p.S679P mutation (Supplementary Fig. 2C). The *VPS35* p.D620N variant segregated with the parkinsonism phenotype within the family. However, the affected mother of the proband carrying the heterozygous *VPS35* p.S679P was deceased; therefore, we were unable to check the segregation of this substitution in this family. The potentially novel *VPS35* p.S679P mutation was not found in the Taiwan Biobank database. Although there are 2 heterozygotes of East Asian descent described in the Genome Aggregation Database (gnomAD; <http://gnomad.broadinstitute.org/gnomAD>), we speculate this rare variant may cause the disease within the family. Two probands had previously reported *MAPT* mutations: 1 carried p.R5H,<sup>39</sup> and another one had a p.R5C mutation.<sup>37,39</sup> Two novel

heterozygous mutations were identified: 1 was p.R1382H in the *DNAJC13* gene, and the other was p.R457Q in the *GIGYF2* gene. The *DNAJC13* p.R1382H was also identified in the affected father of the proband but was absent in the nonaffected sibling. The affected family member of the proband having *GIGYF2* p.R457Q was deceased; therefore, we were unable to check the segregation within the family. Given that the finding of *GIGYF2* variants causing PD has not been replicated yet, the pathogenicity of *GIGYF2* p.R457Q needs further studies to be confirmed. Another 2 patients (1.4%) had heterozygous *GBA* p.L444P mutations.<sup>31</sup> Four probands (2.9% of families) had increased numbers of trinucleotide repeats in genes associated with spinocerebellar ataxia (SCA). Three probands had *SCA3* mutations (64, 64, and 68 repeats), and 1 had an increased repeat number in *SCA17* (46 repeats). Finally, 1 proband had an increased hexanucleotide repeat expansion in the *C9orf72* gene (52 repeats; Supplementary Fig. 3A,B). The abnormal repeat expansions of *C9orf72* cosegregated with parkinsonism or other neurodegenerative phenotypes, such as frontotemporal dementia and motor neuron disorder, within the family (Supplementary Fig. 3A).



**FIG. 3.** *LRRK2* mutations identified in the current study. (A) Pedigrees of the 4 probands carrying *LRRK2* mutations. Affected family members are represented with black circles (female) or squares (male). Arrows indicate the proband. Sanger chromatogram sequences are shown in the lower panel of each family pedigree. (B) Schematic depiction of *LRRK2* structure and associated disease-linked missense mutation. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**FIG. 4.** *UQCRC1* mutation in a family with multiplex PD and an autosomal-dominant inheritance pattern. (A) Pedigree of the family with the *UQCRC1* c.941A > C (p.Y314S) mutation. Open symbol, unaffected; filled symbol, affected; symbol with a diagonal line, deceased; diamond, total number of children, unknown sex; arrow, proband. \*Patients whose whole exomes were sequenced. (B) Sanger sequencing traces confirming the c.941A > C (p.Y314S) variant (RefSeq NM\_003365.2). (C) Alignment of multiple *UQCRC1* orthologs shows conservation of the p.Tyr314 residue across species. (D) Light microscopic images show SH-SY5Y neurite morphology (Nikon Eclipse, 80 $\times$  10 $\times$  magnification) with different genotypes. (E) Quantitative analyses of mean total neurite lengths for the neurons with individual genotypes; n = 25 for each genotype. Data represent the mean  $\pm$  SEM. \*\**P* < 0.01. (F) Percentages of oxygen consumption rate attributable to the activities of complexes I-IV. In each experiment, data were collected and averaged from 4 separate wells for each individual cell line. Each cell line was assayed in at least 3 independent experiments, and means were calculated. Error bars are standard errors of the mean. *P* = 0.01, 1-way ANOVA for *UQCRC1* WT versus *UQCRC1* p.Y314S for complex III activity. \**P* < 0.05. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

