

ARTICLE OPEN



Mutational spectrum and clinical features of *GBA1* variants in a Chinese cohort with Parkinson's disease

Yangjie Zhou¹, Yige Wang¹, Juan Wan², Yuwen Zhao¹, Hongxu Pan¹, Qian Zeng¹, Xun Zhou³, Runcheng He³, Xiaoxia Zhou¹, Yaqin Xiang¹, Zhou Zhou³, Bin Chen¹, Qiying Sun³, Qian Xu¹, Jieqiong Tan⁴, Lu Shen^{1,4,5,6}, Hong Jiang^{1,4,5}, Xinxiang Yan¹, Jinchun Li^{3,4,6}, Jifeng Guo^{1,4,5}, Beisha Tang^{1,2,4,5,6}, Heng Wu^{2,7}✉ and Zhenhua Liu^{1,4,5,6}✉

GBA1 variants are important risk factors for Parkinson's disease (PD). Most studies assessing *GBA1*-related PD risk have been performed in European-derived populations. Although the coding region of the *GBA1* gene in the Chinese population has been analyzed, the sample sizes were not adequate. In this study, we aimed to investigate *GBA1* variants in a large Chinese cohort of patients with PD and healthy control and explore the associated clinical characteristics. *GBA1* variants in 4034 patients and 2931 control participants were investigated using whole-exome and whole-genome sequencing. The clinical features of patients were evaluated using several scales. Regression analysis, chi-square, and Fisher exact tests were used to analyze *GBA1* variants and the clinical symptoms of different groups. We identified 104 variants, including 8 novel variants, expanding the spectrum of *GBA1* variants. The frequency of *GBA1* variants in patients with PD was 7.46%, higher than that in the control (1.81%) ($P < 0.001$, odds ratio [OR] = 4.38, 95% confidence interval [CI]: 3.26–5.89). Among patients, 176 (4.36%) had severe variants, 34 (0.84%) carried mild variants, three (0.07%) had risk variants, and 88 (2.18%) carried unknown variants. Our study, for the first time, found that p.G241R ($P = 0.007$, OR = 15.3, 95% CI: 1.25–261.1) and p.S310G ($P = 0.005$, OR = 4.86, 95% CI: 1.52–28.04) variants increased the risk of PD. Patients with *GBA1* variants exhibited an earlier onset age and higher risk of probable rapid-eye-movement sleep behavior disorder, olfactory dysfunction, depression, and autonomic dysfunction than patients without *GBA1* variants.

npj Parkinson's Disease (2023)9:129; <https://doi.org/10.1038/s41531-023-00571-4>

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative and progressively disabling disease characterized by bradykinesia, tremor, and muscular rigidity¹. A multitude of factors can affect the risk, onset, and progression of PD, including aging as well as environmental conditions, and genetic predisposition¹.

More than twenty genes with different degrees of genetic evidence are mutated in monogenic PD^{2,3}. Variants in the *GBA1* gene, encoding the lysosomal enzyme β -glucocerebrosidase (GCase), are common risk factors⁴. *GBA1* variants confer 5–30 folds increased risk of PD, and across different populations, at least 5–20% of patients with PD have *GBA1* variants⁵. More than 300 PD-related variants have been reported, with distinctive patterns observed in different populations⁶.

Studies that explore the correlation between genotype and phenotype have also illuminated how variants of *GBA1* can influence the characteristics of PD^{7,8}. Patients harboring *GBA1* variants, when compared to those without, exhibit distinct features, including an earlier age at onset (AAO), more severe motor impairment, higher risk of cognitive decline, depression^{7,9,10}, rapid-eye-movement sleep behavior disorder (RBD)¹¹, and reduced survival¹². Furthermore, the clinical attributes resulting from various *GBA1* variants differ. The classification of *GBA1* variants is based on their role in Gaucher's Disease (GD) or PD: mild variants give rise to GD type I, while severe variants lead to GD type II or III⁶. Presence of heterozygous *GBA1* variants,

whether mild or severe, might differentially impact the risk and age at onset of PD^{13–15}. Most studies on *GBA1* variants in patients with PD focused on several *GBA1* variants, such as p.L483P, p.N409S, and p.R159W. Although some case-control studies have investigated the coding region of *GBA1* gene in Chinese population, these studies were limited by their relatively small sample sizes^{15–17}.

