Neurobiology of Aging 76 (2019) 215.e1–215.e7

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Systematically analyzing rare variants of autosomal-dominant genes for sporadic Parkinson's disease in a Chinese cohort

Nannan Yang [a], Yuwen Zhao [a], Zhenhua Liu [a], Rui Zhang [a], Yan He [a], Yangjie Zhou [a], Qian Xu [a,b], Qiying Sun [a,b], Xinxiang Yan [a], Jifeng Guo [a,b,c,d], Beisha Tang [a,b,c,d,e,f,*]

[a] Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan, P.R. China
[b] National Clinical Research Center for Geriatric Medicine, Changsha, Hunan, P.R. China
[c] Key of Hunan Province in Neurodegenerative Disorders, Central South University, Changsha, Hunan, P.R. China
[d] Parkinson's Disease Center of Beijing Institute for Brain Disorders, Beijing, P.R. China
[e] Collaborative Innovation Center for Brain Science, Shanghai, P.R. China
[f] Collaborative Innovation Center for Genetics and Development, Shanghai, P.R. China

ARTICLE INFO

Article history:
Received 6 May 2018
Received in revised form 4 July 2018
Accepted 13 November 2018
Available online 20 November 2018

Keywords:
Sporadic Parkinson's disease
Rare variant of autosomal-dominant PD genes
Burden analysis

ABSTRACT

Studies have shown that rare variants of Mendelian genes for Parkinson's disease (PD) contribute to sporadic PD in the Caucasian population, which lacked confirmation in the Chinese population. Because the autosomal-dominant PD (AD-PD) had a phenotype closely resembling sporadic PD, we performed a systematic analysis of 7 AD-PD genes (*SNCA*, *LRRK2*, *GIGYF2*, *VPS35*, *EIF4G1*, *DNAJC13*, and *CHCHD2*) in 1456 Chinese sporadic PD patients and 1568 controls. Overall, 72 rare variants were identified, 7 of which were classified as likely pathogenic, 63 of which were categorized as of uncertain significance, and 2 of them were predicted to be likely benign. These AD-PD genes represented a clear enrichment of rare variants in PD patients from a burden analysis ($p = 0.003$), and significant differences could still be observed when likely pathogenic variants were removed ($p = 0.027$). The gene-based association testing also reached significance for *LRRK2* ($p = 0.004$) and remained statistically significant after the Bonferroni correction. This report suggested that rare variants of AD-PD genes had a role in the Chinese sporadic PD cohort, especially for those rare variants of *LRRK2*.

1. Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder, with an estimated prevalence of 1% in people aged 65 years or older worldwide (Kalia and Lang, 2015). Most PD cases are sporadic, only approximately 11% of patients with PD have one or more first-degree relatives diagnosed with PD. To date, several genes causing familial PD have been identified through linkage studies, including dominant genes (*SNCA*, *LRRK2*, *GIGYF2*, *VPS35*, *EIF4G1*, *DNAJC13*, *CHCHD2*, and *TMEM230*) and recessive genes (*PARK2, PINK1, DJ-1, ATP13A2, PLA2G6, FBXO7, DNAJC6, SYNJ1,* and *VPS13C*), representing 10% of all the familial cases (Clarimon and Kulisevsky, 2013; Funayama et al., 2015; Hattori, 2012; Krebs et al., 2013; Lesage et al., 2016; Sundal et al., 2012; Vilarino-Guell et al., 2014). Apart from the rare variant studies of familial PD, recent genome-wide association studies have also identified at least 40 common risk loci for sporadic PD, with 2 of the genes uncovered turning out to be familial PD-linked genes: *SNCA* and *LRRK2*, which indicate the linkage between familial PD and sporadic PD and the possibility of learning about the molecular pathogenesis of sporadic PD through investigating the Mendelian genes in sporadic PD (Chang et al., 2017; Nalls et al., 2014).

For genome-wide association studies approaches in sporadic PD, the common risk loci that have been found only accounted for a small part of the genetic factors, while with the advent of sequencing technologies and recent large-scale studies of human variation, rare coding variants that were more prone to affect the function gradually, drew more attention, and were suggested to help explain the so-called "missing heritability" in complex diseases such as sporadic PD (Fu et al., 2013; Maher, 2008; Manolio et al., 2009; Pihlstrom and Toft, 2011; Zuk et al., 2014).

* Corresponding author at: Department of Neurology, Xiangya Hospital, 87 of Xiangya Road, Changsha, Hunan Province, China; National Clinical Research Center for Geriatric Medicine, Changsha, Hunan, P.R. China; Key Laboratory of Hunan Province in Neurodegenerative Disorders, Central South University, Changsha, Hunan, P.R. China; Parkinson's Disease Center of Beijing Institute for Brain Disorders, Beijing, P.R. China; Collaborative Innovation Center for Brain Science, Shanghai, P.R. China; Collaborative Innovation Center for Genetics and Development, Shanghai, P.R. China. Tel.: +86 13974856709; fax: 0731-84327332.
E-mail address: bstang7398@163.com (B. Tang).

0197-4580/$ – see front matter © 2018 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.neurobiolaging.2018.11.012

Studies from the Caucasian population representing rare variants of Mendelian genes for PD showed a clear enrichment in sporadic PD patients (Benitez et al., 2016; Foo et al., 2014; Spataro et al., 2015), and this result was barely confirmed in a large Chinese cohort. Because autosomal-dominant PD (AD-PD) had a phenotype closely resembling sporadic PD (Schulte and Gasser, 2011), we assumed that rare variants from AD-PD genes also contributed to the etiology of sporadic PD in the Chinese population, and we performed a systemic screening of 7 AD-PD genes (*SNCA*, *LRRK2*, *GIGYF2*, *VPS35*, *EIF4G1*, *DNAJC13*, and *CHCHD2*) in a large Chinese cohort of 1456 idiopathic PD patients and 1568 unrelated controls to elucidate this assumption.