A novel gene, mitochondrial ubiquinol-cytochrome c reductase core protein I (*UQCRC1*, MIM 191328), was identified by WES in a family with autosomal-dominant late-onset parkinsonism and polyneuropathy. WES performed on 3 affected members of the family (Fig. 4A) with a coverage of 100× read depth showed 217 nonsynonymous variants with minor allele frequency  $\leq 0.0001$  in the gnomAD. Further comparative analyses identified 3 rare heterozygous variants shared in all exomes that resulted in coding substitutions: *UQCRC1* (c.941A > C, p.Y314S), *CLCN6* (c.1663G > A, p.D555N), and *PTPRG* (c.3141C > T, p.P808S). All variants that differed from the consensus sequence were annotated using the ANNOVAR package. The frequency of the variants in the population were also annotated using the gnomAD exome data set. All 3 variants were predicted to be potentially pathogenic based on PolyPhen-2 and SIFT programs. Only the *UQCRC1* p.Y314S cosegregated with disease within the family and was not present in the Taiwan Biobank database ( $n = 1517$  exomes) or 1000 control subjects (Fig. 4A, B). In addition, the gnomAD ( $n = 123,136$  exome and 15,496 whole-genome sequences) showed *UQCRC1* p.Y314S is a novel variant, although *CLCN6* p.D555N and *PTPRG* p.P808S were found at appreciable allele frequencies,  $2.4 \times 10^{-5}$  and  $4.1 \times 10^{-5}$ , respectively. Therefore, the *UQCRC1* p.Y314S was selected as the candidate in this index family. The *UQCRC1* p.Y314S substitution was conserved across species (Fig. 4C). *UQCRC1* is a subunit of mitochondrial respiratory chain complex III<sup>40</sup> and is highly expressed in brain tissue (<https://www.proteinatlas.org/ENSG00000010256-UQCRC1/tissue>). We therefore selected it as the disease candidate gene. To characterize the impact of the *UQCRC1* p.Y314S substitution on human dopaminergic neurons, we established mutant *UQCRC1* knock-in (KI) SH-SY5Y cell lines with CRISPR-Cas9 technology. Neurite morphology analysis showed that cells that expressed the *UQCRC1* p.Y314S substitution exhibited shortened neurites compared with wild-type (WT) neurons (SH-SY5Y control,  $80.1 \pm 4.9 \mu\text{m}$ ; *UQCRC1* WT,  $79.2 \pm 5.4 \mu\text{m}$ ; *UQCRC1* p.Y314S,  $45.81 \pm 4.3 \mu\text{m}$ ; Fig. 4D; statistics are shown in Fig. 4E; 1-way analysis of variance [ANOVA],  $P = 0.008$  for *UQCRC1* WT vs *UQCRC1* p.Y314S; the number of cells assessed in each genotype was 56, 51, and 48 individually, respectively). These results demonstrated that *UQCRC1* proteins with the p.Y314S variation impaired neurite morphogenesis and may cause neurite degeneration. As *UQCRC1* is a subunit of mitochondrial respiratory complex III protein, we further measured the individual respiratory chain activity in wild-type neurons and *UQCRC1* p.Y314S KI neurons. Treatment with individual mitochondrial respiratory complex inhibitors demonstrated that *UQCRC1* p.Y314S

expression did not influence on mitochondrial complex I, II, or IV activity, but significantly reduced complex III activity (WT vs *UQCRC1* p.Y314S,  $5.87\% \pm 0.23\%$  vs  $3.92\% \pm 0.41\%$ ; 1-way ANOVA,  $P = 0.01$ ; Fig. 4F). This suggests that *UQCRC1* p.Y314S disturbs mitochondrial respiratory chain complex III activity in neurons. Further studies including more families and in vivo functional assays are needed to support the pathogenicity of *UQCRC1* in PD.

### Clinical Phenotypes

A detailed description of all observed major phenotypic features of patients with mutations is summarized in Table 1.

#### Patients Presenting With Levodopa-Responsive Parkinsonism Without Atypical Features

All patients with mutations in *PINK1*, *FBXO7*, *SYNJ1*, *DNAJC13*, and *GIGYF2* presented with early-onset levodopa-responsive parkinsonism. No one reported foot dystonia as an early manifestation. The with-SCA8 mutation presented with left leg slowness and rigidity at age 38 years. He had good responses to levodopa but complicated by peak-dose dyskinesia after 4 years of treatment. The clinical phenotypes among the 4 families with *LRRK2* mutations were heterogeneous. The mean age of onset for levodopa-responsive parkinsonism patients with *LRRK2* mutations was  $58.8 \pm 2.9$  years.

