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 UQRC1 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.

Key Words: early onset; genetics; next-generation sequencing; Parkinson’s disease; parkinsonism
number of genes implicated in both familial and sporadic PD have been identified. Identification of these PD-causative genes provides biological insights into underlying disease processes. Neuromodification therapy by targeting these pathways provides an avenue for future drug discovery to mitigate disease progression.

Evidence has shown that genetic causes can vary depending on the geographic and ethnic backgrounds of the studied populations. For example, the major genetic cause for AD inheritance of parkinsonism is a mutation in leucine-rich repeat kinase 2 (LRRK2) with the LRRK2 p.G2019S mutation having the highest frequency among North African Arab-Berbers and Ashkenazi Jews. However, the LRRK2 p.G2019S mutation is rare in Asian patients. 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. 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. 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, 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 and Hoehn-and-Yahr staging. Cognition was evaluated with the Mini-Mental State Examination, and some patients received complete neuropsychological tests. 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. 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. 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). Patents 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 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.\textsuperscript{19,20}

**Targeted NGS Panel**

A custom-designed NGS panel, including 40 genes associated with parkinsonism (Supplementary Table 1), was performed as previously described.\textsuperscript{21} 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.\textsuperscript{22} We measured mitochondrial respiratory chain activity in wild-type and mutant UQCRCL1 knock-in (KI) SH-SY5Y cells with a Seahorse XFe24 extracellular flux analyzer (Seahorse Bioscience, North Billerica, MA), as previously described.\textsuperscript{23}

**Generation and Quantitative Analysis of Neurites of UQRC1 Knock-In SH-SY5Y Cells**

We generated mutant UQRC1 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.\textsuperscript{23} 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.\textsuperscript{24}

**Measurement of Mitochondrial Respiratory Chain Activity**

We measured mitochondrial respiratory chain activity in wild-type and mutant UQRC1 SH-SY5Y cells with a Seahorse XFe24 extracellular flux analyzer (Seahorse Bioscience, North Billerica, MA), as previously described.\textsuperscript{25}

**Results**

**Genetic Analyses**

The mean age at onset of patients with early-onset parkinsonism was 41.6 ± 6.4 years, and 50.1% were men, whereas the mean age at onset of probands with familial parkinsonism was 54.4 ± 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).\textsuperscript{26,27} We also identified 7 patients with heterozygous *PINK1* mutations (2.2% of patients with early-onset parkinsonism), including 5 missense mutations (2 with p.M341L,\textsuperscript{28} 1 with p.P209A, 1 with p.V350L, 1 with p.L557P), and 2 heterozygous exon 8 deletions; see Figure 2B. The novel *PINK1* p.V350L 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.\textsuperscript{29} 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,\textsuperscript{30} 1 with p.A551V in *SYNJ1* and another is p.G394V in the DNAJC13 gene. An additional 2 patients had heterozygous *GBA* p.L444P mutations.\textsuperscript{31}

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.V350L 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.I1433A mutation.\textsuperscript{32} Nine probands (8.3%) had heterozygous *GBA* p.L444P mutations.\textsuperscript{31}

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,\textsuperscript{33,34} 2 had p.K1441H mutations,\textsuperscript{35,36} and
another had a p.I2012T mutation (Fig. 3A). 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 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 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, and another one had a p.R5C mutation. 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. 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]

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, 80i; 10× 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 ± 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]
A novel gene, mitochondrial ubiquinol-cytochrome c reductase core protein I (UQCRCI, 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 100x read depth showed 217 nonsynonymous variants with minor allele frequency ≤ 0.0001 in the gnomAD. Further comparative analyses identified 3 rare heterozygous variants shared in all exomes that resulted in coding substitutions: UQCRCI (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 UQCRCI 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 UQCRCI p.Y314S is a novel variant, although CLCN6 p. D555N and PTPRG p.P808S were found at appreciable allele frequencies, 2.4 x 10^{-5} and 4.1 x 10^{-5}, respectively. Therefore, the UQCRCI p.Y314S was selected as the candidate in this index family. The UQCRCI p.Y314S substitution was conserved across species (Fig. 4C). UQCRCI is a subunit of mitochondrial respiratory chain complex III protein, we further characterized the impact of the UQCRCI p.Y314S substitution on human dopaminergic neurons, we measured the individual respiratory chain activity in wild-type neurons and UQCRCI p.Y314S KI neurons. Treatment with individual mitochondrial respiratory complex inhibitors demonstrated that UQCRCI p.Y314S expression did not influence on mitochondrial complex I, II, or IV activity, but significantly reduced complex III activity (WT vs UQCRCI p.Y314S, 5.87% ± 0.23% vs 3.92% ± 0.41%; 1-way ANOVA, P = 0.01; Fig. 4F). This suggests that UQCRCI 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 UQCRCI 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, FBX07, SYNJ1, DAAJ13, 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 ± 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 ± 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). Brain MRI scans of patients having homzygous 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.
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.1371V 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. The mean age of onset was 42.3 ± 4.0 years.

An Autosomal-Dominant-Inheritance PD Family With a Novel UQCRCl Mutation

The novel UQCRCl mutation p.Y314S was detected in 5 affected members of the index family (Fig. 4A). All affected UQCRCl 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, UQCRCl 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 UQCRCSI 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. 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% in Korean patients with a mutation frequency of 5% and in Japanese patients with a frequency of 11%. 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. 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. 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 and with the results from a Japanese study with a mutation frequency of 1.05% in patients with early-onset parkinsonism. 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, 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. 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, 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. 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, and p.I2012T mutations, which are on the kinase domain of LRRK2, in our families with PD. Our results reinforce the observations that LRRK2 mutations have ethnic differences. Current LRRK2 GTP-binding inhibitors such as FX2149 and kinase inhibitors such as LRRK2-IN-1 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, 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 UQCRCI, 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 UQCRCSI 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, UQCRCSI 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 UQCRCSI mutations are needed to prove the pathogenicity of UQCRCSI 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.

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