2. Methods

2.1. Participants

The Human Research Ethics Committee of Central South University approved this study, and all the subjects who participated in this study completed informed consent before the original sample collection. The genomic DNA samples were isolated from the peripheral blood leukocytes using standard procedures from all the subjects. The subjects enrolled in this cohort were recruited from the outpatient neurology clinics of Xiangya Hospital between October 2006 and June 2016. In total, 1456 cases of sporadic PD and 1568 age- and sex-matched healthy control subjects were included in this study. PD was diagnosed according to the UK PD Society Brain Bank clinical diagnostic or MDS clinical diagnostic criteria for PD (Hughes et al., 1992; Postuma et al., 2015). The basic information and demographic characteristics are shown in Table 1.

2.2. Targeted resequencing, variant filtering, validation, and analysis

Seven AD-PD genes (*SNCA*, *LRRK2*, *GIGYF2*, *VPS35*, *EIF4G1*, *DNAJC13*, and *CHCHD2)* were selected as targeted genes for capturing and sequencing analyses. Molecular inversion probes (MIPs) were designed to capture all the exons and intron-exon boundaries (5-bp flanking sequences) of the targeted genes, and Supplementary Table S1 shows some MIPs used to sequence those target genes. Detailed methodology is described elsewhere (Hiatt et al., 2013; Nuttle et al., 2014). Briefly, MIPs were first pooled together by gene; next these pooled MIPs were used to capture the genomic DNA. Then, the captured DNA was amplified and subjected to massively parallel sequencing using a 100 paired-end protocol on the HiSeq 3000 platform for the library establishment and high-throughput sequencing stage. Then, reads were mapped to the hg19 human reference genome using Burrows-Wheeler Aligner (Li and Durbin, 2010) and followed the Genome Analysis Toolkit Best Practices for v3 (Van der Auwera et al., 2013). The variants were filtered based on a read depth $\geq$4×, a genotype quality $\geq$20, and proportions of the reads with alternative alleles $\geq$0.3. Four publicly available resources were used to extract the variant frequency data: the 1000 Genomes Project (Oct 2014), the Exome Aggregation Consortium, the dbSNP138, and the Exome Sequencing Project (ESP6500). All the rare variants were validated using PCR and Sanger sequencing.

Table-1
Summary of sample demographics

Series	N	Age at onset mean(AAO) ± standard deviation (SD)in years	Male:Female ratio
Total PD	1456	49.34 ± 13.27	733:723
EOPD (AAO <50)	736	41.06 ± 7.58	381:355
LOPD (AAO ≥50)	720	57.79 ± 6.37	352:368
Controls	1568	50.73 ± 16.68	764:804

2.3. Criteria for pathogenicity of rare variants

For the interpretation of these validated variants, American College of Medical Genetics and Genomics (ACMG) guidance was adopted to classify their pathogenicity, and the variants were classified as pathogenic, likely pathogenic, of uncertain significance, likely benign or benign based on criteria and using typical types of variant evidence (population data, computational predication, function data, and segregation data) (Richards et al., 2015).

2.4. Statistical analysis

The rare variants were defined as variants with a minor allele frequency $\leq$0.1% and were included in the gene-based burden test. The association between rare variants and PD was analyzed by using Fisher's exact test, odd ratios (OR), and 95% confidence intervals (CI). The analyses were performed using the statistical analysis program, R (http://www.r-project.org).

3. Results

We resequenced the protein-coding regions of 7 AD-PD genes in 1456 Chinese sporadic PD patients and 1568 unrelated healthy controls. Overall, the percentages of read depth of the targeted genes were 96%, 92%, and 84% of the bases covered by at least 4X, 10X, and 20X, respectively (Supplementary Table S2, Figure S1). Overall, 72 rare nonsynonymous-coding variants with minor allele frequency $\leq$0.1% were identified in the exon regions of these 7 genes after applying a quality filter. Among them, 31.94% (23/72) of the variants were found in *LRRK2*, 22.22% (16/72) in *GIGYF2*, 19.44% (14/72) in *EIF4G1*, 16.67% (12/72) in *DNAJC13*, 2.78% (2/72) in *CHCHD2* and *VPS35*, and 2.78% (2/72) in *SNCA* (Table 2).

3.1. Single variant analysis

For these 72 rare nonsynonymous-coding variants that were detected in our cohort, ACMG guidelines were further applied to classify their own pathogenicity. In total, 7 variants were categorized as likely pathogenic: 5 from *LRRK2*, 1 from *GIGYF2*, and 1 from *CHCHD2*; 2 variants from *DNAJC13* were classified as likely benign; and the remaining 63 variants were predicted to be of uncertain significance (Table 2; Supplementary Table S3).