This study aimed to characterize the frequency and distribution of *GBA1* variants in a large cohort of 4034 patients with PD and 2931 healthy participants who are Han Chinese using whole-exome sequencing (WES) and whole-genome sequencing (WGS). All variants were validated by polymerase chain reaction and Sanger sequencing. In addition, we analyzed the relationship between *GBA1* variants and phenotypes by comprehensively assessing the clinical manifestations in patients with PD.

RESULTS

Demographic characteristics

This study encompassed a total of 4034 patients and 2931 healthy participants. Among the patients, 1777 (44.1%) individuals were diagnosed with early-onset PD (EOPD, AAO \leq 50 years); parallelly, 1652 healthy participants matched for age and sex were included. Additionally, there were 2257 (55.9%) participants with late-onset PD (LOPD, AAO $>$ 50 years), accompanied by 1279 age and sex matched control participants. Detailed demographic information

¹Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan, China. ²Department of Neurology, & Multi-Omics Research Center for Brain Disorders, The First Affiliated Hospital, University of South China, Hengyang, Hunan, China. ³Department of Geriatrics, Xiangya Hospital, Central South University, Changsha, Hunan, China. ⁴Centre for Medical Genetics & Hunan Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Changsha, Hunan, China. ⁵Key Laboratory of Hunan Province in Neurodegenerative Disorders, Changsha, Hunan, China. ⁶Bioinformatics Center & National Clinical Research Centre for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, Hunan, China. ⁷Clinical Research Center for Immune-Related Encephalopathy of Hunan Province, Hengyang, Hunan, China.

✉email: 2915176817@qq.com; liuzhenhua@csu.edu.cn

When selecting variants with at least ten patients to systematically interrogate the association of single-nucleotide variants (SNVs), we observed significant differences for the p.L483P variant detected in PD patients compared to that in control (2.35% vs. 0.14%, $P < 0.001$, OR = 17.65, 95% CI: 6.48–48.05). Furthermore, our study showed that p.G241R and p.S310G variants contribute to an increased risk of PD. Specifically, p.G241R was present in 10 patients (0.25%) but none in control ($P = 0.007$, OR = 15.3, 95% CI: 1.25–261.1). Similarly, p.S310G was found in twenty patients (0.5%) and only three control participants (0.1%) ($P = 0.005$, OR = 4.86, 95% CI: 1.52–28.04). Conversely, the analysis did not reveal significant difference for the second most common variant in our cohort, the p.R202Q variant, with frequencies of 0.55% in patients and 0.72% in control (Table 1, Supplementary Table 3).

Furthermore, we investigated the frequency of *GBA1* variants in EOPD and LOPD patients. In EOPD patients, 185 (10.41%) carried *GBA1* variants, significantly higher than in LOPD patients, in which only 116 (5.14%) carried *GBA1* variants. A total of 122 (6.87%) EOPD patients and 54 (2.39%) LOPD patients carried severe variants. However, the analysis showed no significant differences for mild, risk, and unknown variants between the two groups. Performing the SNV association, we found that the proportion of EOPD patients with the p.L483P variant was significantly higher than that of LOPD patients. Seventy-four (4.16%) EOPD patients carried p.L483P, while 21 (0.93%) LOPD patients carried p.L483P.

Genotype-Phenotype

We found that patients with non-synonymous *GBA1* variants had an earlier AAO (mean: 50 years, standard deviation (SD): 9.64 years) compared to non-carriers (mean: 54.15 years, SD: 11.01 years), along with a higher Hoehn and Yahr (H-Y) stage (mean: 2.12, SD: 0.76) in contrast to non-carriers (mean: 1.98, SD: 0.76) (Table 2). Furthermore, the postural instability gait difficulty (PIGD) motor subtype was predominant in both groups, but the proportion of PIGD in patients with *GBA1* variants was higher than that in non-carriers (69.93% vs. 57.36%), indicating increased rigidity and less tremor. In the realm of non-motor symptoms, patients with *GBA1* variants demonstrated lower Hyposmia Rating Scale (HRS) scores related to olfactory function than those without *GBA1* variants (18.19 vs. 19.55), and olfactory loss was more prevalent among patients with *GBA1* variants than non-carriers (54.48% vs. 40.95%). Regarding sleep disturbances, patients with *GBA1* variants exhibited a higher rate of probable rapid-eye-movement sleep behavior disorder (pRBD) than those without *GBA1* variants, while no significant differences were observed in excessive daytime sleepiness (EDS) and overall sleep quality. In addition, patients with non-synonymous *GBA1* variants displayed higher rates of constipation and depression than non-carriers. Regarding motor complications, patients with *GBA1* variants had higher freezing of gait (FOG) rate than non-carriers (Fig. 3, Table 2).