#### Patients Presenting With Levodopa-Responsive Parkinsonism With Dystonia

Foot dystonia is a common and sometimes preceding symptom among patients carrying homozygous or compound heterozygous *Parkin* mutations (40%; Table 1). The mean age of onset for patients with homozygous or compound heterozygous *Parkin* mutations was  $28.6 \pm 10.5$  years.

#### Patients Presenting With Levodopa-Responsive Parkinsonism and Psychiatric Features

Anxiety and depression are common symptoms for patients with mutations in *Parkin* and *PLA2G6* genes (Table 1).<sup>41</sup> Brain MRI scans of patients having homozygous *PLA2G6* p.D331Y mutations did not reveal abnormal iron depositions in the basal ganglia (Supplementary Fig. 2B). Of *LRRK2* mutation carriers, 1 proband with an *LRRK2* p.R1441H mutation had prominent psychotic symptoms 6 years after the onset of motor symptoms under the levodopa dosage of 600 mg/day.



**TABLE 1. Clinical characteristics of patients with known PD mutations**

	Parkin	PINK1	PLA2G6	LRRK2	VPS35	MAPT	GBA	C9orf72	SCA
	Homozygous/ Compound Heterozygous (n = 15)	Heterozygous (n = 10)	Homozygous (n = 2)	Heterozygous (n = 9 From 4 Families)	Heterozygous (n = 3 From 2 Families)	Heterozygous (n = 4 From 2 Families)	Heterozygous (n = 13)	Heterozygous (n = 2 From 1 Family)	(n = 5)
AAO (mean ± SD)	28.6 ± 10.5	37.7 ± 5.9	28.0 ± 2.8	58.8 ± 2.9	44.5 ± 3.5	52.5 ± 10.6	52.3 ± 12.9	56.5 ± 2.1	41.8 ± 4.2
Male	53.3%	70.0%	0%	22.2%	33.3%	50%	38.5%	100%	80%
Initial symptoms									
Tremor	46.3 %	60.0%	0%	33.3%	33.3%	25.0%	53.8%	0%	0%
Disease progression	Slow	Slow	Fast	Variable	Slow	Variable	Variable	Fast	Fast
Associate symptoms	Foot dystonia Anxiety Depression	NA	Anxiety Depression Psychosis	FTD Anxiety Psychosis	NA	FTD Depression	Dementia	Dementia MND	Ataxia Dementia Polyneuropathy

AAO, age at onset of symptoms; SD, standard deviation; NA, not applicable; FTD, frontotemporal dementia; MND, motor neuron disorder. The fast disease progression is defined as a mean annual rate of progression of more than 5 points on the motor subscore of the Unified Parkinson's Disease Rating Scale (UPDRS part III).

**Patients Presenting With Levodopa-Unresponsive Parkinsonism and FTD**

Late-onset rapid progressive parkinsonism with poor levodopa responses was observed in the proband with an *LRRK2* p.1371 V mutation, 2 probands with *MAPT* mutations, and 1 proband with a hexanucleotide repeat expansion in *C9orf72*.

**Patients Presenting With Motor Neuron Disorders and Parkinsonism With Poor Levodopa Responses**

The proband with a hexanucleotide repeat expansion in *C9orf72* had progressive leg weakness with fasciculations at the age of 58, and amyotrophic lateral sclerosis was diagnosed. The patient then developed a left-sided parkinsonism feature and cognition impairment with a neuropsychological test demonstrating behavior variant of frontotemporal dementia (FTD). Brain MRI showed asymmetrical atrophy over the right frontotemporal lobe, and Tc-99 m TRODAT images revealed decreased uptake over the right putamen (Supplementary Fig. 3C). His brother also had a *C9orf72* mutation and developed FTD at age 55 years, followed by rapidly progressive parkinsonism, and was bedridden within 5 years.