3.1.1. LRKK2

A total of 23 rare variants were found in *LRRK2*; 5 of them were classified as likely pathogenic (p.E255K, p.V414I, p.R1067Q, p.I1192M, and p.I1548V), and the locations of the observed variants are depicted in Fig. 1. Among them,

Table 2
Summary of the rare variants found in the cohort

Gene	Total rare variants	Likely pathogenic	Uncertain significance	Benign
SNCA	2	0	2	0
LRRK2	23	5	18	0
GIGYF2	16	1	15	0
VPS35	3	0	3	0
EIF4G1	14	0	14	0
DNAJC13	12	0	10	2
CHCHD2	2	1	1	0
Total	72	7	63	2

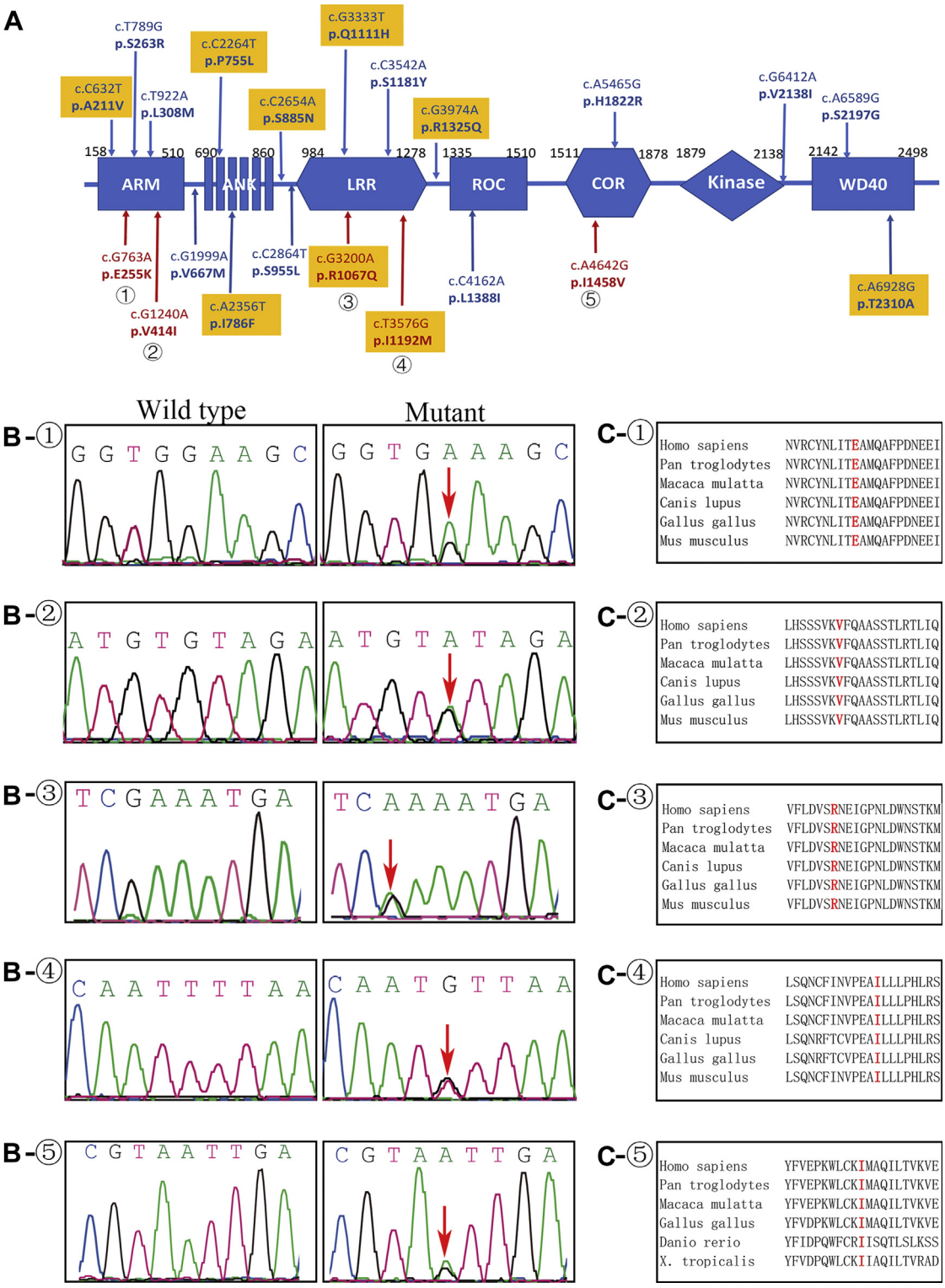

Fig. 1. Variants detected in *LRRK2* (A) Schematic representations of the lrrk2 protein with blue boxes indicating different domains. Variants found in present study were indicated by arrows and variants that were classified as of likely pathogenic were highlighted in red. Variants that have been previously reported were highlighted in yellow. (B) Electropherograms of the sequence of the likely pathogenic variants (C) the position and surroundings of those likely pathogenic *LRRK2* variants were highly conserved across different species. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

p.R1067Q and p.I1192M have previously been reported as pathogenic mutations, whereas the other 3 were novel variants. All of them were located in mutational hot spots, determined as disease causing by Polyphen-2 and CADD, and remained conserved by the prediction of GERP++. In addition, these variants were absent in our gender-matched healthy control cohort, as well as the 1000G database and the ExAC database (Table 3).