Since the most important variant we found was p.L483P, we specifically analyzed the clinical characteristics of patients with p.L483P. Compared with non-carriers, cases with p.L483P had an earlier AAO and a higher H-Y stage. Compared with non-carriers, olfactory loss and pRBD were more prevalent in those with p.L483P (Fig. 3, Table 2).

Additionally, we analyzed the clinical characteristics of both EOPD and LOPD cases with *GBA1* variants. When comparing EOPD cases with and without *GBA1* variants, we found no significant differences between the two groups in age and AAO. However, patients with *GBA1* variants displayed a higher H-Y stage. Moreover, compared to non-carriers, patients with *GBA1* variants exhibited a higher prevalence of olfactory loss, pRBD, and constipation. While when comparing LOPD cases with and without *GBA1* variants, those with *GBA1* variants exhibited an earlier AAO. Furthermore, *GBA1* variants carriers within the LOPD

group displayed a higher occurrence of olfactory loss, depression, pRBD and constipation (Supplementary Table 4).

Finally, we compared the clinical characteristics of different types of *GBA1* variants including Severe-PD and Mild-PD. Notably, Severe-PD cases displayed an earlier AAO and higher levodopa equivalent daily dose (LEDD) compared to Mild-PD cases. However, we did not identify significant differences in other clinical characteristics (Supplementary Table 5).

DISCUSSION

This study represents the largest endeavor to comprehensively analyze *GBA1* coding variants within a Chinese cohort of patients with PD and control. This investigation identified 104 variants, including 8 novel variants, thereby expanding the spectrum of *GBA1* variants. Notably, the frequency of *GBA1* variants among patients with PD was 7.46%, significantly higher than that in control (1.81%). This observed frequency among patients with PD was within the reported range (5.4–10.72%) in other Chinese studies to date^{15–17,19,20}. Moreover, when categorizing these variants according to their deduced and observed phenotypic impact on GD or PD, a significant proportion of patients with PD carried the *GBA1* variants associated with phenotypic effects.

Although globally, four missense variants-p.E365K, p.T408M, p.N409S, and p.L483P-account for >80% of PD alleles²¹, we found no patients with p.T408M, and only two patients had p.N490S and p.E365K, respectively. Moreover, these three variants are very rare among East Asians and are present in up to only 0.2% of South Asians based on the gnomAD database. The p.L483P variant was prominent among our Chinese patients with PD, which, together with p.R202Q, p.S310G, and p.G241R, accounted for half of the *GBA1* cases in this group. Our study showed that p.L483P, p.S310G, and p.G241R increased the risk of PD. The p.L483P variant has also been reported to be the most common *GBA1* variant in other Asian and Hispanic populations^{16,20}. While among Ashkenazi Jews, the *GBA1* variants in PD patients were mainly p.N409S²². The difference in mutation frequency indicates that it may be affected by factors such as environment, region, ethnicity, etc. Interestingly, our study firstly found p.S310G and p.G241R may be relatively specific variants for increasing the risk of PD in Chinese population.

In terms of clinical characteristics, *GBA1* variant carriers had an average of 4 years earlier in age at onset than non-carriers in our study, consistent with previous studies that found PD patients with *GBA1* variants were younger and had 1–11 years earlier in age at onset than PD patients without *GBA1* variants^{23–25}. In addition, we found that the tremor score of PD patients with *GBA1* variants was relatively lower and the PIGD motor subtype was more common in PD patients with *GBA1* variants than in patients without *GBA1* variants, indicating that PD patients with *GBA1* variants may belong to the non-tremor-dominant phenotype of PD. This finding is consistent with findings from a recent study that patients with *GBA1* variants were more likely to present with the PIGD phenotype compared with non-carriers²⁶. In addition, one study found that patients with PD who carried *GBA1* variants displayed a faster decline in PIGD scores but not tremor scores²⁷.