**Patients Presenting With Parkinsonism and Cerebellar Ataxia**

Mild ataxic gait with asymmetrical onset of akinetic-rigidity was observed in 3 patients with increased numbers of trinucleotide repeats in *SCA3* and in 1 patient with a mutation in *SCA17*.<sup>42</sup> The mean age of onset was 42.3 ± 4.0 years.

**An Autosomal-Dominant-Inheritance PD Family With a Novel UQCRC1 Mutation**

The novel *UQCRC1* mutation p.Y314S was detected in 5 affected members of the index family (Fig. 4A). All affected *UQCRC1* heterozygotic carriers presented with asymmetrical onset tremor-predominant levodopa-responsive parkinsonism, and the mean age of onset was 54.2 ± 8.7 years. In addition to parkinsonian features, *UQCRC1* p.Y314S heterozygotes also had intrinsic muscle atrophy in the hands and toes, and nerve conduction studies revealed axonal type sensorimotor polyneuropathy.

**Discussion**

We identified the genetic causes for early-onset and familial parkinsonism in a large Taiwanese cohort using an integrated genetic approach and established the relationships between genotypes and clinical phenotypes. Our results showed that 9.3% of patients with early-onset parkinsonism have mutations in *Parkin* and *PINK1*, along with fewer than 1% of patients with

mutations in *PLA2G6*, *FBXO7*, *SYNJ1*, *SCA8*, and *DNAJC13* genes. Of the AR inheritance families, 26.6% had mutations in *Parkin*, *PINK1*, *GBA*, or *HTRA2*. Of the AD inheritance families, 12.3% had mutations in *LRRK2*, *VPS35*, *MAPT*, *GBA*, *DNAJC13*, *C9orf72*, *SCA3*, or *SCA17*. A potential novel mutation in the *UQCRC1* gene was identified in a family with AD inheritance of parkinsonism. Our study represents the most extensive survey of the genetic etiology of early-onset parkinsonism and within families with parkinsonism in eastern Asia to date and indicates a different mutational spectrum for PD than that seen in white populations.

*Parkin* was the most commonly mutated gene in patients with early-onset parkinsonism or probands with AR inheritance of parkinsonism. Exon deletions were the most prevalent mutation types. Concordantly, most of the deleted exons fell into the genomic region between exons 2 and 5, further confirming this region as a mutational hot spot across populations.<sup>43</sup> Although biallelic *Parkin* mutations only account for 1.3% of patients with early-onset parkinsonism with an onset age younger than 50 and the mutation frequency increases to 4.3% by including heterozygous variants, the biallelic *Parkin* mutations account for 10.1% of patients with an onset age younger than 40 and 44.4% of patients with an onset before the age of 20 in our population. Familial cases with an AR inheritance pattern were more likely to have biallelic mutations in *Parkin* than patients with sporadic early-onset parkinsonism, with a proportion of 10.1% versus 1.3% in our cohort. The *Parkin* mutation rate seen in our early-onset parkinsonism cases was comparable with that seen in Norwegian patients, with 2.8% of patients with early-onset PD (age at onset, <45 years) have biallelic *Parkin* mutations, and by including heterozygous variants, the mutation frequency increases to 4.6%<sup>44</sup> in Korean patients with a mutation frequency of 5% and in Japanese patients with a frequency of 11%.<sup>45</sup> However, our *Parkin* mutation rate is lower than that in some Western populations. A study from a European consortium showed that *Parkin* mutations account for 18% of early-onset parkinsonism with onset before age 45 and 50% of families with AR inheritance of parkinsonism.<sup>46</sup> The reasons that contribute to the differences in mutation frequency may come from fewer consanguineous marriages in our society because of legal regulations or ethnic differences.

