doi:10.1093/brain/awaa167 BRAIN 2020: 143; 2220–2234 | 2220

BRAIN
A JOURNAL OF NEUROLOGY

The role of genetics in Parkinson's disease: a large cohort study in Chinese mainland population

Yuwen Zhao,[1,2,*] Lixia Qin,[1,2,*] Hongxu Pan,[1] Zhenhua Liu,[1,2] Li Jiang,[1] Yan He,[1] Qian Zeng,[1] Xun Zhou,[1] Xiaoxia Zhou,[1] Yangjie Zhou,[1] Zhenghuan Fang,[3] Zheng Wang,[4] Yaqin Xiang,[1] Honglan Yang,[1] Yige Wang,[1] Kailin Zhang,[1] Rui Zhang,[1] Runcheng He,[1] Xiaoting Zhou,[1] Zhou Zhou,[1] Nannan Yang,[1] Dongxiao Liang,[1] Juan Chen,[1] Xuxiang Zhang,[1] Yao Zhou,[1] Hongli Liu,[1] Penghui Deng,[1] Kun Xu,[1] Ke Xu,[1] Chaojun Zhou,[1] Junfei Zhong,[1] Qian Xu,[1] Qiying Sun,[4] Bin Li,[2] Guihu Zhao,[2] Tao Wang,[5] Ling Chen,[6] Huifang Shang,[7] Weiguo Liu,[8] Piu Chan,[9,10] Zheng Xue,[11] Qing Wang,[12] Li Guo,[13] Xuejing Wang,[14] Changshui Xu,[15] Zhentao Zhang,[16] Tao Chen,[17] Lifang Lei,[18] Hainan Zhang,[19] Chunyu Wang,[19] Jieqiong Tan,[3] Xinxiang Yan,[2] Lu Shen,[1,2] Hong Jiang,[1,2] Zhuohua Zhang,[3] Zhengmao Hu,[3] Kun Xia,[3] Zhenyu Yue,[20] Jinchen Li,[1,2,3,4] Jifeng Guo[1,2,3] and Beisha Tang,[1,2,3,4]

*These authors contributed equally to this work.

See Morris (doi:10.1093/brain/awaa185) for a scientific commentary on this article.

This study aimed to determine the mutational spectrum of familial Parkinson's disease and sporadic early-onset Parkinson's disease (sEOPD) in a mainland Chinese population and the clinical features of mutation carriers. We performed multiplex ligation-dependent probe amplification assays and whole-exome sequencing for 1676 unrelated patients with Parkinson's disease in a mainland Chinese population, including 192 probands from families with autosomal-recessive Parkinson's disease, 242 probands from families with autosomal-dominant Parkinson's disease, and 1242 sEOPD patients (age at onset $\leqslant$ 50). According to standards and guidelines from the American College of Medical Genetics and Genomics, pathogenic/likely pathogenic variants in 23 known Parkinson's disease-associated genes occurred more frequently in the autosomal-recessive Parkinson's disease cohort (65 of 192, 33.85%) than in the autosomal-dominant Parkinson's disease cohort (10 of 242, 4.13%) and the sEOPD cohort (57 of 1242, 4.59%), which leads to an overall molecular diagnostic yield of 7.88% (132 of 1676). We found that *PRKN* was the most frequently mutated gene ($n = 83$, 4.95%) and present the first evidence of an *SNCA* duplication and *LRRK2* p.N1437D variant in mainland China. In addition, several novel pathogenic/likely pathogenic variants including *LRRK2* (p.V1447M and p.Y1645S), *ATP13A2* (p.R735X and p.A819D), *FBXO7* (p.G67E), *LRP10* (c.322dupC/p.G109Rfs*51) and *TMEM230* (c.429delT/p.P144Qfs*2) were identified in our cohort. Furthermore, the age at onset of the 132 probands with genetic diagnoses (median, 31.5 years) was about 14.5 years earlier than that of patients without molecular diagnoses (i.e. non-carriers, median 46.0 years). Specifically, the age at onset of Parkinson's disease patients with pathogenic/likely pathogenic variants in *ATP13A2*, *PLA2G6*, *PRKN*, or *PINK1* was significantly lower than that of non-carriers, while the age at onset of carriers with other gene pathogenic/likely pathogenic variants was similar to that of non-carriers. The clinical spectrum of Parkinson's disease-associated gene carriers in this mainland Chinese population was similar to that of other populations. We also detected 61 probands with *GBA* possibly pathogenic variants (3.64%) and 59 probands with *GBA* p.L444P (3.52%). These results shed insight into the genetic spectrum and clinical manifestations of Parkinson's disease in mainland China and expand the existing repertoire of pathogenic or likely

Received October 3, 2019. Revised March 19, 2020. Accepted April 6, 2020. Advance access publication July 2, 2020

pathogenic variants involved in known Parkinson's disease-associated genes. Our data highlight the importance of genetic testing in Parkinson's disease patients with age at onset < 40 years, especially in those from families with a recessive inheritance pattern, who may benefit from early diagnosis and treatment.

1 Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan 410008, China
2 National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, Hunan 410008, China
3 Centre for Medical Genetics and Hunan Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Changsha, Hunan 410008, China
4 Department of Geriatrics, Xiangya Hospital, Central South University, Changsha, Hunan 410008, China
5 Department of Neurology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430022, China
6 Department of Neurology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong 510080, China
7 Department of Neurology, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China
8 Department of Neurology, Affiliated Brain Hospital of Nanjing Medical University, Nanjing, Jiangsu 210029, China
9 Department of Neurobiology, Xuanwu Hospital of Capital Medical University, Beijing 100053, China
10 Parkinson's Disease Center, Beijing Institute for Brain Disorders, Beijing 100101, China
11 Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430022, China
12 Department of Neurology, Zhujiang Hospital of Southern Medical University, Guangzhou, Guangdong 510282, China
13 Department of Neurology, The First Affiliated Hospital of Jinan University, Guangzhou, Guangdong 510632, China
14 Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450047, China
15 Department of Neurology, Henan provincial people's hospital, Zhengzhou, Henan 450003, China
16 Department of Neurology, Renmin Hospital of Wuhan University, Wuhan, Hubei 430060, China
17 Department of Neurology, Hainan General Hospital, Haikou, Hainan 570311, China
18 Department of Neurology, The Third Xiangya Hospital, Central South University, Changsha, Hunan 410013, China
19 Department of Neurology, The Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China
20 Departments of Neurology and Neuroscience, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

*Correspondence to: Beisha Tang
Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan Province 410008, China
E-mail: bstang7398@163.com

*Correspondence may also be addressed to: Jifeng Guo
E-mail: guojifeng2003@163.com

*Jinchen Li
National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, Hunan 410008, China
E-mail: lijinchen@csu.edu.cn

Keywords: Parkinson's disease; age at onset; whole-exome sequencing; disease-associated gene; pathogenic or likely pathogenic variant

Abbreviations: AAO = age at onset; ADPD = autosomal-dominant Parkinson's disease; ARPD = autosomal-recessive Parkinson's disease; CNV = copy-number variant; P/LP = pathogenic/likely pathogenic; sEOPD = sporadic early-onset Parkinson's disease; VUS = variants of uncertain significance

Introduction

As the second most frequent neurodegenerative disorder, Parkinson's disease is characterized by motor symptoms, such as bradykinesia, resting tremor, muscle stiffness, and postural instability, as well as non-motor symptoms, including olfactory dysfunction, sleep disorders, constipation and dysautonomia, which are due to the loss of neurons in several brain areas and may occur before or after the loss of dopaminergic neurons (He *et al.*, 2018). Parkinson's disease is thought to be caused by a combination of ageing, as well as genetic and environmental risk factors (Guo *et al.*, 2018*a*; Lim *et al.*, 2019; Bandres-Ciga *et al.*, 2020), and ~5–10% of patients with Parkinson's disease have rare Mendelian

variants. To date, 23 genes with different degrees of genetic evidence (Blauwendraat *et al.*, 2019*b*) have been found to be mutated in monogenic Parkinson's disease (defined as Parkinson's disease-associated genes in this study), and variants in the glucocerebrosidase (*GBA*) gene were found to be an important risk factor in Parkinson's disease (Sidransky *et al.*, 2009; Sun *et al.*, 2010; Velez-Pardo *et al.*, 2019). The 23 genes included 10 genes with recessive inheritance patterns and 13 genes with dominant inheritance patterns (Puschmann, 2013; Singleton and Hardy, 2016, 2019; Sudhaman *et al.*, 2016; Quadri *et al.*, 2018*a*; Blauwendraat *et al.*, 2019*b*).

Recently, several genetic-testing studies have been conducted with Parkinson's disease patients (Bandres-Ciga *et al.*, 2019; Lin *et al.*, 2019; Tan *et al.*, 2019*a*; Trinh *et al.*, 2019; Youn *et al.*, 2019). These studies revealed that the mutational spectrum does vary across different populations, even within the same genes, which provided evidence of genetic heterogeneity in Parkinson's disease due to geographic and ethnic differences, among the studied populations (Lim *et al.*, 2019). For example, the p.G2019S variation accounts for most patients with *LRRK2* mutations from the UK or other European population-based cohorts (Montaut *et al.*, 2018; Tan *et al.*, 2019*b*), whereas very few Asian patients with Parkinson's disease have been found to carry the p.G2019S (Lin *et al.*, 2019; Shu *et al.*, 2019; Youn *et al.*, 2019). In addition to the ethnic and geographical heterogeneities of the subjects, other characteristics such as the age at onset (AAO) and family history of Parkinson's disease are also associated with the prevalence of specific mutations (Alcalay *et al.*, 2010). Most disease-associated genes are first identified in familial Parkinson's disease patients (Deng *et al.*, 2016; Quadri *et al.*, 2018*a*) and there is evidence that the earlier the age at onset, the more likely a genetic cause will be found (Xu *et al.*, 2017; Guo *et al.*, 2018*a*; Kasten *et al.*, 2018; Blauwendraat *et al.*, 2020), in addition to the correlation between age at onset and common polygenic alleles (Escott-Price *et al.*, 2015; Blauwendraat *et al.*, 2019*a*).