Previous studies have reported that, PD patients carrying *GBA1* variants showed worse cognitive function than PD patients who did not carry *GBA1* variants²⁶. Some studies also showed that there was no significant difference in cognitive function between PD patients with *GBA1* variants and PD patients without *GBA1* variants in the early stages of the disease. As the disease progresses, PD patients with *GBA1* variants progress to dementia faster²⁸. In our study, there was a statistical difference in cognitive function between PD patients with *GBA1* variants and PD patients without *GBA1* variants, while the difference of Mini-Mental State Examination (MMSE) scores was marginal. This could be attributed to the average course duration of our patients, which was only about

Table 1. All GBA1 non-synonymous variations identified in this study.

Severity	Chromosomal Position	Exon	cDNA Change	AA Change	Variant Type	Frequency in gnomAD_EAS		CADD	Total		EOPD		LOPD	
						Case	Control		Case	Control	Case	Control	Case	Control
Severe	Chr1:155209424	4	c.437 C > T	p.S146L	Missense	-	-	29.8D	1	1	1	1	1	1
	Chr1:155208421	5	c.475 C > T	p.R159W	Missense	0	0	28.6D	3	1	1	2	2	2
	Chr1:155208420	5	c.476 G > A	p.R159Q	Missense	-	-	29.3D	2	2	2	2	2	2
	Chr1:155208006	6	c.[680 A > G;681 T > G]*	PN227R	Missense	-	-	-	3	1	1	1	2	2
	Chr1:155208006	6	c.680 A > G	p.N227S	Missense	0	0	0.013:T	7	2	2	5	5	5
	Chr1:155208005	6	c.681 T > G	p.N227K	Missense	0	0	18.28:T	1	1	1	1	1	1
	Chr1:155207965	6	c.721 G > A	p.G241R	Missense	-	-	24.4D	10	4	4	6	6	6
	Chr1:155207932	6	c.754 T > A	p.F252I	Missense	0	0	23.6D	2	2	2	2	2	2
	Chr1:155207322	7	c.809 C > G*	p.T270R	Missense	-	-	26.5D	1	1	1	1	1	1
	Chr1:155206170	8	c.1090 G > A	p.G364R	Missense	-	-	18.92:T	1	1	1	1	1	1
	Chr1:155205614	9	c.1246 G > A	p.G416S	Missense	0	0	33D	2	2	2	2	2	2
	Chr1:155205548	9	c.1312 G > A	p.D438N	Missense	-	-	26.1D	3	3	3	3	3	3
	Chr1:155205518	9	c.1342 G > C	p.D448H	Missense	0.0006	0.0006	22.7D	8	5	5	3	3	3
	Chr1:155205499	9	c.1361 C > G	p.P454R	Missense	-	-	26.6D	1	1	1	1	1	1
	Chr1:155205043	10	c.1448 T > C	p.L483P	Missense	0.0012	0.0012	24.8D	95	4	4	21	21	21
	Chr1:155210876	1	c.27+1 G > T		Splicing	-	-	23.7D	1	1	1	1	1	1
	Chr1:155210420	2	c.115+1 G > A		Splicing	0	0	25D	3	2	2	1	1	1
	Chr1:155210420	2	c.115+1 G > T		Splicing	-	-	25.1D	1	1	1	1	1	1
	Chr1:155209405	4	c.454+2 T > A		Splicing	-	-	21.6D	1	1	1	1	1	1
	Chr1:155207370	7	c.762-1 G > C		Splicing	0.0006	0.0006	23.3D	8	2	2	1	1	1
Chr1:155205471	9	c.1388+1 G > A		Splicing	-	-	25.5D	1	1	1	1	1	1	
Chr1:155209728	3	c.256 C > T	p.R86X	Stopgain	-	-	34D	1	1	1	1	1	1	
Chr1:155207942	6	c.744 G > A	p.W248X	Stopgain	-	-	38D	5	3	3	2	2	2	
Chr1:155207330	7	c.801 G > A	p.W267X	Stopgain	-	-	37D	2	2	2	2	2	2	
Chr1:155207245	7	c.886 C > T	p.R296X	Stopgain	-	-	36D	1	1	1	1	1	1	
Chr1:155206059	8	c.1201 C > T	p.Q401X	Stopgain	-	-	36D	1	1	1	1	1	1	
Chr1:155205056	10	c.1435 C > T*	p.Q479X	Stopgain	-	-	28.7D	1	1	1	1	1	1	
Chr1:155209827	3	c.157delG		Frameshift	-	-	-	1	1	1	1	1	1	
Chr1:155209817	3	c.166_167delTG		Frameshift	-	-	-	1	1	1	1	1	1	
Chr1:155209780	3	c.203dupC		Frameshift	-	-	-	1	1	1	1	1	1	
Chr1:155209690	3	c.293dupA		Frameshift	-	-	-	1	1	1	1	1	1	
Chr1:155207255	7	c.876delT		Frameshift	-	-	-	1	1	1	1	1	1	
Chr1:155207141	7	c.983_990del		Frameshift	-	-	-	1	1	1	1	1	1	
Chr1:155205488	9	c.1372delC		Frameshift	-	-	-	1	1	1	1	1	1	
Chr1:155205029	10	c.1447_1466del TG ins		Frameshift	-	-	-	4	2	2	2	2	2	
Mild	Chr1:155209758	3	c.226 T > G	p.F76V	Missense	-	-	12.81:T	1	1	1	1	1	1
	Chr1:155209725	3	c.259 C > T	p.R87W	Missense	-	-	24.8D	1	1	1	1	1	1
	Chr1:155208412	5	c.484 A > G	p.M162V	Missense	-	-	10.47:T	2	2	2	2	2	2