*PINK1* is the second most common gene causing early-onset parkinsonism.<sup>47</sup> Familial cases with an AR inheritance pattern were more likely to have *PINK1* mutations than patients with sporadic early-onset parkinsonism, with a proportion of 2.8% versus 2.2% in our cohort. Our findings are consistent with a previous study performed in a Taiwanese population reporting that the frequency of *PINK1* mutations was 2% in patients with early-onset parkinsonism<sup>48</sup> and with the results from a Japanese study with a mutation

frequency of 1.05% in patients with early-onset parkinsonism.<sup>49</sup> However, the identified mutations were in a heterozygous state in our population. Although evidence has suggested that heterozygous mutations in recessive genes such as *PINK1* are indeed susceptibility factors for disease development,<sup>50,51</sup> we suggest that additional modifier genes in combination with environmental exposures may contribute to disease susceptibility in our population. We also identified a homozygous *PLA2G6* p.D331Y mutation in 2 patients with early-onset parkinsonism and parental consanguinity. This mutation was previously reported in a Chinese family with AR inheritance of early-onset parkinsonism.<sup>29</sup> Our findings suggest that *PLA2G6* mutations should be considered for patients with early-onset parkinsonism in our ethnicity.

The genetic causes of AD-inheritance parkinsonism pedigrees are heterogeneous and include *LRRK2*, *VPS35*, *MAPT*, *GBA*, *DNAJC13*, *C9orf72*, *SCA3*, *SCA8*, and *SCA17*, and the phenotypic spectrums were also variable, ranging from levodopa-responsive parkinsonism to rapid-progressive parkinsonism mixed with other neurodegenerative symptoms, including FTD, motor neuron disorders, and ataxia. Our findings support previous observations that patients with increased trinucleotide repeats in *SCA* genes, especially *SCA* 2, 3, 8, and 17, may present with parkinsonism, especially in Asia.<sup>52-54</sup> We suggest that screening of abnormal trinucleotide expansions in *SCA*-related genes should be considered in patients with parkinsonism in our population.

Our results also identified 4 families with *LRRK2* mutations. Although the most common *LRRK2* mutation in Western populations is p.G2019S on the *LRRK2* kinase domain, we identified p.I1371V and p.R144H mutations, which are on the ROC domain of *LRRK2*,<sup>55</sup> and p.I2012T mutations, which are on the kinase domain of *LRRK2*, in our families with PD.<sup>56-58</sup> Our results reinforce the observations that *LRRK2* mutations have ethnic differences. Current *LRRK2* GTP-binding inhibitors such as FX2149<sup>59</sup> and kinase inhibitors such as *LRRK2-IN-1*<sup>60</sup> have been studied in cellular or animal models carrying common mutations in Western populations and may also have therapeutic potential for Asian *LRRK2* mutation carriers. We also identified a family presenting with left hemiparkinsonism mixed with motor neuron disorder and FTD, with an increased number of hexanucleotide repeats in the *C9orf72* gene. Although mutations in *C9orf72* are rarely reported in Asian patients,<sup>18,61,62</sup> *C9orf72* repeat expansions should be considered for patients with parkinsonism that have overt symptoms of FTD or motor neuron disorders in our population. Finally, we identified a mutation in the potential novel parkinsonism-causing gene *UQCRC1*, which encodes a mitochondria respiratory complex III protein, in a family with AD-inheritance parkinsonism. Further studies including



more families and functional assays are needed to support the pathogenicity of *UQCRC1* in PD.

Although we established a comprehensive genetic screening in patients with early-onset or familial parkinsonism, there are some limitations in the current study. First, MLPA may occasionally fail to detect variations in the gene dosage, and MLPA is also unable to detect balanced rearrangements. Second, we identified a potential novel mutation, *UQCRC1* p.Y314S, which may contribute to familial parkinsonism in our ethnicity. However, because of the rarity of the affected family, future functional analyses using animal models and enrollment of more families having *UQCRC1* mutations are needed to prove the pathogenicity of *UQCRC1* mutations in the disease pathogenesis. In conclusion, this study presents a systemic genetic analysis of a large Taiwanese cohort of patients to elucidate the genetic architecture of early-onset and familial parkinsonism in our population. Our results have the potential to facilitate accurate molecular diagnosis and future tailored treatments for susceptible individuals carrying genetic mutations. ■

**Acknowledgments:** The authors are grateful to the patients who participated in this study. We are very grateful to Dr. Matthew J. Farrer and Dr. Zbigniew K. Wszolek, who helped us to initiate the genetic study in young-onset and familial parkinsonism in the Taiwanese population. We also thank the staff of the Second Core Lab, Department of Medical Research, National Taiwan University Hospital, for technical support during the study.