Several large-scale studies have been conducted to screen for Parkinson's disease-associated genes in cohorts based on UK (Tan *et al.*, 2019*b*), Norwegian (Gustavsson *et al.*, 2017), European (Alcalay *et al.*, 2010) and Taiwanese populations (Lin *et al.*, 2019). In addition, some relatively small, but more systematic studies were conducted with German (Trinh *et al.*, 2019) and Korean populations (Youn *et al.*, 2019). Previously, we performed mutation analysis of *PRKN*, *PINK1*, *DJ1*, *ATP13A2* (Guo *et al.*, 2008), *PLA2G6* (Shi *et al.*, 2011), *CHCHD2* (Liu *et al.*, 2015), *RAB39B* (Kang *et al.*, 2016), *TMEM230* (Yan *et al.*, 2017) and other genes associated with familial Parkinson's disease or early-onset Parkinson's disease, using a patient cohort from mainland China. We also applied trio-based whole exome sequencing to reveal the association between *NUS1* and early onset Parkinson's disease in Han Chinese individuals (Guo *et al.*, 2018*a*). Some possible limitations were associated with these previous studies. First, some of these studies (Lin *et al.*, 2019; Trinh *et al.*, 2019) might have focused on patients diagnosed as atypical parkinsonism, or on disease-associated genes associated with other movement disorders. Second, most of these studies (Alcalay *et al.*, 2010; Tan *et al.*, 2019*b*; Youn *et al.*, 2019), including our previous studies, used techniques such as multigene panel testing or Sanger sequencing, which only included a certain set of genes, thereby limiting the comprehensiveness and extensibility of genetic testing. Therefore, it was necessary to systematically study the mutational spectrum of the Parkinson's disease population in mainland China.

In this study, we comprehensively analysed the mutational spectrum of known Parkinson's disease-associated genes for patients with familial Parkinson's disease and sporadic early-onset Parkinson's disease (sEOPD) in a mainland Chinese population, using gene dosage analysis and whole-exome sequencing. First, we systematically identified pathogenic and likely pathogenic (P/LP) variants of known Parkinson's disease-associated genes, including known variants and novel variants, and summarized and compared the frequency among patients with autosomal-recessive familial Parkinson's disease (ARPD), autosomal-dominant familial Parkinson's disease (ADPD), or sEOPD. Second, we compared the frequency of P/LP variants among patients with different AAOs and among different genes separately, in order to determine differences in the molecular diagnosis, and to depict the AAO spectrum for these disease-associated genes in our population. Third, we generalized the clinical manifestations of Parkinson's disease patients with certain mutated genes important for Parkinson's disease pathogenesis, thereby contributing to genetic counselling, early diagnosis, and early intervention.

Materials and methods

Subjects

Participants with sEOPD (AAO $\leqslant$ 50 years old) or from families with ARPD and ADPD were recruited at the Xiangya Hospital (Central South University) between October 2006 and January 2019 and at other co-operating hospitals of Parkinson's Disease and Movement Disorders Multicenter Database and Collaborative Network in China (PD-MDCNC, http://pd-mdcnc.com : 3111/) established by our team. The ARPD families were consanguineous, or the probands in these families had two or more affected siblings but no affected family members within two consecutive generations, whereas Parkinson's disease was diagnosed in ADPD family members for two or more generations. Patients with sporadic, early-onset Parkinson's disease lacked a family history of Parkinson's disease and had an AAO of no more than 50 years old. Of them, 15 probands were found to have certain mutations in specific target genes by real-time quantitative PCR or Sanger sequencing analysis, in our previous studies (Tang *et al.*, 2006; Guo *et al.*, 2008, 2010; Shi *et al.*, 2011). All participants fulfilled the UK Parkinson's disease Society Brain Bank clinical diagnostic criteria (Gibb and Lees, 1988) or Movement Disorders Society (MDS) clinical diagnostic criteria for Parkinson's disease (Postuma *et al.*,

2015). Patients with detectable polynucleotide repeats (SCA3, SCA2, SCA1, SCA17 etc.) were identified as described in our previous study (Wang *et al.*, 2009), and excluded from this study. The study was approved by the Ethics Committee of Xiangya Hospital (Central South University), and written informed consent was collected from all participants. Genomic DNA was prepared from peripheral blood leucocytes according to standard procedures. A comprehensive dataset of basic demographic data, including the subjects' age, gender, family history, disease duration, and clinical features (including motor and non-motor manifestations, shown in Supplementary material), was collected from the Parkinson's disease patients enrolled in this study and inputted into the PD-MDCNC.

Multiplex ligation-dependent probe amplification and quantification PCR

The Salsa Multiplex Ligation-Dependent Probe Amplification Kit (P051-c1; MRC) was used to detect exon deletions or duplications [copy-number variants (CNVs)] of several Parkinson's disease-associated genes, including *PRKN*, *PINK1*, *ATP13A2*, *DJ1* and *SNCA*, according to the manufacturer's instructions. Furthermore, patients with *SNCA* duplications were later confirmed by real-time quantitative PCR (Klein *et al.*, 2005).

Whole-exome sequencing and analysis

Genomic DNA were isolated from peripheral blood leucocytes. Whole-exome DNA was capture using the SureSelect Human All Exon Kit V6 (Agilent) and high-throughput sequencing was conducted using the Illumina ×10 platform with an average 123× coverage. Paired-end sequence reads were aligned with the Burrow-Wheeler Aligner (Li, 2014) against the reference human genome (UCSC hg19). The Picard tool (http://broadinstitute.github.io/picard/) was used to remove duplicate reads, generate the converse format, and index the sequencing data. Base quality-score recalibration, local realignments around possible insertions/deletions (indels), variant calling, and filtering were performed with the Genome Analysis Toolkit (GATK) (McKenna *et al.*, 2010). ANNOVAR (Wang *et al.*, 2010*a*; Yang and Wang, 2015) and VarCards (Li *et al.*, 2018*a*) was used to systematically annotate the variants with RefSeq (UCSC hg19), including gene regions, amino acid alterations, functional effects, and allele frequencies in East Asian (EAS) population (GnomAD database, ExAC database, and an in-house generated Chinese control database, which included 1279 Chinese controls without neurological disease). Among the recessive disease-associated genes, the minor allele frequency (MAF) of the variants was limited to 0.01 for the above population database, whereas the MAF of variants in dominant disease-associated genes was limited to 0.001.

Only predicted damaging missense and loss-of-function variants (nonsense variants, frameshift indels, and splicing-site variants) of 23 known Parkinson's disease-associated genes were included for further analysis. Non-frameshift indels in the *PRKN* gene were also included in our study. Recently, our group developed the ReVe (Li *et al.*, 2018*b*) programme, which was used in this study to predict deleterious missense variants. Rare damaging variants, including CNVs, single-nucleotide variants (SNVs), and indels, were further classified as pathogenic, likely pathogenic or of uncertain significance, according to the standards and guidelines from the American College of Medical Genetics and Genomics (ACMG) (Richards *et al.*, 2015; Li *et al.*, 2018*b*).

We searched the Gene4PD database (http://www.genemed.tech/gene4pd/), which was recently developed by our group, the MDSgene database (https://www.mdsgene.org/g4d), and PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) to determine which, if any, variants had been reported previously. Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) were also carried out in cases where affected or unaffected samples from families who carried P/LP variants were available, as described in our previous study (Guo *et al.*, 2018*a*). The study workflow is shown in Fig. 1.

Statistical analyses

Wilcoxon's rank-sum test was used to compare the AAO and mutation statuses of the patients. Linear regression was used to compare demographic data with covariate adjustment. The relationship between clinical manifestations and the genetic status (P/LP variant carriers or non-carriers) was assessed by performing linear regressions of continuous scores versus the genetic status, adjusting for age at entry, disease duration, and gender. We performed binary logistic regression of the symptom status (motor complication) against the genetic status to assess correlations between symptom status and genetic status. The Hoehn and Yahr stage and motor subtype were analysed by ordered logistic regression and multinomial logistic regression, where the tremor-dominant group was used as the referent group, respectively. All *P*-values were two-tailed. Statistical analysis of the data was conducted using SPSS software, version 24.0.

Data availability

The datasets supporting the results and conclusions of this manuscript are included within the article and its Supplementary material. All other datasets used and analysed during the current study are available from the corresponding authors on reasonable request.

Results

Cohort description

We included 1676 probands in this study, and they were divided into the ARPD ($n = 192$), ADPD ($n = 242$) and sEOPD cohorts ($n = 1242$), based on the family history and AAO. The baseline characteristics of the demographic data, and the motor and non-motor manifestations of patients from the ARPD, ADPD, sEOPD cohorts, and entire cohort are presented in Table 1. Briefly, the mean AAO of probands from the ARPD, ADPD, and sEOPD cohorts were 45.30 ± 16.47, 51.60 ± 10.93 and 43.58 ± 6.43 years, respectively. The proportions of male subjects in each group were 51.0%, 54.5% and 54.8%, respectively.

We carried out whole-exome sequencing for all 1676 probands. On average, we obtained 11.66 GB clean data with a mean sequencing depth of 123-fold. Over 99.83%,

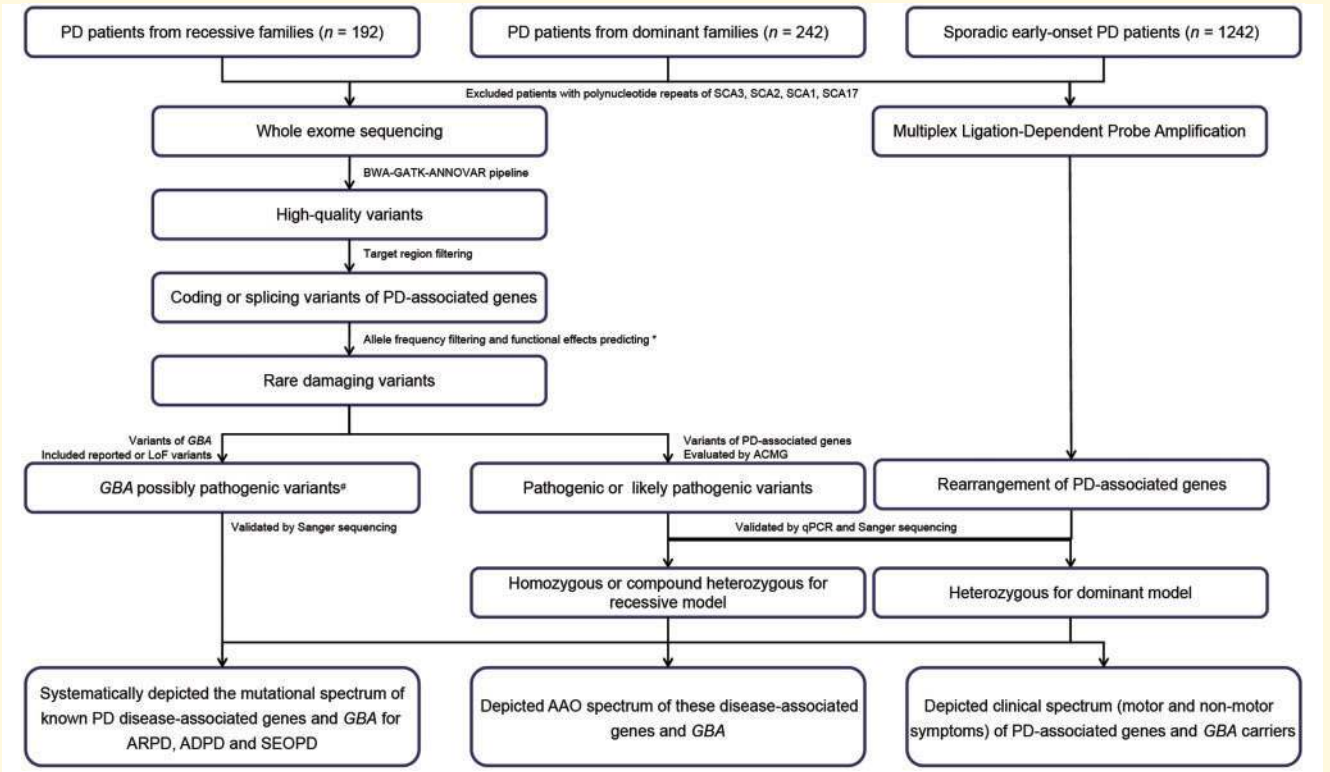

Figure 1 Workflow of this study. *Standard for allele frequency filtering: MAF < 1% for the recessive model, MAF < 1‰ for the dominant model. Functional-effect predictions: loss-of-function variants or missense variants predicted to be damaging by ReVe. PD = Parkinson's disease.