Table 3
Summary of variants classified as likely pathogenic

Gene	*LRRK2* p.E255 K	*LRRK2* p.V414I	*LRRK2* p.R1067Q＼1	*LRRK2* p.I1192 M＼1	*LRRK2* p.I1548 V＼1	*GIGYF2* p.S566P＼1	*CHCHD2* p.A79S＼1
Position	40637408	40646770	40692148	40696670	40707879	233671257	56171984
Freq. patient	0.0002749	0.00028	0.0008621	0.0002775	0.0002758	0.0002744	0.000274
Freq. control	0	0	0	0	0	0	0
Freq. 1000G	NA	NA	NA	NA	NA	NA	NA
Freq. ExAC	1.65E-05	NA	3.30E-05	8.24E-06	NA	NA	NA
dbSNP ID	NA	NA	rs111341148	NA	NA	NA	NA
SIFT score	0.31	0.21	0.2	0.03	0.03	0.01	0.11
Polyphen score	0.873	1	1	1	0.73	0.999	1
CADD score	29.5	16.17	34	16.36	16.36	20.8	34
GERP++score	5.95	5.21	5.85	2.45	5.83	5.84	5.62
ACMG	PM1/PM2/PM6/PP3	PM1/PM2/PM6/PP3	PM1/PM2/PP3/PP5	PM1/PM2/PP3/PP5	PM1/PM2/PP3/PM6	PMI/PM2/PP2/PP3	PMI/PM2/PP2/PP3
Sex	Female	Female	Male	Male	Male	Female	Female	Male
AAO	61	35	53	45	61	70	64	42
IS	T	T	PI	B	B	B	B	T
B	+	+	+	+	+	+	+	+
R	+	+	+	+	+	+	+	+
T	+	+	+	−	−	−	+	+
PI	+	−	+	+	−	−	+	−
L-DOPA RESPONSIVE	Good	Good	Good	Good	Not good	Good	Not treated	Good
DK	+	+	+	−	−	−	−	−
DM	−	−	−	−	−	+	−	−

Threshold values for deleteriousness: SIFT-less than 0.05; Polyphen-2 greater than 0.86; CADD-greater than 12.35; GERP++ is a score for the conservation of the amino acid; scores>3 can be considered as highly conserved.
Key: NA, not found; AAO, age at onset; IS, initial symptoms; B, bradykinesia; R, rigidity; T, resting tremor; PI, postural instability; DK, dyskinesia; DM, dementia; +, positive; −, negative.

3.1.2. *GIGYF2*

One of the 16 rare variants that found in *GIGYF2*, p.S566P, was classified as likely pathogenic. This novel variant was located in the GYF domain and was highly conserved across different species; it is predicted to disrupt the binding between the GYF domain and its interacting ligands (Fig. 2, Table 3).

3.1.3. *CHCHD2*

One novel rare variant, p.A79S, was classified as likely pathogenic. It was located in a highly conserved domain and was predicted to be disease causing by CADD, Polyphen-2, and SIFT (Fig. 3); it was not found in our gender-matched 1565 healthy control individuals and was absent in the 1000G and ExAc databases (Table 4).

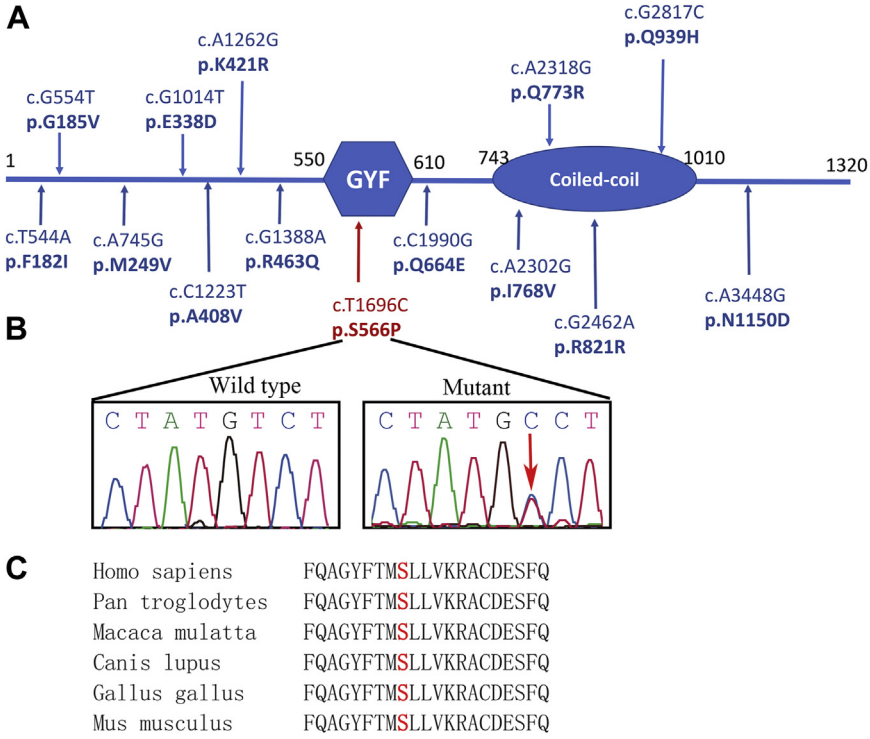

Fig. 2. Variants detected in *GIGYF2*. (A) Schematic representations of the gifyf2 protein with blue boxes indicating different domains. Variants found in present study were indicated by arrows and variants that were classified as of likely pathogenic were highlighted in red. (B) Electropherograms of the sequence of *GIGYF2* p.S566P. (C) The position and surrounding of this likely pathogenic *GIGYF2* p.S566P was highly conserved across different species. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