## References

- Kalia LV, Lang AE. Parkinson's disease. *Lancet* 2015;386:896-912.
- Hardy J, Lewis P, Revesz T, Lees A, Paisan-Ruiz C. The genetics of Parkinson's syndromes: a critical review. *Curr Opin Genet Dev* 2009;19:254-265.
- Singleton A, Hardy J. The evolution of genetics: Alzheimer's and Parkinson's diseases. *Neuron* 2016;90:1154-1163.
- Lang AE, Espay AJ. Disease Modification in Parkinson's Disease: Current Approaches, Challenges, and Future Considerations. *Mov Disord* 2018;33:660-677.
- Hardy J, Cai H, Cookson MR, Gwinn-Hardy K, Singleton A. Genetics of Parkinson's disease and parkinsonism. *Ann Neurol* 2006;60:389-398.
- Di Fonzo A, Rohe CF, Ferreira J, et al. A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease. *Lancet* 2005;365:412-415.
- Kachergus J, Mata IF, Hulihan M, et al. Identification of a novel LRRK2 mutation linked to autosomal dominant parkinsonism: evidence of a common founder across European populations. *Am J Hum Genet* 2005;76:672-680.
- Lesage S, Ibanez P, Lohmann E, et al. G2019S LRRK2 mutation in French and North African families with Parkinson's disease. *Ann Neurol* 2005;58:784-787.
- Ozelius LJ, Senthil G, Saunders-Pullman R, et al. LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. *N Engl J Med* 2006;354:424-425.
- Tan EK, Shen H, Tan LC, et al. The G2019S LRRK2 mutation is uncommon in an Asian cohort of Parkinson's disease patients. *Neurosci Lett* 2005;384:327-329.
- Alessi DR, Sammler E. LRRK2 kinase in Parkinson's disease. *Science*. 2018;360:36-37.
- Wu RM, Bounds R, Lincoln S, et al. Parkin mutations and early-onset parkinsonism in a Taiwanese cohort. *Arch Neurol* 2005;62:82-87.
- Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry* 1992;55:181-184.
- Goetz CG, Tilley BC, Shaftman SR, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov Disord* 2008;23:2129-2170.
- Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. *Neurology* 1967;17:427-442.
- Folstein MF, Folstein SE, McHugh PR. "Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;12:189-198.
- Yu RL, Wu RM, Tai CH, Lin CH, Cheng TW, Hua MS. Neuropsychological profile in patients with early stage of Parkinson's disease in Taiwan. *Parkinsonism Relat Disord* 2012;18:1067-1072.
- Lin CH, Chen TF, Chiu MJ, Lin HI, Wu RM. Lack of c9orf72 repeat expansion in Taiwanese patients with mixed neurodegenerative disorders. *Front Neurol* 2014;5:59.
- Orr HT, Chung MY, Banfi S, et al. Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. *Nat Genet* 1993;4:221-226.
- Lin CH, Hwu WL, Chiang SC, Tai CH, Wu RM. Lack of mutations in spinocerebellar ataxia type 2 and 3 genes in a Taiwanese (ethnic Chinese) cohort of familial and early-onset parkinsonism. *Am J Med Genet B Neuropsychiatr Genet* 2007;144B:434-438.
- Fan TS, Wu RM, Chen PL, et al. Clinical heterogeneity of LRRK2 p.I2012T mutation. *Parkinsonism Relat Disord* 2016;33:36-43.
- Bentley SR, Bortnick S, Guella I, et al. Pipeline to gene discovery - Analysing familial Parkinsonism in the Queensland Parkinson's Project. *Parkinsonism Relat Disord* 2018;49:34-41.
- Ran FA, Hsu PD, Wright J, et al. Genome engineering using the CRISPR-Cas9 system. *Nat Protoc* 2013;8:2281-2308.
- Lin CH, Lin HI, Chen ML, et al. Lovastatin protects neurite degeneration in LRRK2-G2019S parkinsonism through activating the Akt/Nrf pathway and inhibiting GSK3β activity. *Hum Mol Genet* 2016;25:1965-1978.
- Salabei JK, Gibb AA, Hill BG. Comprehensive measurement of respiratory activity in permeabilized cells using extracellular flux analysis. *Nat Protoc* 2014;9:421-438.
- Nuytemans K, Theuns J, Cruts M, Van Broeckhoven C. Genetic etiology of Parkinson disease associated with mutations in the SNCA, PARK2, PINK1, PARK7, and LRRK2 genes: a mutation update. *Hum Mutat* 2010;31:763-780.
- Funayama M, Li Y, Tsoi TH, et al. Familial Parkinsonism with digenic parkin and PINK1 mutations. *Mov Disord* 2008;23:1461-1465.
- Lee MJ, Mata IF, Lin CH, et al. Genotype-phenotype correlates in Taiwanese patients with early-onset recessive Parkinsonism. *Mov Disord* 2009;24:104-108.
- Shi CH, Tang BS, Wang L, et al. PLA2G6 gene mutation in autosomal recessive early-onset parkinsonism in a Chinese cohort. *Neurology* 2011;77:75-81.
- Lin CH, Chen ML, Lai TT, Tai CH, Wu RM. Mutational analysis of FBXO7 gene in Parkinson's disease in a Taiwanese population. *Neurobiol Aging* 2013;34:1713 e1711-1714.
- Aharon-Peretz J, Rosenbaum H, Gershoni-Baruch R. Mutations in the glucocerebrosidase gene and Parkinson's disease in Ashkenazi Jews. *N Engl J Med* 2004;351:1972-1977.
- Lin CH, Chen ML, Chen GS, Tai CH, Wu RM. Novel variant Pro143Ala in HTRA2 contributes to Parkinson's disease by inducing hyperphosphorylation of HTRA2 protein in mitochondria. *Hum Genet* 2011;130:817-827.
- Seki N, Takahashi Y, Tomiyama H, et al. Comprehensive mutational analysis of LRRK2 reveals variants supporting association with autosomal dominant Parkinson's disease. *J Hum Genet* 2011;56:671-675.