99.72%, or 99.32% of the targeted regions were covered at least 1-fold, 4-fold, or 10-fold, respectively (Supplementary Table 1). Specifically, our study focused on known Parkinson's disease-associated genes and high-risk gene *GBA* (Supplementary Table 2). The workflow of this study is presented in Fig. 1.

Mutational spectrum of members of the ARPD cohort

In ARPD families, we detected 37 homozygous variants and 28 compound heterozygous variants of Parkinson's disease-associated genes in 65 of 192 probands (33.85%). Interestingly, the molecular diagnosis was associated with the AAO of the patients, which showed that the molecular diagnostic rate was 93.02%, 78.38%, or 60.19% for ARPD probands with an AAO of ⩽ 30 years, 40 years, or 50 years, respectively. Of note, we did not detect any P/LP variants in 84 ARPD probands with an AAO >50 years (Fig. 2A and Supplementary Table 3).

Of the 65 probands with P/LP variants, *PRKN* was the most frequently mutated gene, accounting for 50 (26.04%; of 192 ARPD probands) molecularly diagnosed probands, including 27 patients with homozygous variants and 23 patients with compound heterozygous variants (Supplementary Table 3). Among these P/LP variants, *PRKN* CNVs were detected in 42 probands (21.88%; of 192 ARPD probands), including 20 patients with homozygous CNVs, eight patients with compound heterozygous CNVs, and 14 patients with a combination of *PRKN* CNVs and SNVs/indels. Exon deletions represented the most prevalent type of mutations, especially in exon 3 ($n = 18$) and exon 4 ($n = 19$). The remaining 21 probands carried *PRKN* P/PL SNVs/indels. We noted that two known variants, p.G284R ($n = 7$) and p.M1T ($n = 3$), were the most frequent *PRKN* P/PL SNVs in our ARPD cohort (Supplementary Fig. 1A and Supplementary Table 4A). Interestingly, since we included patients with loss-of-function variants and non-frameshift variants of *PRKN*, we found that two probands carried a novel *PRKN* splicing SNV (c.619-1G>C) and two other probands carried the reported *PRKN* non-frameshift deletion (c.968_973del/p.C323_V324del) (Guo *et al.*, 2008), which were extremely rare variants that were absent from the East Asian populations of GnomAD exome, GnomAD genome, the ExAC database, and our in-house Chinese control cohort. Of note, we found that the splicing mutation, *PRKN* c.619-1G>C, caused exon 6 skipping and generated a truncated protein where exon 6-encoded amino acids were replaced with an abnormal 28 amino acid sequence followed by premature stop codons (Supplementary material and Supplementary Fig. 2D). Besides, *PRKN* c.968_973del was found to cause the protein to lose mitochondrial localization in CCCP-induced mitophagy (Supplementary material and

Table 1 Summary of clinical features of the Parkinson's disease patients in this study

Clinical features	ARPD (*n* = 192)	ADPD (*n* = 242)	sEOPD (*n* = 1242)	All (*n* = 1676)
Age at onset, years	45.30 ± 16.47	51.60 ± 10.93	43.58 ± 6.43	44.94 ± 9.31
Male (%)	51.0	54.5	54.8	54.4
Age at assessment, years	53.50 ± 14.65	56.93 ± 11.22	50.14 ± 7.66	51.51 ± 9.60
Disease duration, years	8.20 ± 7.57	5.33 ± 3.95	6.56 ± 5.46	6.57 ± 5.61
Motor manifestations				
UPDRS-Part I score	2.62 ± 2.23	2.47 ± 2.11	2.41 ± 2.10	2.44 ± 2.11
UPDRS-Part II score	12.40 ± 7.44	11.60 ± 7.00	11.87 ± 6.77	11.89 ± 6.88
UPDRS-Part III score	29.45 ± 16.69	26.88 ± 15.46	27.33 ± 15.96	27.51 ± 15.98
Tremor score	4.21 ± 4.11	4.32 ± 3.97	3.65 ± 3.73	3.81 ± 3.82
Stiffness score	5.75 ± 4.32	5.23 ± 4.09	5.65 ± 4.28	5.60 ± 4.26
Bradykinesia score	10.78 ± 6.51	9.88 ± 6.47	10.21 ± 6.68	10.22 ± 6.63
Postural instability score	4.73 ± 3.47	3.99 ± 3.31	3.99 ± 3.14	4.07 ± 3.21
Hoehn and Yahr stage (%)				
0–1.5	20.3	31.0	32.5	30.9
2 or 2.5	48.4	46.7	43.2	44.3
3 +	31.3	22.3	24.3	24.8
Dyskinesia	17.9	11.7	17.5	16.7
Freezing gait	30.9	27.5	27.3	27.7
Motor subtype (%)				
Tremor-dominant	27.10	34.7	24.9	26.6
Intermediate	17.20	16.9	16.9	16.9
PIGD-dominant	55.70	48.4	58.2	56.5
Non-motor manifestations				
Cognition: total MMSE score	26.29 ± 3.68	26.57 ± 3.42	27.29 ± 3.00	27.06 ± 3.18
PDSS score	120.13 ± 22.53	113.41 ± 31.04	115.92 ± 30.08	116.06 ± 29.43
RBDQ-HK score	13.36 ± 15.67	15.70 ± 17.68	12.73 ± 15.55	13.26 ± 15.93
ESS score	7.84 ± 5.95	7.76 ± 6.41	7.24 ± 6.08	7.40 ± 6.11
HAMD score	5.96 ± 5.34	5.99 ± 6.35	5.79 ± 5.48	5.84 ± 5.61
HRS score	21.04 ± 5.58	19.37 ± 7.09	19.91 ± 6.30	19.96 ± 6.36
PFS score	45.85 ± 18.81	42.97 ± 19.56	44.29 ± 18.98	44.29 ± 19.04
SCOPA-AUT score	7.53 ± 6.48	8.66 ± 7.36	7.00 ± 6.80	7.32 ± 6.87
PDQ39 score	33.63 ± 28.14	30.02 ± 27.09	29.69 ± 25.67	30.23 ± 26.22

Scores in the last four columns are mean ± SD; gender, motor complication (dyskinesia, wearing-off, freezing gait), motor subtype, restless legs syndrome and constipation are shown as numbers and proportions (%).Tremor score was measured by adding up score of tremors at rest and action and postural tremor of hands from UPDRS score, while bradykinesia score was calculated by score on finger taps, hand movements, rapid alternating movements of hand, and leg agility. And rigidity score was added up by the score on rigidity of neck, hands and fee. Disease motor subtype was classified as tremor-dominant (TD) phenotype when the ratio of tremor score and postural instability and gait difficulty (PIGD) score was no less than 1.5, while patients with a ratio of no more than 1.0 were defined to PIGD phenotype and rest of patients belonged to the indeterminate phenotype. ADPD = PD families with autosomal dominant inheritance; All = ARPD + ADPD + sEOPD; ARPD = PD families with autosomal recessive inheritance; ESS = Epworth Sleepiness Score; HAMD = 17-item Hamilton Depression Rating Scale; HRS = Hyposmia Rating Scale; PDQ39 = The 39-item Parkinson's disease Questionnaire; PDSS = Parkinson's disease sleep scale; PFS = Parkinson's disease fatigue scale; RBDQ-HK = REM sleep behaviour disorder questionnaire-Hong Kong; SCOPA-AUT = Scales for Outcomes in Parkinson's disease-Autonomic; sEOPD = patients with sporadic Parkinson's disease with AAO younger than 50 years old; UPDRS = Unified Parkinson's Disease Rating Scale.

Supplementary Fig. 2E). Both of them supported that they could be deleterious and cause Parkinson's disease. Nevertheless, other unreported P/LP variants still deserve further replication in larger cohorts or experimental studies in the future.

We identified five probands carrying homozygous *PINK1* P/LP variants and one proband carrying a compound heterozygous *PINK1* variant. In addition, another nine probands were identified with P/LP variants in other recessive Parkinson's disease-associated genes, including *PLA2G6* (*n* = 6), *ATP13A2* (*n* = 2), and *DJ1* (*n* = 1). Of the *ATP13A2* gene carriers, two novel homozygous variants (p.R735X and p.A819D) were identified in two consanguineous families, in which two probands were initially diagnosed with Parkinson's disease, and their follow-up diagnosis (after molecular testing) was considered as Parkinsonism, due to the presence of a red flag sign (patients with p.R735X showed pyramidal tract signs) or the clinical manifestation of absolute exclusion criteria (parkinsonian features of patients with p.A819D restricted to the lower limbs for more than 3 years) in MDS clinical-diagnostic criteria for Parkinson's disease (Postuma *et al.*, 2015).