Table 1 continued

Severity	Chromosomal Position	Exon	cDNA Change	AA Change	Variant Type	Frequency in gnomAD_EAS	CADD	Total	EOPD	LOPD
	Chr1:155207992	6	c.694 G>A	p.G232R	Missense	-	26.1:D	1		1
	Chr1:155207224	7	c.907 C>A	p.L303I	Missense	-	24.2:D	8	7	1
	Chr1:155207203	7	c.928 A>G	p.S310G	Missense	0.0006	14.81:T	20	3	8
	Chr1:155205634	9	c.1226 A>G	p.N409S	Missense	0	22.7:D	1	12	1
	Chr1:155204793	11	c.1604G>A	p.R535H	Missense	0	15.69:T	2	1	1
Risk	Chr1:155206167	8	c.1093 G>A	p.E365K	Missense	0	17.33:T	1	1	1
	Chr1:155205581	9	c.1279 G>A	p.E427K	Missense	0	23.2:D	2	1	1
Unknown	Chr1:155210496	2	c.40 C>A	p.P14T	Missense	-	0.01:T	1		1
	Chr1:155210478	2	c.58 A>G	p.I20V	Missense	0.0006	0.001:T	1	1	1
	Chr1:155210438	2	c.98 C>T	p.A33V	Missense	0.0006	17.03:T	1	1	1
	Chr1:155210421	2	c.115 G>C	p.G39R	Missense	-	24.3:D	3	3	1
	Chr1:155209860	3	c.124 C>G	p.P42A	Missense	-	13.28:T	1		1
	Chr1:155209836	3	c.148 T>C	p.Y50H	Missense	-	4.628:T	1		1
	Chr1:155209814	3	c.170 G>C	p.C57S	Missense	-	26:D	1		1
	Chr1:155209685	3	c.299 C>T	p.T100M	Missense	-	21.7:D	1	1	1
	Chr1:155209461	4	c.400 G>T	p.A134S	Missense	-	8.905:T	1	1	1
	Chr1:155209431	4	c.430 C>A	p.L144I	Missense	-	23.3:D	1		1
	Chr1:155208414	5	c.482 C>G	p.P161R	Missense	-	24.2:D	1	1	1
	Chr1:155208409	5	c.487 G>A	p.A163T	Missense	-	27.6:D	2	2	2
	Chr1:155208375	5	c.521 A>G	p.Y174C	Missense	-	23.9:D	1		1
	Chr1:155208081	6	c.605 G>A	p.R202Q	Missense	0.0025	20.4:D	22	8	14
	Chr1:155208060	6	c.626 G>A	p.R209H	Missense	0	17.53:T	1	1	5
	Chr1:155207983	6	c.703 T>C	p.S235P	Missense	0	11.51:T	2	1	1
	Chr1:155207979	6	c.707 T>C	p.L236P	Missense	-	25.5:D	2	2	1
	Chr1:155207974	6	c.712 G>A	p.G238R	Missense	-	28.1:D	1	1	1
	Chr1:155207366	7	c.765 C>G	p.F255L	Missense	-	23.8:D	1	1	1
	Chr1:155207355	7	c.776 A>G	p.Y259C	Missense	-	25.4:D	1	1	1
	Chr1:155207272	7	c.859 T>C	p.C287R	Missense	-	25.1:D	1		1
	Chr1:155207230	7	c.901 C>T	p.R301C	Missense	-	24.9:D	1	1	1
	Chr1:155207229	7	c.902 G>A	p.R301H	Missense	0	17.54:T	1		1
	Chr1:155207224	7	c.907 C>G	p.L303V	Missense	-	23.4:D	3	3	1
	Chr1:155207209	7	c.922 G>A	p.A308T	Missense	-	19.73:T	1		1
	Chr1:155207202	7	c.929 G>C	p.S310T	Missense	-	17.67:T	1		1
	Chr1:155207193	7	c.938 A>G	p.H313R	Missense	-	0.002:T	1	2	1
	Chr1:155206248	8	c.1012 C>A	p.P338T	Missense	-	14.00:T	1	2	1
	Chr1:155206194	8	c.1066 C>G	p.L356V	Missense	-	10.91:T	1	1	1
	Chr1:155206181	8	c.1079 A>G	p.K360R	Missense	-	15.02:T	1	1	1
	Chr1:155206173	8	c.1087 C>A	p.L363I	Missense	-	23.3:D	1	1	1
	Chr1:155206158	8	c.1102 C>G	p.R368G	Missense	-	22.7:D	1		1
	Chr1:155206157	8	c.1103 G>A	p.R368H	Missense	-	22.6:D	1	1	1