34. Jankovic MZ, Kresojevic ND, Dobricic VS, et al. Identification of novel variants in LRRK2 gene in patients with Parkinson's disease in Serbian population. *J Neurol Sci* 2015;353:59-62.
35. Ross OA, Soto-Ortolaza AI, Heckman MG, et al. Association of LRRK2 exonic variants with susceptibility to Parkinson's disease: a case-control study. *Lancet Neurol* 2011;10:898-908.
36. Lin CH, Tzen KY, Yu CY, Tai CH, Farrer MJ, Wu RM. LRRK2 mutation in familial Parkinson's disease in a Taiwanese population: clinical, PET, and functional studies. *J Biomed Sci* 2008;15:661-667.
37. Vilarino-Guell C, Wider C, Ross OA, et al. VPS35 mutations in Parkinson disease. *Am J Hum Genet* 2011;89:162-167.
38. Chen YF, Chang YY, Lan MY, Chen PL, Lin CH. Identification of VPS35 p.D620N mutation-related Parkinson's disease in a Taiwanese family with successful bilateral subthalamic nucleus deep brain stimulation: a case report and literature review. *BMC Neurol* 2017; 17:191.
39. Hayashi S, Toyoshima Y, Hasegawa M, et al. Late-onset frontotemporal dementia with a novel exon 1 (Arg5His) tau gene mutation. *Ann Neurol* 2002;51:525-530.
40. Hoffman GG, Lee S, Christiano AM, et al. Complete coding sequence, intron/exon organization, and chromosomal location of the gene for the core 1 protein of human ubiquinol-cytochrome c reductase. *J Biol Chem* 1993;268:21113-21119.
41. Lu CS, Lai SC, Wu RM, et al. PLA2G6 mutations in PARK14-linked young-onset parkinsonism and sporadic Parkinson's disease. *Am J Med Genet B Neuropsychiatr Genet* 2012;159B:183-191.
42. Lin IS, Wu RM, Lee-Chen GJ, Shan DE, Gwinn-Hardy K. The SCA17 phenotype can include features of MSA-C, PSP and cognitive impairment. *Parkinsonism Relat Disord* 2007;13:246-249.
43. Kilariski LL, Pearson JP, Newsday V, et al. Systematic review and UK-based study of PARK2 (parkin), PINK1, PARK7 (DJ-1) and LRRK2 in early-onset Parkinson's disease. *Mov Disord* 2012;27: 1522-1529.
44. Gustavsson EK, Trinh J, McKenzie M, et al. Genetic Identification in Early Onset Parkinsonism among Norwegian Patients. *Mov Disord Clinical Practice* 2017; doi:<https://doi.org/10.1002/mdc3.12501>.
45. Hattori N, Kitada T, Matsumine H, et al. Molecular genetic analysis of a novel Parkin gene in Japanese families with autosomal recessive juvenile parkinsonism: evidence for variable homozygous deletions in the Parkin gene in affected individuals. *Ann Neurol* 1998;44:935-941.
46. 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* 2000;342:1560-1567.
47. Bonifati V, Rohe CF, Breedveld GJ, et al. Early-onset parkinsonism associated with PINK1 mutations: frequency, genotypes, and phenotypes. *Neurology* 2005;65:87-95.