Mutational spectrum of members of the ADPD cohort

In the ADPD families, 10 of 242 probands (4.13%) carried P/LP variants of Parkinson disease-associated genes including *LRRK2* (*n* = 5), *SNCA* (*n* = 4), and *LRP10* (*n* = 1) (Fig. 2B and Supplementary Table 3). Five probands (2.07%) carried

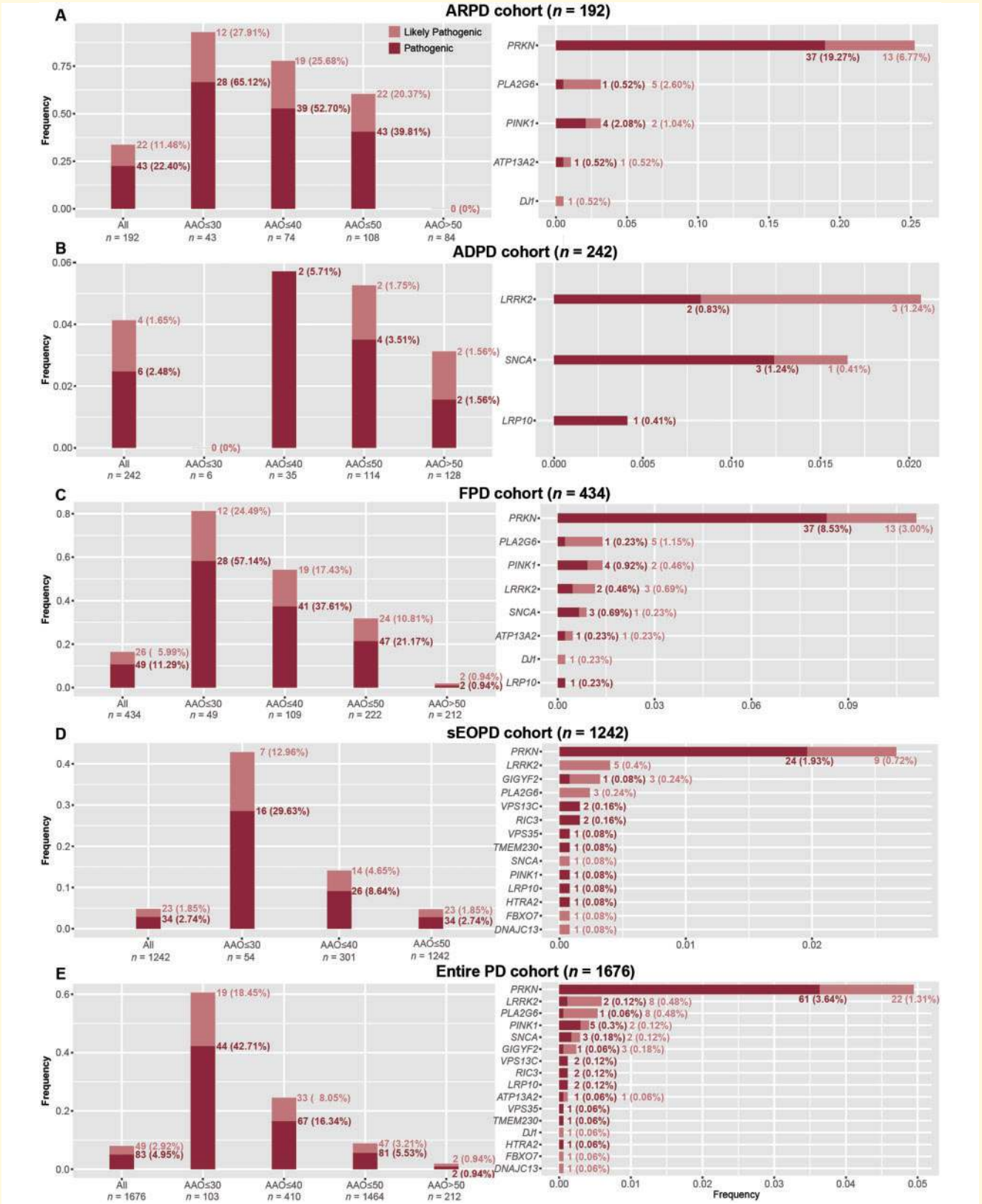

Figure 2 Mutational frequencies of known Parkinson's disease-associated genes in Parkinson's disease cohorts. Mutational frequencies of all (*left*) and each (*right*) known Parkinson's disease-associated gene in the different cohorts. (**A**) ARPD cohort. (**B**) ADPD cohort. (**C**) All familial Parkinson's disease (FPD) cohort. (**D**) The sEOPD cohort. (**E**) Entire Parkinson's disease (PD) cohort. *Variants that were classified as pathogenic or likely pathogenic according to the standards and guidelines of the ACMG. 'Pathogenic' means that the patients had pathogenic variants in known Parkinson's disease-associated genes, and 'likely pathogenic' means that the patients had likely pathogenic variants in known Parkinson's disease-associated genes.

four *LRRK2* missense variants: p.N1437D ($n = 2$), p.R1441C, p.R1441H, and p.K616R ($n = 1$ each) (Wang *et al.*, 2010*b*) (Supplementary Tables 3 and 4A). *LRRK2* p.N1437D, a missense variant absent from East Asian populations of public databases and our in-house control cohort, was first identified in two families of our cohort; this variation segregated with the parkinsonism phenotype within one family, but one unaffected member (a 65-year-old female) from another family also carried this variant. The novel p.N1437D variant occurred at the same position as another pathogenic missense change (p.N1437H), which was only reported previously in two Norwegian families (Aasly *et al.*, 2010). Moreover, we performed an *in vitro* kinase assay to assess the respective enzymatic properties of wild-type protein, LRRK2 p.G2019S, p.N1437H, and p.N1437D. Compared to wild-type protein, LRRK2 p.N1437D and p.N1437H display a significantly higher proportion of LRRK2 kinase compared to wild-type LRRK2 ($P < 0.01$), consistent with the property observed for the p.G2019S LRRK2 pathogenic substitution (Supplementary material and Supplementary Fig. 2A and B). The increased enzyme activity caused by the p.N1437D further supported its pathogenicity. In the family identified with *LRRK2* p.R1441C, two unaffected members (a 74-year-old male and a 71-year-old female) also carried the variant; these two unaffected carriers may be explained by incomplete penetrance as described in a previous report (Haugarvoll *et al.*, 2008) (Supplementary Fig. 1B). Of the family identified with *LRRK2* p.R1441H, two unaffected members (a 59-year-old male and a 56-year-old male) carried this variant, which may also be explained by incomplete penetrance. The *LRRK2* p.R1441C and p.R1441H variants were detected in two families in the ADPD cohort, indicating that these two variants had prevalence of 0.12% separately. This finding was in agreement with other studies showing the rarity of *LRRK2* p.R1441C in European ancestry (0–0.3%) (Zabetian *et al.*, 2005; Tan *et al.*, 2006; Moller *et al.*, 2008).

We found that four probands carried *SNCA* P/LP variants, including three patients with *SNCA* duplications and one patient with an unreported missense variant (p.P117S). Furthermore, Triton-X soluble and insoluble α-synuclein lysates assay indicated that the p.P117S mutation may also alter α-synuclein protein solubility (as described in the Supplementary material and Supplementary Fig. 2C), which also suggested its pathogenicity. Nevertheless, further functional analyses using animal models is needed, in addition to the recruitment of more families with this mutation, to validate the pathogenicity. Of these three families with CNVs, one of them segregated with the parkinsonism phenotype; in another two families, two of the probands' unaffected siblings were identified as having *SNCA* duplications, but they currently remain asymptomatic and are 42 and 47 years old, which is still below the median AAO of 48 years (the penetrance remains incomplete even at 79 years) (Nishioka *et al.*, 2009; Konno *et al.*, 2016). To our knowledge, this study is the first to identify an *SNCA* duplication in young-onset or familial Parkinson's disease in mainland China, although the frequency is slightly lower than it was in other populations (Bonifati, 2014; Schneider and Alcalay, 2017). In addition, an unreported insertion mutation (c.322dupC/p.G109Rfs*51) was identified in *LRP10*, which segregated with parkinsonism in the corresponding family (Supplementary Fig. 2). As a recently reported disease-associated gene, the pathogenicity of *LRP10* remains controversial (Quadri *et al.*, 2018*b*), yet the presence of this deletion mutation in our cohort provides more evidence of its pathogenicity.

Mutational spectrum of members of the sEOPD cohort

In the sEOPD cohort, we detected P/LP variants of Parkinson's disease-associated genes in 57 of 1242 (4.59%) patients (Fig. 2D and Supplementary Table 3). We found that a lower AAO was associated with a higher molecular diagnosis rate. When the AAO decreased from 40 to 30 years, the molecular diagnosis rate increased from 13.29% to 42.59%. It was noted that molecular diagnosis was much more frequently established with the ARPD cohort ($n = 65$ of 192; 33.85%) than with the ADPD cohort ($n = 10$ of 242; 4.13%) and the sEOPD cohort ($n = 57$ of 1242; 4.59%). This indicated that the P/LP variants occurred more frequently in patients with a family history of Parkinson's disease, especially those with a pattern of recessive inheritance.

PRKN was also the most frequently mutated gene in the sEOPD cohort, accounting for 33 (2.66%; of 1242 probands) of 57 molecularly diagnosed probands. Exon deletions in *PRKN* also represented the most common mutation type, especially in exon 3 ($n = 19$) and exon 2 ($n = 16$). Exon 3 was further confirmed as a hotspot mutation site between the ARPD and sEOPD cohorts, similar to findings with Hispanic populations (exon 3–4 deletions were more common) (Marder *et al.*, 2010). Furthermore, we noted that the most frequent variant in *PRKN* P/PL SNVs was p.C441R ($n = 3$), which was reported previously (Supplementary Table 4A). Probands (26.04%) from the ARPD cohort were more likely to have biallelic mutations in *PRKN* than patients with sEOPD (2.66%) in our cohort.

We detected five (0.40%) P/LP variants in *LRRK2*, the second most frequently mutated gene in the sEOPD cohort. We identified three patients with the *LRRK2* p.S1181Y variant, and the rest of the variants were p.V1447M ($n = 1$), or p.Y1645S ($n = 1$). Familial cases with Alzheimer's disease inheritance were more likely to have *LRRK2* P/LP variants than patients from the sEOPD cohort, with relative proportions of 2.07% versus 0.40%, respectively, in our cohorts. In general, P/LP variants occur more frequently in familial Parkinson's disease patients (Di Fonzo *et al.*, 2005; Nichols *et al.*, 2005) than in sporadic cases (Gilks *et al.*, 2005). However, the p.G2019S was not found in our cohort, although it is common in Europe (Bonifati, 2007) and North Africa (Puschmann, 2013).

Numerous P/LP variants of other genes were also identified, including *GIGYF2* ($n = 4$), *PLA2G6* ($n = 3$), *VPS13C*

(*n* = 2), *RIC3* (*n* = 2), *VPS35* (*n* = 1), *TMEM230* (*n* = 1), *SNCA* (*n* = 1), *PINK1* (*n* = 1), *LRP10* (*n* = 1), *HTRA2* (*n* = 1), *FBXO7* (*n* = 1), and *DNAJC13* (*n* = 1) (Supplementary Table 3). Furthermore, two patients in our sEOPD cohort were identified with the *GIGYF2* p.D1075 missense variant, which is absent from Eastern Asian populations of public databases and our in-house control cohort.