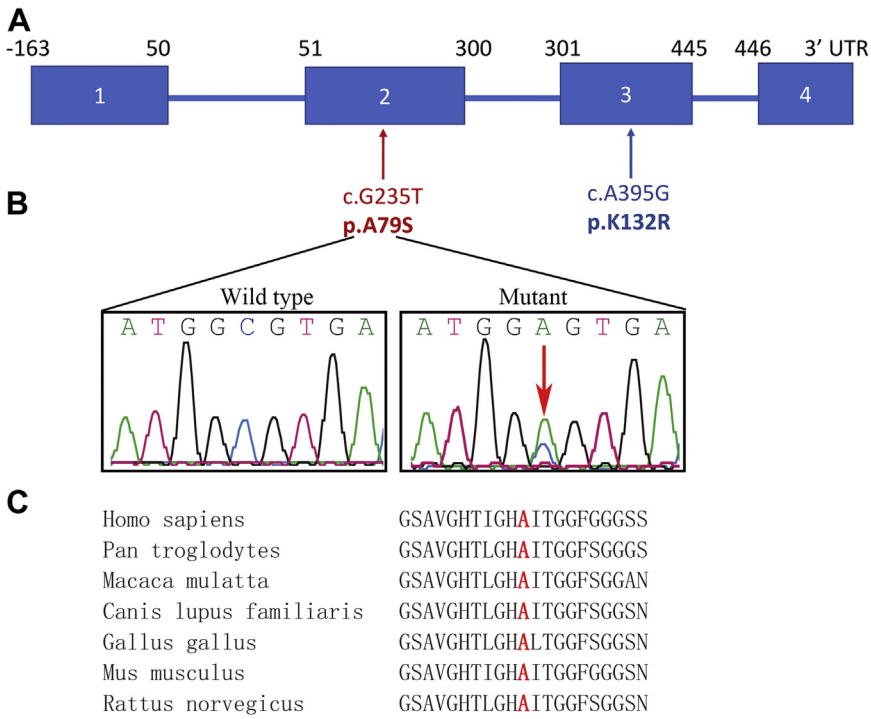

Fig. 3. Variants detected in *CHCHD2*. (A) Schematic representations of the *CHCHD2* gene with blue boxes indicating exons 1-4. Variants found in present study were indicated by arrows and variants that were classified as of likely pathogenic were highlighted in red. (B) Electropherograms of the sequence of *CHCHD2* p.A79S. Pathogenic variants (C) the position and surrounding of *CHCHD2* p.A79S was highly conserved across different species. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.1.4. Other genes

A total of 2 variants in *DNAJC13* were categorized as likely benign; all the variants in *EIF4G1*, *DNAJC13*, *Omi/HTRA2*, *VPS35*, and *SNCA* were categorized as of uncertain significance (Supplementary Table S2).

3.2. Gene-based burden analysis

To further investigate whether rare variants in these 7 AD-PD genes contribute collectively to sporadic PD risk, we performed a gene-based burden test using the Fisher's exact test (Nicolae, 2016), and a clear general enrichment was detected in PD patients (p = 0.003; OR = 1.760; 95% CI = 1.201–2.579) (Table 4). In addition, significant differences could still be observed between PD patients and controls when we conducted another association test after removing the deleterious 8 rare variants, (p = 0.027; OR = 1.550; 95% CI = 1.047–2.294) (Table 4).

To uncover whether specific genes account for this observed rare variant association in our cohort, we then assessed the accumulation of these rare variants for each gene in the patients and the controls. The gene-based association testing reached significance for *LRRK2* (p = 0.004; OR = 2.643; 95% CI = 1.316–5.309) (Table 4) and remained statistically significant after the Bonferroni correction (α = 0.05/9 = 0.0055). Moderate enrichment was also observed for *GIGYF2* (p = 0.035; OR = 2.568; 95% CI = 1.059–5.966) (Table 4), but the significance failed to reach the Bonferroni-corrected threshold (α =0.0055). No significant differences were found for *SNCA*, *VPS35*, *EIF4G1*, *DNAJC13*, and *CHCHD2* (Table 4).

4. Discussion

This was the first study to systematically screen rare variants of AD-PD genes in a large sporadic PD Chinese cohort. Overall, 72 rare nonsynonymous-coding variants with a frequency ≤0.1% were

Table 4
Summary of the gene-based burden test for unavailable

Gene	All rare variants involved				Remove deleterious variants			
	Cases/1456	Control/1568	*p*-value	OR (95% CI)	Cases/1456	Control/1568	*p*-value	OR (95% CI)
LRRK2	27	11	0.004[a]	2.643 (1.316–5.309)	21	11	0.047[b]	2.056 (1.001–4.249)
GIGYF2	16	7	0.035[b]	2.568 (1.059–5.966)	-	-	-	
DNAJC13	9	8	0.628		-	-	-	
VPS35	1	2	0.629		-	-	-	
EIF4G1	11	11	0.784		-	-	-	
CHCHD2	2	1	0.907		-	-	-	
SNCA	1	1	0.934		-	-	-	
Total	67	41	0.003[a]	1.760 (1.201–2.579)	59	41	0.027[a]	1.550 (1.047–2.294)

[a] For statistically significant.
[b] For moderately significant.

identified in the exon regions of these genes. When we utilized the latest ACMG guidelines to classify their pathogenicity, 7 of these 72 variants were categorized as likely pathogenic, 63 of them were clustered to be of uncertain significance, and 2 of them were predicted to be likely benign.

Five of these likely pathogenic variants were detected in *LRRK2*, which encoded a large, multidomain protein, with pathogenic mutations mostly occurring in several functional domains (Hernandez et al., 2016). In our present study, those 5 likely pathogenic variants were located in different domains: p.E255K and p.V414I in armadillo repeats (ARM), p.R1067Q and p.I1192M in leucine rich repeat (LRR), and p.I1458V in the C-terminal of roc domain (COR). In total, 6 patients were found to carry those variants, and they showed typical parkinsonian symptoms. Most of them progressed slowly, responded well to levodopa treatment and were less likely to develop dementia, which was in accordance with previous studies both in Chinese and Caucasian populations (Table 3) (Haugarvoll and Wszolek, 2009; Peng et al., 2017; Wang et al., 2010).

In our present study, one novel p.S566P in *GIGYF2* was discovered in our 1565 sporadic PD cohort and it was predicted to be likely pathogenic, which might contradict the current main opinion about this gene because subsequent studies found mutations in controls or not cosegregating with the PD phenotype, and its pathogenicity was still in doubt and needed to be confirmed (Nichols et al., 2009; Puschmann, 2013). As far as this study was concerned, it was still plausible because we had a larger sample number than previous studies, but this gene might make limited contributions to sporadic PD because the variants was so rare and could barely be detected.