48. Weng YH, Chou YH, Wu WS, et al. PINK1 mutation in Taiwanese early-onset parkinsonism: clinical, genetic, and dopamine transporter studies. *J Neurol* 2007;254:1347-1355.
49. Kumazawa R, Tomiyama H, Li Y, et al. Mutation analysis of the PINK1 gene in 391 patients with Parkinson disease. *Arch Neurol* 2008;65:802-808.
50. Puschmann A, Fiesel FC, Caulfield TR, et al. Heterozygous PINK1 p.G411S increases risk of Parkinson's disease via a dominant-negative mechanism. *Brain* 2017;140:98-117.
51. Klein C, Westenberger A. Genetics of Parkinson's disease. *Cold Spring Harb Perspect Med* 2012;2:a008888.
52. Park H, Kim HJ, Jeon BS. Parkinsonism in spinocerebellar ataxia. *Biomed Res Int* 2015;2015:125273.
53. Wu YR, Lin HY, Chen CM, et al. Genetic testing in spinocerebellar ataxia in Taiwan: expansions of trinucleotide repeats in SCA8 and SCA17 are associated with typical Parkinson's disease. *Clin Genet* 2004;65:209-214.
54. Kim JS, Son TO, Youn J, Ki CS, Cho JW. Non-ataxic phenotypes of SCA8 mimicking amyotrophic lateral sclerosis and Parkinson disease. *J Clin Neurol* 2013;9:274-279.
55. West AB, Moore DJ, Choi C, et al. Parkinson's disease-associated mutations in LRRK2 link enhanced GTP-binding and kinase activities to neuronal toxicity. *Hum Mol Genet* 2007;16:223-232.
56. Hatano T, Funayama M, Kubo SI, et al. Identification of a Japanese family with LRRK2 p.R1441G-related Parkinson's disease. *Neurobiol Aging* 2014;35:2656.e17-2656.e23.
57. Wu YR, Chang KH, Chang WT, et al. Genetic variants of LRRK2 in Taiwanese Parkinson's disease. *PLoS One*. 2013;8:e82001.
58. Tomiyama H, Li Y, Funayama M, et al. Clinicogenetic study of mutations in LRRK2 exon 41 in Parkinson's disease patients from 18 countries. *Mov Disord* 2006;21:1102-1108.
59. Li T, He X, Thomas JM, et al. A novel GTP-binding inhibitor, FX2149, attenuates LRRK2 toxicity in Parkinson's disease models. *PLoS One* 2015;10:e0122461.
60. Deng X, Dzamko N, Prescott A, et al. Characterization of a selective inhibitor of the Parkinson's disease kinase LRRK2. *Nat Chem Biol* 2011;7:203-205.
61. Chen X, Chen Y, Wei Q, et al. C9orf72 repeat expansions in Chinese patients with Parkinson's disease and multiple system atrophy. *J Neural Transm (Vienna)* 2016;123:1341-1345.
62. Jiao B, Guo JF, Wang YQ, et al. C9orf72 mutation is rare in Alzheimer's disease, Parkinson's disease, and essential tremor in China. *Front Cell Neurosci* 2013;7:164.

## Supporting Data

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