Age at onset spectrum

We found that the median AAO of patients with molecular diagnosis (median 31.50 years; average 32.27 years) was approximately 14.5 years earlier than the median AAO of patients without molecular diagnosis (median 46.00 years; average 46.02 years) across the entire Parkinson's disease cohort (Fig. 3C and Supplementary Table 5). These results indicated that the median AAO was significantly lower in Parkinson's disease patients with P/LP variants, especially in patients with a family history (Fig. 3A). The median AAO ranking in carriers in the FPD cohort with P/LP variants in known Parkinson's disease-associated genes was similar to that in the entire Parkinson's disease cohort, except for *LRRK2* (Fig. 3A). The median AAO ranking in carriers in the sEOPD cohort with P/LP variants in these Parkinson's disease-associated genes was consistent across the entire Parkinson's disease cohort, except for *PLA2G6* (Fig. 3B). The subtle difference of median AAO ranking in patients with *LRRK2* P/LP variants may be caused by late-onset Parkinson's disease with reported *LRRK2* P/LP variants in the ADPD cohort, which would be consistent with previous studies.

These results indicated that the top five genes in our cohorts were autosomal-recessive genes (Fig. 3C and Supplementary Table 5), which is in line with the opinion that recessive genes usually exist in patients with early AAO (Kasten *et al.*, 2018). Specifically, the median AAOs of subjects with *ATP13A2*, *PLA2G6*, *PRKN*, and *PINK1* P/LP variants were significantly lower than those of non-carriers, whereas the median AAOs of subjects with *VPS13C* and *RIC3* P/LP variants were slightly lower and the median AAOs of subjects with *LRRK2*, *SNCA*, and *GIGYF2* P/LP variants were similar to those of non-carriers (Fig. 3C).

Clinical manifestations in subjects with Parkinson's disease-associated gene variants

We then analysed associations between five Parkinson's disease-associated genes and phenotypes based on genes with P/LP variants in five or more patients. We found that carriers of *PRKN*, *PLA2G6*, and *PINK1* P/LP variants had earlier AAOs than non-carriers, after adjusting for gender and disease duration, and carriers of *PRKN* and *PINK1* P/LP variants also had longer disease durations than non-carriers, after adjusting for age at assessment and gender (Table 2; comparison of all clinical features shown in Supplementary Table 6A). *PRKN* P/LP variant carriers associated with milder motor manifestations, especially in terms of stiffness, milder autonomic dysfunction, and lighter depression, but they were associated with severe sleep disorders, a higher rate of dyskinesias, and a lower rate of wearing-off than non-carriers, after adjusting for age at assessment, gender and disease duration. *PLA2G6* P/LP-variant carriers showed more severe motor symptoms, especially in terms of postural instability symptoms, more severe autonomic dysfunction,

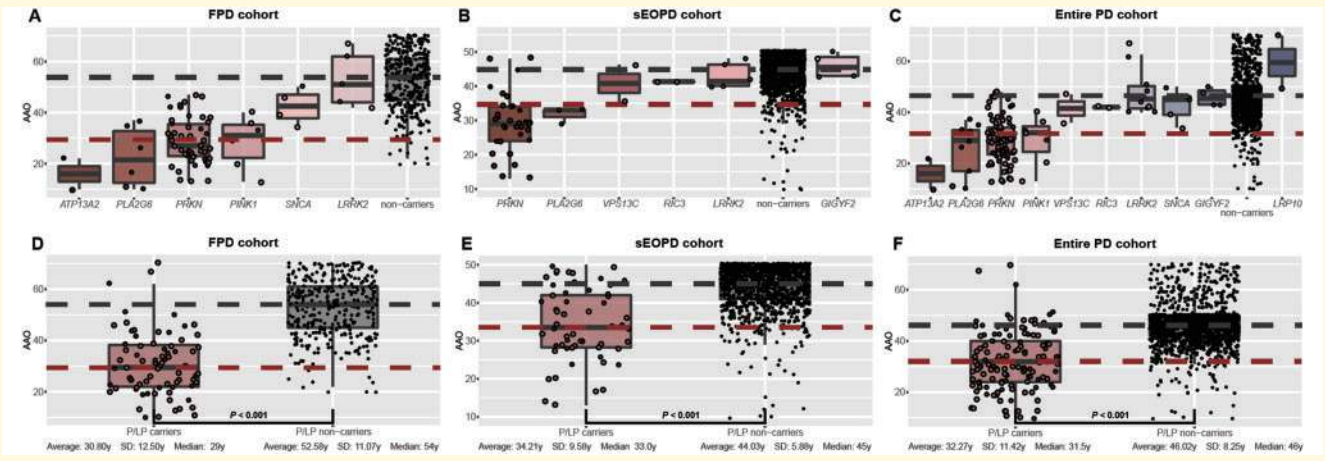

Figure 3 AAO spectrum of Parkinson's disease-associated genes. Spectrum of AAO in patients with P/LP variants of each Parkinson's disease-associated gene (only gene with no less than two patients carried was included in) and in patients without P/LP variants of known Parkinson's disease-associated genes: (**A**) Familial Parkinson's disease (FPD) cohort. (**B**) The sEOPD cohort. (**C**) Entire Parkinson's disease (PD) cohort. Comparison of AAO in all patients with P/LP variants of known Parkinson's disease-associated genes and patients without P/LP variants in known Parkinson's disease-associated genes. (**D**) FPD cohort. (**E**) sEOPD cohort. (**F**) Entire Parkinson's disease cohort. *The dashed red line refers to the median AAO of all patients with P/LP variants in known Parkinson's disease-associated genes, whereas the dashed grey line refers to the median AAO of patients without P/LP variants in known Parkinson's disease-associated genes in the corresponding cohort.

Table 2 Differential clinical features between carriers of P/LP variants in Parkinson's disease-associated genes and mutation-negative patients

Mutation carriers	Clinical features	Non-carriers (*n* = 1544)	Carriers	Beta	*P*-value
PRKN carriers *n* = 83 biallelic	Age at onset, years	46.02 ± 8.25	29.07 ± 8.78	–0.372	**<0.001**[a]
	Age at assessment, years	52.25 ± 8.92	41.43 ± 11.49	–0.360	**<0.001**[a]
	Disease duration, years	6.24 ± 5.16	12.36 ± 9.24	0.353	**<0.001**[b]
	Motor manifestations				
	UPDRS-Part II score	11.97 ± 6.87	10.02 ± 6.76	–0.104	**<0.001**
	Stiffness score	5.61 ± 4.25	4.99 ± 4.32	–0.054	**0.045**
	Dyskinesia (%)	16.2	22.2	0.190	**0.007**
	Non-motor manifestations				
	PDSS score	115.77 ± 29.69	122.46 ± 26.93	0.082	**0.010**
	RBDQ-HK score	13.43 ± 16.02	9.85 ± 12.21	–0.072	**0.024**
	HAMD score	5.87 ± 5.62	4.57 ± 5.15	–0.095	**0.003**
	SCOPA-AUT score	7.39 ± 6.90	4.24 ± 5.09	–0.121	**<0.001**
	PDQ39 score	30.11 ± 26.00	28.50 ± 29.07	–0.107	**<0.001**
PLA2G6 carriers *n* = 9 biallelic	Age at onset, years	46.02 ± 8.25	25.67 ± 10.43	–0.187	**<0.001**[a]
	Age at assessment, years	52.25 ± 8.92	30.56 ± 12.61	–0.173	**<0.001**[a]
	Disease duration, years	6.24 ± 5.16	4.89 ± 4.14	0.057	**0.015**[b]
	Motor manifestations				
	UPDRS-Part I score	2.44 ± 2.08	3.33 ± 3.08	0.060	**0.018**
	UPDRS-Part II score	11.97 ± 6.87	15.89 ± 9.06	0.086	**<0.001**
	Postural instability score	4.06 ± 3.20	6.56 ± 5.22	0.101	**<0.001**
	Hoehn and Yahr stage			0.053	**0.045**
	0–1.5 (%)	32.0	22.2		
	2 or 2.5 (%)	43.8	44.4		
	3 + (%)	24.2	33.3		
	Freezing gait (%)	27.3	77.8	–1.223	**0.001**
	Non-motor manifestations				
	SCOPA-AUT score	7.39 ± 6.90	18.00 ± 9.90	0.075	**0.018**
	PDQ39 score	30.11 ± 26.00	77.00 ± 19.80	0.069	**0.016**
PINK1 carriers *n* = 7 biallelic	Age at onset, years	46.02 ± 8.25	29.00 ± 9.42	–0.124	**<0.001**[a]
	Age at assessment, years	52.25 ± 8.92	40.86 ± 7.49	–0.115	**<0.001**[a]
	Disease duration, years	6.24 ± 5.16	11.86 ± 7.56	0.108	**<0.001**[b]
	PDQ39 score	30.11 ± 26.00	60.25 ± 14.66	0.058	**0.044**
SNCA dup carriers *n* = 3 heterozygous	Cognition: total MMSE score	27.03 ± 3.19	23.00 ± 7.07	–0.070	**0.014**
	HAMD score	5.87 ± 5.62	15.50 ± 9.19	0.081	**0.007**
	PFS score	44.28 ± 18.92	70.00 ± 14.14	0.074	**0.035**
	SCOPA-AUT score	7.39 ± 6.90	14.00 ± 1.41	0.064	**0.043**

Only clinical manifestations with significant differences between mutational carriers and non-carriers are shown, and the complete information is provided in the Supplementary material. Non-carriers were patients without any Parkinson's disease associated genes P/LP variants. Mutation-negative = patients without any pathogenic or likely pathogenic variants of Parkinson's disease-associated genes. Scores in the first four columns are mean ± SD, except for gender, initial symptoms, motor subtype, constipation and RLS, which are shown as *n* or proportions (%). Increasing scores and increasing beta-values for motor and non-motor variables are associated with worse symptoms, except for the Mini-Mental State Examination (MMSE) test scores. Increasing scores for the MMSE test are associated with better cognition. HAMD = 17-item Hamilton Depression Rating Scale; PDQ39 = The 39-item Parkinson's disease Questionnaire; PDSS = Parkinson's disease sleep scale; PFS = Parkinson's disease fatigue scale; RBDQ-HK = REM sleep behaviour disorder questionnaire-Hong Kong; SCOPA-AUT = Scales for Outcomes in Parkinson's disease-Autonomic; UPDRS = Unified Parkinson's Disease Rating Scale.
[a]Adjusting for gender and disease duration at entry.
[b]Adjusting for gender and age at entry.

and tended to have a higher rate of freezing gait problems (Table 2 and Supplementary Table 6A). We did not find any differences in clinical manifestations between *LRRK2* and *SNCA* P/LP-variant carriers and non-carriers (Table 2 and Supplementary Table 6B). Specifically, we then individually analysed the *SNCA* duplication carriers, and found that they showed more severe cognitive dysfunction, depression, fatigue, and automatic dysfunction. We also further assessed the cognitive function (Montreal Cognitive Assessment) of three probands with *SNCA* duplication, and they scored 23 (duration = 1 year), 16 (duration = 3.5 years), and 11 (duration = 11 years), respectively. Our results showed that the clinical manifestations of subjects with Parkinson's disease-associated genes were similar with those of other populations or studies (Kasten *et al.*, 2018; Trinh *et al.*, 2018).