The last deleterious variant was detected in *CHCHD2*, which encoded a coiled-coil-helix-coiled-coil-helix domain containing protein and may be linked to cytochrome oxidase activity and mitochondrial functions (Funayama et al., 2015). To date, 7 mutations have been found to be linked with PD (Funayama et al., 2015; Jansen et al., 2015; Koschmidder et al., 2016). In our study, none of the 7 known mutations were found, but a novel likely pathogenic p.A79S variant was discovered. Similar to other typical PD patients, the male patient who carried this variant manifested resting tremors at the age of 43 years, gradually developed bradykinesia and mask face in a year and was diagnosed with PD. The patient also responded well to levodopa treatment (Table 3).

Overall, these 7 likely pathogenic variants accounted for 0.48% in the present Chinese sporadic PD cohort, explaining some of the genetic factors. However, there were still another 63 rare variants, which remained to be of uncertain significance, and their roles in PD are unknown to some extent. Recently, one European-originated Caucasian cohort reported a clear general enrichment of Mendelian genes of rare functional variants in PD patients, suggesting that rare variants of Mendelian genes for PD may have a role in sporadic forms (Spataro et al., 2015). The autosomal-dominant PD had a phenotype closely resembling sporadic PD, so we hypothesized that rare variants of AD-PD genes may also contribute to the risk of sporadic PD in the Chinese cohort and could help shed light on the pathogenesis of sporadic forms of this disease. First, we conducted a total analysis of the accumulation of those rare variants in PD patients and controls, and PD patients turned out to be more abundant with more rare variants than controls ($p = 0.003$; OR = 1.760; 95% CI = 1.201–2.579), indicating that those dominant genes did have a role in Chinese sporadic PD. However, those likely pathogenic variants were also included in this first collective analysis, which may greatly contribute to this enrichment. To further explore the role of those 63 variants of uncertain significance in PD, we performed another gene-based burden test after removal of those 8 deleterious rare variants, and significant differences could still be observed between patients and controls ($p = 0.027$; OR = 1.550; 95% CI =1.047–2.294), showing that these additional variants also contribute to sporadic PD.

According to the first stage analysis, we demonstrated that those rare variants showed significant enrichment in PD patients, confirming their influence on the risk of PD, and was in accordance with studies from the Caucasian population. However, which gene played the most important part during this enrichment remained to be explored, so we proceeded to assess the aggregation difference in PD patients and controls for each gene. Among those 7 genes, *LRRK2* was significantly different among the patients and controls ($p = 0.004$; OR = 2.643; 95% CI = 1.316–5.309) and managed to reach the Bonferroni-corrected threshold ($\alpha = 0.05/9 = 0.0055$). The result was consistent with the East Asian population PD cohort and one European American PD cohort, during which significant enrichment of *LRRK2* rare variants were both observed in PD patients (Benitez et al., 2016; Foo et al., 2014), demonstrating that *LRRK2* was involved in sporadic PD. Moderate enrichment was also observed for *GIGYF2* ($p = 0.035$; OR = 2.568; 95% CI = 1.059–5.996), but the significance failed to reach the Bonferroni-corrected threshold (α=0.0055), which suggested that they might make very limited contributions to PD and that follow-up studies would be needed to determine the exact risk associated with these variants.

5. Conclusion

In conclusion, 72 rare variants in AD-PD genes were identified in this large case-control study; 7 of them were classified as likely pathogenic, 63 variants were categorized as of uncertain significance, and 2 of them were predicted to be likely benign. These AD-PD genes represented a clear enrichment of rare variants in PD patients by burden analysis ($p = 0.003$), and significant differences could still be observed when likely pathogenic variants were removed ($p = 0.027$), indicating that those variants of uncertain significance also contribute to the risk of sporadic PD. The gene-based association testing reached significance for *LRRK2* ($p = 0.004$) and reached statistical significance after the Bonferroni correction, showing that *LRRK2* may account for most of the rare variants enrichment of the 7 AD-PD genes. Our findings suggested that rare variants of AD-PD genes played a role in the Chinese sporadic PD cohort, especially for those rare variants in *LRRK2*.

Disclosure statement

The authors have no actual or potential conflicts of interest.

Acknowledgements

The authors are grateful to all subjects for their participation in our study. This study was supported by grants from the National Natural Science Foundation of China (No. 81430023 to Beisha Tang), the National Key Plan for Scientific Research and Development of China grants (No. 2016YFC1306000 to Beisha Tang).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2018.11.012.

References

Benitez, B.A., Davis, A.A., Jin, S.C., Ibanez, L., Ortega-Cubero, S., Pastor, P., Choi, J., Cooper, B., Perlmutter, J.S., Cruchaga, C., 2016. Resequencing analysis of five