Potential variations meriting further study

We further identified variants of uncertain significance (VUSs) and heterozygous variants of autosomal-recessive

genes in the 23 Parkinson's disease-associated genes, which may be worth investigating further. First, we identified 68 probands carrying VUSs, including 26 VUSs in *POLG*, 13 VUSs in *DNAJC13* and 13 VUSs in *LRRK2*. The other VUSs were identified in *EIF4G1*, *GIGYF2*, *LRP10*, *SNCA*, *RIC3*, *TMEM230* and *VPS35*. We did not observe significant differences in the AAO, gender, and proportion of patients with a family history of Parkinson's disease between patients with VUSs and patients without P/LP variants and VUSs. Further exploration and periodical reassessment may be necessary to investigate whether any of the VUSs play critical roles in Parkinson's disease (Supplementary Tables 4B and 7). Second, 195 patients carried heterozygous variants of autosomal-recessive Parkinson's disease-associated genes. Of note, eight patients carried two heterozygous variants from two different recessive inheritance genes (Supplementary Table 4C). Some studies have supported that the oligogenic inheritance of rare Mendelian variants may be important in Parkinson's disease (Tang *et al.*, 2006; Lubbe *et al.*, 2016), but this has not been replicated on a large scale, which needs further exploration. Of all of these heterozygous variants, variants in *VPS13C* were the most common ($n = 70$), followed by *PRKN* variants ($n = 49$).

Mutational frequency of GBA in all cohorts

In the ARPD families, we detected six possibly pathogenic variants of *GBA* in six families ($n = 6$ of 192; 3.15%) and two VUSs in two other families. In the ADPD families, we detected 10 possibly pathogenic variants in 10 families ($n = 10$ of 242; 4.13%) and two VUSs in two other families. In the sEOPD cohort, we detected 53 variants: 45 patients carried possibly pathogenic variants ($n = 45$ of 1242; 3.62%) (Fig. 4 and Supplementary Table 4D), whereas eight patients carried VUSs. Of note, no homozygous or compound heterozygous *GBA* mutations was found in our cohorts.

Further, we also found 59 probands who carried p.L444P, including two probands from ARPD families ($n = 2$ of 192; 1.04%), nine probands from ADPD families ($n = 9$ of 242; 3.72%), and 48 patients from the sEOPD cohort ($n = 48$ of 1,242; 3.86%), respectively. This gives p.L444P an overall frequency of 3.52% (59 of 1676) in the entire FPD and sEOPD cohort (Fig. 4). However, we did not find any patients that carry p.N370S in our cohort.

Next, we analysed the relationship between *GBA* possibly pathogenic variants and phenotypes, and the relationship between *GBA* p.L444P and phenotypes (Supplementary Table 8). We found that patients with *GBA* possibly pathogenic variants were associated with more severe rapid eye movement sleep disorder, depression and autonomic dysfunction. Moreover, patients with *GBA* p.L444P were associated with younger age at onset, more severe motor symptoms, slighter tremor symptoms, more severe stiffness symptoms, more severe rapid eye movement sleep disorder, and autonomic dysfunction.

Discussion

With the development of precision medicine, an improved understanding of the genetic mechanism underlying Parkinson's disease is becoming increasingly important (Charvin *et al.*, 2018). In this study, we determined the mutational spectrum of 23 known Parkinson's disease-associated genes in purely clinically diagnosed Parkinson's disease patients with an early AAO or with a family history of Parkinson's disease, with a population from mainland China. Our results indicated that Parkinson's disease patients with an AAO of <40 years, especially those from autosomal-recessive families, may benefit from genetic counselling, and we identified patients with familial or early-onset Parkinson's disease who progressed relatively faster or slower.

Above all, this study enabled us to systematically and comprehensively estimate the prevalence of P/LP variants in populations from mainland China with early-onset and familial Parkinson's disease. As the most common recurrent gene, *PRKN* P/LP variants were identified in 4.95% of all patients (26.04% in the ARPD cohort and 2.66% in the sEOPD cohort). In addition, *LRRK2* p.G2019S accounts for 3–6% of familial Parkinson's disease cases in European populations and nearly 14% in Ashkenazi Jews (Shu *et al.*, 2019), but this variant was absent from our cohort, and 11 other variants were identified (0.60% in the entire Parkinson's disease cohort, 2.07% in the ADPD cohort, and 0.40% in the sEOPD cohort), which was similar to previous data for Korean (1.43% in early-onset Parkinson's disease) (Youn *et al.*, 2019) and Taiwanese populations (2.9% in patients with autosomal-dominant inheritance of parkinsonism) (Lin *et al.*, 2019). We also identified *PLA2G6* P/LP variants in six probands from the ARPD cohort and three probands in the sEOPD cohort, which suggested that *PLA2G6* was indispensable for molecular diagnosis in the mainland Chinese population, especially among patients with recessive inheritance or early-onset Parkinson's disease. We not only confirmed that *PRKN*, *LRRK2* and *PLA2G6* variants were most frequently found in Parkinson's disease cohorts, but our findings also implied that these genes should be screened for in patients from mainland China. In addition, our findings showed that families with dominant gene P/LP variants did not always segregate with the phenotype successfully, but recessive families almost always did; this may be due to the incomplete penetrance of dominant genes and the late AAO of the carriers. Specifically, *GBA* possibly pathogenic variants were identified in 3.64% (61 of 1676) of all patients, whereas *GBA* p.L444P was identified in 3.52% of all patients. The previous studies have also shown that heterozygous functional coding variants of *GBA* were identified in 4–15% of Parkinson's disease patients from different populations (Sidransky *et al.*, 2009; Lesage *et al.*, 2011; Gan-Or *et al.*, 2015*a*; Blauwendraat *et al.*, 2020). However, the pathogenicity of *GBA* possibly pathogenic variants still needs further validation using functional experiments or larger cohorts, especially the loss-of-function variants. Of note, though some genetic variants of low

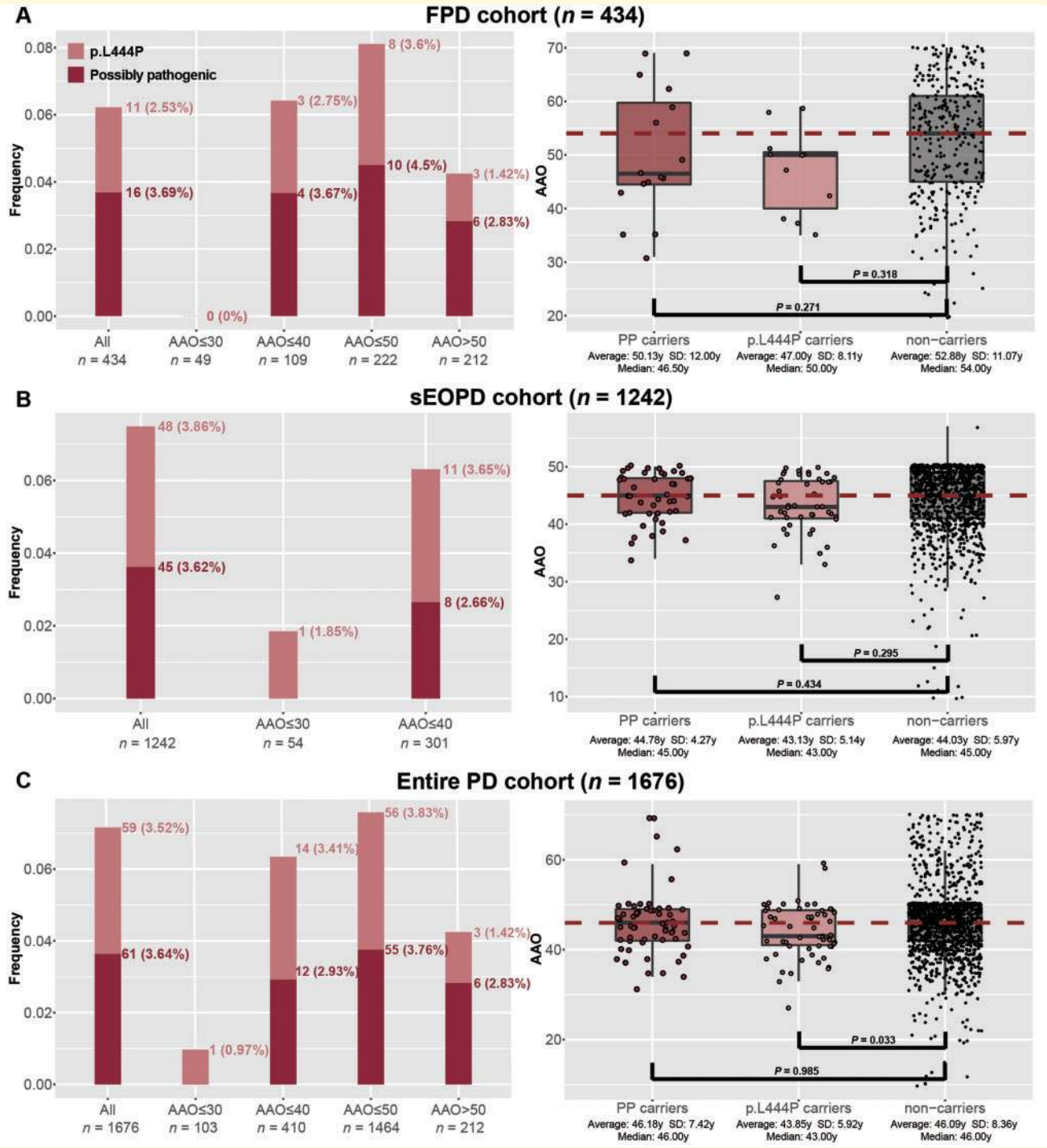

Figure 4 Mutational frequencies and AAO spectrum of *GBA* gene in the Parkinson's disease cohorts. Mutational frequencies of *GBA* possibly pathogenic (PP) variants and p.L444P variant in the different cohorts, spectrum of AAO in patients with *GBA* variants and p.L444P variant. (**A**) All familial Parkinson's disease (FPD) cohort. (**B**) The sEOPD cohort. (**C**) Entire Parkinson's disease (PD) cohort. *The dashed red line refers to the median AAO of patients without *GBA* possibly pathogenic variants, *GBA* p.L444P and P/LP variants in known Parkinson's disease-associated genes in the corresponding cohort. And *P*-value was adjusted for gender and disease duration at entry.

confidence genes (*GIGYF2*, *HTRA2*, *TMEM230*, *DNAJC13*, *LRP10* and *RIC3*) were evaluated as P/LP or VUS variants according to the same criteria of high confidence genes, the pathogenicity of low-confidence genes still needs to be replicated in larger multi-ethnic populations and further functional validations.

The clinical spectrum of Parkinson's disease-associated genes P/LP-variant subjects in our study was similar to the

subjects reported for other populations (Kasten *et al.*, 2017; Guo *et al.*, 2018*b*), suggesting that the pathogenicity of P/LP variants in Parkinson's disease-associated genes may conceal population differences in patients with different clinical phenotypes. In our study, we found that carriers of *PRKN*, *PLA2G6* and *PINK1* variants had an earlier AAO, that *PRKN* variants were associated with less severe motor symptoms, and that *PLA2G6* was associated with more severe motor symptoms, especially in terms of postural instability symptoms and freezing gait problems. The patients with an *SNCA* duplication in our cohorts showed worse cognitive dysfunction, more severe depression and were more prone to freezing gait. No special clinical manifestations were found in patients with *LRRK2* P/LP variants, which may indicate that *LRRK2*-linked Parkinson's disease has a similar clinical course to typical Parkinson's disease patients. The clinical symptoms found in patients with *GBA* L444P were similar to other studies (Zhang *et al.*, 2018; Yahalom *et al.*, 2019); however, motor symptoms of *GBA* L444P carriers are not significantly more severe compared to the non-carriers, and there is no cognitive decline, which may be due to the early-onset and relatively shorter disease duration of the carriers. Further prospective studies may be needed to confirm these associations. However, no differences of motor symptoms were found in *GBA* possibly pathogenic variants, which may further indicate that certain variants may not be relevant to the risk or phenotype of Parkinson's disease. Further functional experiments are needed to verify this. Moreover, other studies have also supported that *GBA* variants are associated with rapid eye movement sleep behaviour disorder (Gan-Or *et al.*, 2015*b*; Gamez-Valero *et al.*, 2018), which may also warrant further mechanistic studies. The similarity with other populations suggests that the associations between mutated genotypes and phenotypes in other populations could also be applied to our own populations; however, given the small samples of certain groups, further replication in a larger cohort is also needed to verify this viewpoint.

In this study, we also detected VUSs of dominant genes and heterozygous variants of recessive genes. The potential value of VUSs and heterozygous variants of recessive genes are presently uncertain; thus, they should be studied further to verify their contributions to Parkinson's disease. In line with the rapid pace of increasing knowledge of genetic causes of diseases, reassessing uncertain findings may improve current interpretations, as more data would become available through follow-up research, replication, or functional testing in the future. In this context, it is important to realize that whole-exome sequencing data should be considered a sustainable source of potential diagnostic clues, and should be re-examined at appropriate intervals, especially when the initial results are ambiguous (Liu *et al.*, 2019).

This study represents a comprehensive and systematic screening of Parkinson's disease-associated genes in early-onset and familial Parkinson's disease patients from mainland China, although the current study has some limitations. First, we only focused on known Parkinson's disease-associated genes, not susceptibility genes, risk loci, or new candidate genes, which may play important roles in early-onset Parkinson's disease and familial Parkinson's disease. Second, we screened for SNVs/indels by whole-exome sequencing and screened for CNVs in *PRKN*, *PINK1*, *ATP13A2*, *DJ1*, and *SNCA* by multiplex ligation-dependent probe amplification; yet we did not explore other unknown CNVs, structural variants, or other complex genetic abnormalities, which may need further exploration by whole-genome sequencing or even third-generation sequencing. Third, we only analysed coding region variations, but not non-coding regions such as regulatory elements, which may play important roles in the pathogenicity of Parkinson's disease. Lastly, we tried our best to collect data from family members of P/LP variants probands, but incomplete data for several families were collected, as they were settled in other places and may have lost touch with the probands, or refused to undergo genetic testing. Briefly, along with identifying pathogenic CNVs of known Parkinson's disease-associated genes, susceptibility genes, and risk loci, and discovering new Parkinson's disease-associated genes, it should also be possible to improve the molecular diagnostics of Parkinson's disease patients in the future (Liu *et al.*, 2019).

Conclusion

In conclusion, we conducted the most systematic survey of the mutational spectrum of sEOPD and familial Parkinson's disease patients in a mainland Chinese population. Our data indicate that Parkinson's disease patients with an AAO of <40 years may benefit from genetic counselling, especially those from families with a recessive inheritance pattern. Our findings also expand the existing repertoire of known variants in Parkinson's disease-associated genes and emphasized the potential of genetic testing to accurately guide patients to relevant clinical trials and targeted therapies. The similarity of clinical spectrum with those of other populations suggested that some findings from correlation studies of genotypes and phenotypes in other populations can also be applied to our own populations.

Acknowledgements

We are indebted to the participation of the patients and their family members in this study.

Funding

This study was supported by grants from the National Key Plan for Scientific Research and Development of China grants (Grant No. 2016YFC1306000, No. 2017YFC0909101, No.2017YFC0840100 and 2017YFC0840104), the National Natural Science Foundation of China (Grant No. 81430023), the Central Public-Interest Scientific Institution Basal Research

Fund of Chinese Academy of Medical Sciences (Grant No. 2018-12M-HL-025), the Young Elite Scientist Sponsorship Program by CAST (Grant No. 2018QNRC001), the Innovation-Driven Project of Central South University (Grant No. 20180033040004), Hunan Science Funds for Distinguished Young Scholar (Grant No. 2017JJ1037), the innovative team program from Department of Science & Technology of Hunan Province (Grant No. 2019RS1010), and the innovation-driven team project from Central South University (Grant No. 2020CX016).

Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

References

Aasly JO, Vilarino-Guell C, Dachsel JC, Webber PJ, West AB, Haugarvoll K, et al. Novel pathogenic LRRK2 p.Asn1437His substitution in familial Parkinson's disease. Mov Disord 2010; 25: 2156–63.

Alcalay RN, Caccappolo E, Mejia-Santana H, Tang MX, Rosado L, Ross BM, et al. Frequency of known mutations in early-onset Parkinson disease: implication for genetic counseling: the consortium on risk for early onset Parkinson disease study. Arch Neurol 2010; 67: 1116–22.

Bandres-Ciga S, Ahmed S, Sabir MS, Blauwendraat C, Adarmes-Gomez AD, Bernal-Bernal I, et al. The genetic architecture of parkinson disease in spain: characterizing population-specific risk, differential haplotype structures, and providing etiologic insight. Mov Disord 2019; 34: 1851–63.

Bandres-Ciga S, Diez-Fairen M, Kim JJ, Singleton AB. Genetics of Parkinson's disease: an introspection of its journey towards precision medicine. Neurobiol Dis 2020; 137: 104782.

Blauwendraat C, Heilbron K, Vallerga CL, Bandres-Ciga S, von Coelln R, Pihlstrom L, et al. Parkinson's disease age at onset genome-wide association study: defining heritability, genetic loci, and alpha-synuclein mechanisms. Mov Disord 2019a; 34: 866–75.

Blauwendraat C, Nalls MA, Singleton AB. The genetic architecture of Parkinson's disease. Lancet Neurol 2019b; 19: 170–8.

Blauwendraat C, Reed X, Krohn L, Heilbron K, Bandres-Ciga S, Tan M, et al. Genetic modifiers of risk and age at onset in GBA associated Parkinson's disease and Lewy body dementia. Brain 2020; 143: 234–48.

Bonifati V. LRRK2 low-penetrance mutations (Gly2019Ser) and risk alleles (Gly2385Arg)-linking familial and sporadic Parkinson's disease. Neurochem Res 2007; 32: 1700–8.

Bonifati V. Genetics of Parkinson's disease–state of the art, 2013. Parkinsonism Relat Disord 2014; 20: S23–8.

Charvin D, Medori R, Hauser RA, Rascol O. Therapeutic strategies for Parkinson disease: beyond dopaminergic drugs. Nat Rev Drug Discov 2018; 17: 844.

Deng HX, Shi Y, Yang Y, Ahmeti KB, Miller N, Huang C, et al. Identification of TMEM230 mutations in familial Parkinson's disease. Nat Genet 2016; 48: 733–9.

Di Fonzo A, Rohe CF, Ferreira J, Chien HF, Vacca L, Stocchi F, et al. A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease. Lancet 2005; 365: 412–5.

Escott-Price V, Nalls MA, Morris HR, Lubbe S, Brice A, et al. Polygenic risk of Parkinson disease is correlated with disease age at onset. Ann Neurol 2015; 77: 582–91.

Gamez-Valero A, Iranzo A, Serradell M, Vilas D, Santamaria J, Gaig C, et al. Glucocerebrosidase gene variants are accumulated in idiopathic REM sleep behavior disorder. Parkinsonism Relat Disord 2018; 50: 94–8.

Gan-Or Z, Amshalom I, Kilarski LL, Bar-Shira A, Gana-Weisz M, Mirelman A, et al. Differential effects of severe vs mild GBA mutations on Parkinson disease. Neurology 2015a; 84: 880–7.

Gan-Or Z, Mirelman A, Postuma RB, Arnulf I, Bar-Shira A, Dauvilliers Y, et al. GBA mutations are associated with rapid eye movement sleep behavior disorder. Ann Clin Transl Neurol 2015b; 2: 941–5.

Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988; 51: 745–52.

Gilks WP, Abou-Sleiman PM, Gandhi S, Jain S, Singleton A, Lees AJ, et al. A common LRRK2 mutation in idiopathic Parkinson's disease. Lancet 2005; 365: 415–6.

Guo JF, Xiao B, Liao B, Zhang XW, Nie LL, Zhang YH, et al. Mutation analysis of Parkin, PINK1, DJ-1 and ATP13A2 genes in Chinese patients with autosomal recessive early-onset Parkinsonism. Mov Disord 2008; 23: 2074–9.

Guo JF, Zhang L, Li K, Mei JP, Xue J, Chen J, et al. Coding mutations in NUS1 contribute to Parkinson's disease. Proc Natl Acad Sci U S A 2018a; 115: 11567–72.

Guo JF, Zhang XW, Nie LL, Zhang HN, Liao B, Li J, et al. Mutation analysis of Parkin, PINK1 and DJ-1 genes in Chinese patients with sporadic early onset parkinsonism. J Neurol 2010; 257: 1170–5.

Guo YP, Tang BS, Guo JF. PLA2G6-associated neurodegeneration (PLAN): review of clinical phenotypes and genotypes. Front Neurol 2018b; 9: 1100.

Gustavsson EK, Trinh J, McKenzie M, Bortnick S, Petersen MS, Farrer MJ, et al. Genetic Identification in Early Onset Parkinsonism among Norwegian Patients. Mov Disord Clin Pract 2017; 4: 499–508.