Mendelian genes and the top genes from genome-wide association studies in Parkinson's Disease. Mol. Neurodegen. 11, 29.
Chang, D., Nalls, M.A., Hallgrimsdottir, I.B., Hunkapiller, J., van der Brug, M., Cai, F., Kerchner, G.A., Ayalon, G., Bingol, B., Sheng, M., Hinds, D., Behrens, T.W., Singleton, A.B., Bhangale, T.R., Graham, R.R., 2017. A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. Nat. Genet. 49, 1511–1516.
Clarimon, J., Kulisevsky, J., 2013. Parkinson's disease: from genetics to clinical practice. Curr. Genomics 14, 560–567.
Foo, J.N., Tan, L.C., Liany, H., Koh, T.H., Irwan, I.D., Ng, Y.Y., Ahmad-Annuar, A., Au, W.L., Aung, T., Chan, A.Y., Chong, S.A., Chung, S.J., Jung, Y., Khor, C.C., Kim, J., Lee, J., Lim, S.Y., Mok, V., Prakash, K.M., Song, K., Tai, E.S., Vithana, E.N., Wong, T.Y., Tan, E.K., Liu, J., 2014. Analysis of non-synonymous-coding variants of Parkinson's disease-related pathogenic and susceptibility genes in East Asian populations. Hum. Mol. Genet. 23, 3891–3897.
Fu, W., O'Connor, T.D., Jun, G., Kang, H.M., Abecasis, G., Leal, S.M., Gabriel, S., Rieder, M.J., Altshuler, D., Shendure, J., Nickerson, D.A., Bamshad, M.J., Akey, J.M., 2013. Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants. Nature 493, 216–220.
Funayama, M., Ohe, K., Amo, T., Furuya, N., Yamaguchi, J., Saiki, S., Li, Y., Ogaki, K., Ando, M., Yoshino, H., Tomiyama, H., Nishioka, K., Hasegawa, K., Saiki, H., Satake, W., Mogushi, K., Sasaki, R., Kokubo, Y., Kuzuhara, S., Toda, T., Mizuno, Y., Uchiyama, Y., Ohno, K., Hattori, N., 2015. CHCHD2 mutations in autosomal dominant late-onset Parkinson's disease: a genome-wide linkage and sequencing study. Lancet Neurol. 14, 274–282.
Hattori, N., 2012. Autosomal dominant parkinsonism: its etiologies and differential diagnoses. Parkinsonism Relat. Disord. 18 (Suppl 1), S1–S3.
Haugarvoll, K., Wszolek, Z.K., 2009. Clinical features of LRRK2 parkinsonism. Parkinsonism Relat. Disord. 15 (Suppl 3), S205–S208.
Hernandez, D.G., Reed, X., Singleton, A.B., 2016. Genetics in Parkinson disease: Mendelian versus non-Mendelian inheritance. J. Neurochem. 139 (Suppl 1), 59–74.
Hiatt, J.B., Pritchard, C.C., Salipante, S.J., O'Roak, B.J., Shendure, J., 2013. Single molecule molecular inversion probes for targeted, high-accuracy detection of low-frequency variation. Genome Res. 23, 843–854.
Hughes, A.J., Daniel, S.E., Kilford, L., Lees, A.J., 1992. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J. Neurol. Neurosurg. Psychiatry 55, 181–184.
Jansen, I.E., Bras, J.M., Lesage, S., Schulte, C., Gibbs, J.R., Nalls, M.A., Brice, A., Wood, N.W., Morris, H., Hardy, J.A., Singleton, A.B., Gasser, T., Heutink, P., Sharma, M., 2015. CHCHD2 and Parkinson's disease. Lancet Neurol. 14, 678–679.
Kalia, L.V., Lang, A.E., 2015. Parkinson's disease. Lancet (London, England) 386, 896–912.
Koschmidder, E., Weissbach, A., Bruggemann, N., Kasten, M., Klein, C., Lohmann, K., 2016. A nonsense mutation in CHCHD2 in a patient with Parkinson disease. Neurology 86, 577–579.
Krebs, C.E., Karkheiran, S., Powell, J.C., Cao, M., Makarov, V., Darvish, H., Di Paolo, G., Walker, R.H., Shahidi, G.A., Buxbaum, J.D., De Camilli, P., Yue, Z., Paisan-Ruiz, C., 2013. The Sac1 domain of SYNJ1 identified mutated in a family with early-onset progressive Parkinsonism with generalized seizures. Hum. Mutat. 34, 1200–1207.
Lesage, S., Drouet, V., Majounie, E., Deramecourt, V., Jacoupy, M., Nicolas, A., Cormier-Dequaire, F., Hassoun, S.M., Pujol, C., Ciura, S., Erpapazoglou, Z., Usenko, T., Maurage, C.A., Sahbatou, M., Liebau, S., Ding, J., Bilgic, B., Emre, M., Erginel-Unaltuna, N., Guven, G., Tison, F., Tranchant, C., Vidailhet, M., Corvol, J.C., Krack, P., Leutenegger, A.L., Nalls, M.A., Hernandez, D.G., Heutink, P., Gibbs, J.R., Hardy, J., Wood, N.W., Gasser, T., Durr, A., Deleuze, J.F., Tazir, M., Destee, A., Lohmann, E., Kabashi, E., Singleton, A., Corti, O., Brice, A., 2016. Loss of VPS13C function in autosomal-recessive parkinsonism causes mitochondrial dysfunction and increases PINK1/Parkin-dependent mitophagy. Am. J. Hum. Genet. 98, 500–513.
Li, H., Durbin, R., 2010. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics (Oxford, England) 26, 589–595.
Maher, B., 2008. Personal genomes: the case of the missing heritability. Nature 456, 18–21.
Manolio, T.A., Collins, F.S., Cox, N.J., Goldstein, D.B., Hindorff, L.A., Hunter, D.J., McCarthy, M.I., Ramos, E.M., Cardon, L.R., Chakravarti, A., Cho, J.H., Guttmacher, A.E., Kong, A., Kruglyak, L., Mardis, E., Rotimi, C.N., Slatkin, M., Valle, D., Whittemore, A.S., Boehnke, M., Clark, A.G., Eichler, E.E., Gibson, G., Haines, J.L., Mackay, T.F., McCarroll, S.A., Visscher, P.M., 2009. Finding the missing heritability of complex diseases. Nature 461, 747–753.