Haugarvoll K, Rademakers R, Kachergus JM, Nuytemans K, Ross OA, Gibson JM, et al. Lrrk2 R1441C parkinsonism is clinically similar to sporadic Parkinson disease. Neurology 2008; 70: 1456–60.

He R, Yan X, Guo J, Xu Q, Tang B, Sun Q. Recent Advances in Biomarkers for Parkinson's Disease. Front Aging Neurosci 2018; 10: 305.

Kang JF, Luo Y, Tang BS, Wan CM, Yang Y, Li K, et al. RAB39B gene mutations are not linked to familial Parkinson's disease in China. Sci Rep 2016; 6: 34502.

Kasten M, Hartmann C, Hampf J, Schaake S, Westenberger A, Vollstedt EJ, et al. Genotype-Phenotype Relations for the Parkinson's Disease Genes Parkin, PINK1, DJ1: MDSGene Systematic Review. Mov Disord 2018; 33: 730–41.

Kasten M, Marras C, Klein C. Nonmotor Signs in Genetic Forms of Parkinson's Disease. Int Rev Neurobiol 2017; 133: 129–78.

Klein C, Djarmati A, Hedrich K, Schafer N, Scaglione C, Marchese R, et al. PINK1, Parkin, and DJ-1 mutations in Italian patients with early-onset parkinsonism. Eur J Hum Genet 2005; 13: 1086–93.

Konno T, Ross OA, Puschmann A, Dickson DW, Wszolek ZK. Autosomal dominant Parkinson's disease caused by SNCA duplications. Parkinsonism Relat Disord 2016; 22: S1–6.

Lesage S, Anheim M, Condroyer C, Pollak P, Durif F, Dupuits C, et al. Large-scale screening of the Gaucher's disease-related glucocerebrosidase gene in Europeans with Parkinson's disease. Hum Mol Genet 2011; 20: 202–10.

Li H. Toward better understanding of artifacts in variant calling from high-coverage samples. Bioinformatics 2014; 30: 2843–51.

Li J, Shi L, Zhang K, Zhang Y, Hu S, Zhao T, et al. VarCards: an integrated genetic and clinical database for coding variants in the human genome. Nucleic Acids Res 2018a; 46: D1039–48.

Li J, Zhao T, Zhang Y, Zhang K, Shi L, Chen Y, et al. Performance evaluation of pathogenicity-computation methods for missense variants. Nucleic Acids Res 2018b; 46: 7793–804.

Lim SY, Tan AH, Ahmad-Annuar A, Klein C, Tan LCS, Rosales RL, et al. Parkinson's disease in the Western Pacific Region. Lancet Neurol 2019; 18: 865–79.

Lin CH, Chen PL, Tai CH, Lin HI, Chen CS, Chen ML, et al. A clinical and genetic study of early-onset and familial parkinsonism in taiwan: an integrated approach combining gene dosage analysis and next-generation sequencing. Mov Disord 2019; 34: 506–15.

Liu P, Meng L, Normand EA, Xia F, Song X, Ghazi A, et al. Reanalysis of Clinical Exome Sequencing Data. N Engl J Med 2019; 380: 2478–80.

Liu Z, Guo J, Li K, Qin L, Kang J, Shu L, et al. Mutation analysis of CHCHD2 gene in Chinese familial Parkinson's disease. Neurobiol Aging 2015; 36: 3117 e7–e8.

Lubbe SJ, Escott-Price V, Gibbs JR, Nalls MA, Bras J, Price TR, et al. Additional rare variant analysis in Parkinson's disease cases with and without known pathogenic mutations: evidence for oligogenic inheritance. Hum Mol Genet 2016; 25: 5483–9.

Marder KS, Tang MX, Mejia-Santana H, Rosado L, Louis ED, Comella CL, et al. Predictors of parkin mutations in early-onset Parkinson disease: the consortium on risk for early-onset Parkinson disease study. Arch Neurol 2010; 67: 731–8.

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 2010; 20: 1297–303.

Moller JC, Rissling I, Mylius V, Hoft C, Eggert KM, Oertel WH. The prevalence of the G2019S and R1441C/G/H mutations in LRRK2 in German patients with Parkinson's disease. Eur J Neurol 2008; 15: 743–5.

Montaut S, Tranchant C, Drouot N, Rudolf G, Guissart C, Tarabeux J, et al. Assessment of a targeted gene panel for identification of genes associated with movement disorders. JAMA Neurol 2018; 75: 1234–45.

Nichols WC, Pankratz N, Hernandez D, Paisan-Ruiz C, Jain S, Halter CA, et al. Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease. Lancet 2005; 365: 410–2.

Nishioka K, Ross OA, Ishii K, Kachergus JM, Ishiwata K, Kitagawa M, et al. Expanding the clinical phenotype of SNCA duplication carriers. Mov Disord 2009; 24: 1811–9.

Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, et al. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord 2015; 30: 1591–601.

Puschmann A. Monogenic Parkinson's disease and parkinsonism: clinical phenotypes and frequencies of known mutations. Parkinsonism Relat Disord 2013; 19: 407–15.

Quadri M, Mandemakers W, Grochowska MM, Masius R, Geut H, Fabrizio E, et al. LRP10 genetic variants in familial Parkinson's disease and dementia with Lewy bodies: a genome-wide linkage and sequencing study. Lancet Neurol 2018a; 17: 597–608.

Quadri M, Mandemakers W, Kuipers D, Breedveld GJ, Bonifati V. LRP10 in alpha-synucleinopathies-Authors' reply. Lancet Neurol 2018b; 17: 1035–6.

Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17: 405–24.

Schneider SA, Alcalay RN. Neuropathology of genetic synucleinopathies with parkinsonism: review of the literature. Mov Disord 2017; 32: 1504–23.

Shi CH, Tang BS, Wang L, Lv ZY, Wang J, Luo LZ, et al. PLA2G6 gene mutation in autosomal recessive early-onset parkinsonism in a Chinese cohort. Neurology 2011; 77: 75–81.

Shu L, Zhang Y, Sun Q, Pan H, Tang B. A Comprehensive Analysis of Population Differences in LRRK2 Variant Distribution in Parkinson's Disease. Front Aging Neurosci 2019; 11: 13.

Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. N Engl J Med 2009; 361: 1651–61.

Singleton A, Hardy J. The evolution of genetics: Alzheimer's and Parkinson's Diseases. Neuron 2016; 90: 1154–63.

Singleton A, Hardy J. Progress in the genetic analysis of Parkinson's disease. Hum Mol Genet 2019; 28: R215–R218.

Sudhaman S, Muthane UB, Behari M, Govindappa ST, Juyal RC, Thelma BK. Evidence of mutations in RIC3 acetylcholine receptor chaperone as a novel cause of autosomal-dominant Parkinson's disease with non-motor phenotypes. J Med Genet 2016; 53: 559–66.

Sun QY, Guo JF, Wang L, Yu RH, Zuo X, Yao LY, et al. Glucocerebrosidase gene L444P mutation is a risk factor for Parkinson's disease in Chinese population. Mov Disord 2010; 25: 1005–11.

Tan MMX, Malek N, Lawton MA, Hubbard L, Pittman AM, Joseph T, et al. Genetic analysis of Mendelian mutations in a large UK population-based Parkinson's disease study. Brain 2019a; 142: 2828–44.

Tan MMX, Malek N, Lawton MA, Hubbard L, Pittman AM, Joseph T, et al. Genetic analysis of Mendelian mutations in a large UK population-based Parkinson's disease study. Brain 2019b.

Tan EK, Skipper L, Chua E, Wong MC, Pavanni R, Bonnard C, et al. Analysis of 14 LRRK2 mutations in Parkinson's plus syndromes and late-onset Parkinson's disease. Mov Disord 2006; 21: 997–1001.

Tang B, Xiong H, Sun P, Zhang Y, Wang D, Hu Z, et al. Association of PINK1 and DJ-1 confers digenic inheritance of early-onset Parkinson's disease. Hum Mol Genet 2006; 15: 1816–25.

Trinh J, Lohmann K, Baumann H, Balck A, Borsche M, Bruggemann N, et al. Utility and implications of exome sequencing in early-onset Parkinson's disease. Mov Disord 2019; 34: 133–7.

Trinh J, Zeldenrust FMJ, Huang J, Kasten M, Schaake S, Petkovic S, et al. Genotype-phenotype relations for the Parkinson's disease genes SNCA, LRRK2, VPS35: MDSGene systematic review. Mov Disord 2018; 33: 1857–70.

Velez-Pardo C, Lorenzo-Betancor O, Jimenez-Del-Rio M, Moreno S, Lopera F, Cornejo-Olivas M, et al. The distribution and risk effect of GBA variants in a large cohort of Parkinson's disease patients from Colombia and Peru. Parkinsonism Relat Disord 2019; 63: 204–8.

Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 2010a; 38: e164.

Wang L, Guo JF, Nie LL, Xu Q, Zuo X, Sun QY, et al. A novel LRRK2 mutation in a mainland Chinese patient with familial Parkinson's disease. Neurosci Lett 2010b; 468: 198–201.

Wang JL, Xiao B, Cui XX, Guo JF, Lei LF, Song XW, et al. Analysis of SCA2 and SCA3/MJD repeats in Parkinson's disease in mainland China: genetic, clinical, and positron emission tomography findings. Mov Disord 2009; 24: 2007–11.

Xu Q, Li K, Sun Q, Ding D, Zhao Y, Yang N, et al. Rare GCH1 heterozygous variants contributing to Parkinson's disease. Brain 2017; 140: e41.

Yahalom G, Greenbaum L, Israeli-Korn S, Fay-Karmon T, Livneh V, Ruskey JA, et al. Carriers of both GBA and LRRK2 mutations, compared to carriers of either, in Parkinson's disease: Risk estimates and genotype-phenotype correlations. Parkinsonism Relat Disord 2019; 62: 179–84.

Yan W, Tang B, Zhou X, Lei L, Li K, Sun Q, et al. TMEM230 mutation analysis in Parkinson's disease in a Chinese population. Neurobiol Aging 2017; 49: 219 e1–19 e3.

Yang H, Wang K. Genomic variant annotation and prioritization with ANNOVAR and wANNOVAR. Nat Protoc 2015; 10: 1556–66.

Youn J, Lee C, Oh E, Park J, Kim JS, Kim HT, et al. Genetic variants of PARK genes in Korean patients with early-onset Parkinson's disease. Neurobiol Aging 2019; 75: 224 e9–e15.

Zabetian CP, Samii A, Mosley AD, Roberts JW, Leis BC, Yearout D, et al. A clinic-based study of the LRRK2 gene in Parkinson disease yields new mutations. Neurology 2005; 65: 741–4.

Zhang Y, Shu L, Zhou X, Pan H, Xu Q, Guo J, et al. A meta-analysis of GBA-related clinical symptoms in Parkinson's disease. Parkinsons Dis 2018; 2018: 3136415.