Nalls, M.A., Pankratz, N., Lill, C.M., Do, C.B., Hernandez, D.G., Saad, M., DeStefano, A.L., Kara, E., Bras, J., Sharma, M., Schulte, C., Keller, M.F., Arepalli, S., Letson, C., Edsall, C., Stefansson, H., Liu, X., Pliner, H., Lee, J.H., Cheng, R., Ikram, M.A., Ioannidis, J.P., Hadjigeorgiou, G.M., Bis, J.C., Martinez, M., Perlmutter, J.S., Goate, A., Marder, K., Fiske, B., Sutherland, M., Xiromerisiou, G., Myers, R.H., Clark, L.N., Stefansson, K., Hardy, J.A., Heutink, P., Chen, H., Wood, N.W., Houlden, H., Payami, H., Brice, A., Scott, W.K., Gasser, T., Bertram, L., Eriksson, N., Foroud, T., Singleton, A.B., 2014. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. Nat. Genet. 46, 989–993.
Nichols, W.C., Kissell, D.K., Pankratz, N., Pauciulo, M.W., Elsaesser, V.E., Clark, K.A., Halter, C.A., Rudolph, A., Wojcieszek, J., Pfeiffer, R.F., Foroud, T., 2009. Variation in GIGYF2 is not associated with Parkinson disease. Neurology 72, 1886–1892.
Nicolae, D.L., 2016. Association tests for rare variants. Annu. Rev. Genomics Hum. Genet. 17, 117–130.
Nuttle, X., Itsara, A., Shendure, J., Eichler, E.E., 2014. Resolving genomic disorder-associated breakpoints within segmental DNA duplications using massively parallel sequencing. Nat. Protoc. 9, 1496–1513.
Peng, F., Sun, Y.M., Chen, C., Luo, S.S., Li, D.K., Wang, Y.X., Yang, K., Liu, F.T., Zuo, C.T., Ding, Z.T., An, Y., Wu, J.J., Wang, J., 2017. The heterozygous R1441C mutation of leucine-rich repeat kinase 2 gene in a Chinese patient with Parkinson disease: a five-year follow-up and literatures review. J. Neurol. Sci. 373, 23–26.
Pihlstrom, L., Toft, M., 2011. Parkinson's disease: what remains of the "missing heritability"? Mov. Disord. 26, 1971–1973.
Postuma, R.B., Berg, D., Stern, M., Poewe, W., Olanow, C.W., Oertel, W., Obeso, J., Marek, K., Litvan, I., Lang, A.E., Halliday, G., Goetz, C.G., Gasser, T., Dubois, B., Chan, P., Bloem, B.R., Adler, C.H., Deuschl, G., 2015. MDS clinical diagnostic criteria for Parkinson's disease. Mov. Disord. 30, 1591–1601.
Puschmann, A., 2013. Monogenic Parkinson's disease and parkinsonism: clinical phenotypes and frequencies of known mutations. Parkinsonism Relat. Disord. 19, 407–415.
Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W.W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H.L., 2015. 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. 17, 405–424.
Schulte, C., Gasser, T., 2011. Genetic basis of Parkinson's disease: inheritance, penetrance, and expression. Appl. Clin. Genet. 4, 67–80.
Spataro, N., Calafell, F., Cervera-Carles, L., Casals, F., Pagonabarraga, J., Pascual-Sedano, B., Campolongo, A., Kulisevsky, J., Lleo, A., Navarro, A., Clarimon, J., Bosch, E., 2015. Mendelian genes for Parkinson's disease contribute to the sporadic forms of the disease. Hum. Mol. Genet. 24, 2023–2034.
Sundal, C., Fujioka, S., Uitti, R.J., Wszolek, Z.K., 2012. Autosomal dominant Parkinson's disease. Parkinsonism Relat. Disord. 18 (Suppl 1), S7–S10.
Van der Auwera, G.A., Carneiro, M.O., Hartl, C., Poplin, R., Del Angel, G., Levy-Moonshine, A., Jordan, T., Shakir, K., Roazen, D., Thibault, J., Banks, E., Garimella, K.V., Altshuler, D., Gabriel, S., DePristo, M.A., 2013. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr. Protoc. Bioinformatics 43, 11.10.1-11.10.33.
Vilarino-Guell, C., Rajput, A., Milnerwood, A.J., Shah, B., Szu-Tu, C., Trinh, J., Yu, I., Encarnacion, M., Munsie, L.N., Tapia, L., Gustavsson, E.K., Chou, P., Tatarnikov, I., Evans, D.M., Pishotta, F.T., Volta, M., Beccano-Kelly, D., Thompson, C., Lin, M.K., Sherman, H.E., Han, H.J., Guenther, B.L., Wasserman, W.W., Bernard, V., Ross, C.J., Appel-Cresswell, S., Stoessl, A.J., Robinson, C.A., Dickson, D.W., Ross, O.A., Wszolek, Z.K., Aasly, J.O., Wu, R.M., Hentati, F., Gibson, R.A., McPherson, P.S., Girard, M., Rajput, M., Rajput, A.H., Farrer, M.J., 2014. DNAJC13 mutations in Parkinson disease. Hum. Mol. Genet. 23, 1794–1801.
Wang, L., Guo, J.F., Nie, L.L., Xu, Q., Zuo, X., Sun, Q.Y., Yan, X.X., Tang, B.S., 2010. A novel LRRK2 mutation in a mainland Chinese patient with familial Parkinson's disease. Neurosci. Lett. 468, 198–201.
Zuk, O., Schaffner, S.F., Samocha, K., Do, R., Hechter, E., Kathiresan, S., Daly, M.J., Neale, B.M., Sunyaev, S.R., Lander, E.S., 2014. Searching for missing heritability: designing rare variant association studies. Proc. Natl. Acad. Sci. U. S. A. 111, E455–E464.