Table 1 continued

Severity	Chromosomal Position	Exon	cDNA Change	AA Change	Variant Type	Frequency in gnomAD_EAS	CADD	Total	EOPD	LOPD			
	Chr1:155206104	8	c.1156T > C	p.F386L	Missense	-	16.68:T	1	1	1			
	Chr1:155206101	8	c.1159T > G	p.W387G	Missense	-	17.66:T	1	1	1			
	Chr1:155206088	8	c.1172T > G	p.V391G	Missense	-	26.1:D	1	1	1			
	Chr1:155206086	8	c.1174C > T	p.R392W	Missense	-	26:D	1	1	1			
	Chr1:155206043	8	c.1217T > A	p.I406N	Missense	-	28.8:D	1	1	1			
	Chr1:155206040	8	c.1220T > A	p.I407N	Missense	-	28.4:D	1	1	1			
	Chr1:155205611	9	c.1249T > A	p.W417R	Missense	-	26.9:D	2	2	2			
	Chr1:155205599	9	c.1261A > G	p.N421D	Missense	-	26.2:D	1	1	1			
	Chr1:155205595	9	c.1265T > G	p.L422R	Missense	-	28.4:D	5	3	2			
	Chr1:155205592	9	c.1268C > T	p.A423V	Missense	-	23.6:D	1	1	1			
	Chr1:155205585	9	c.1275C > A	p.N425K	Missense	-	27:D	1	1	1			
	Chr1:155205574	9	c.1286G > T	p.G429V	Missense	-	33:D	2	2	2			
	Chr1:155205568	9	c.1292A > G	p.N431S	Missense	-	23.4:D	4	1	3			
	Chr1:155205563	9	c.1297G > A	p.V433M	Missense	-	33:D	2	2	2			
	Chr1:155205560	9	c.1300C > T	p.R434C	Missense	-	34:D	2	2	2			
	Chr1:155205088	10	c.1403A > G	p.E468G	Missense	-	25.2:D	1	1	1			
	Chr1:155205043	10	c.1448T > G	p.L483R	Missense	-	24.6:D	2	1	1			
	Chr1:155205008	10	c.1483G > C	p.A495P	Missense	0	23:D	4	5	3			
	Chr1:155204996	10	c.1495G > A	p.V499M	Missense	0	27.8:D	2	1	1			
	Chr1:155204996	10	c.1495G > T	p.V499L	Missense	-	20.7:D	1	1	1			
	Chr1:155204812	11	c.1585C > T	p.H529Y	Missense	-	23:D	1	1	1			
	Chr1:155210882	1	c.22A > G*	p.R8G	Missense	-	14.41:T	1	1	1			
	Chr1:155209813	3	c.171C > G*	p.C57W	Missense	-	26.1:D	1	1	1			
	Chr1:155207997	6	c.689T > G*	p.V230G	Missense	0	0.206:T	1	1	1			
	Chr1:155207302	7	c.829G > C*	p.A277P	Missense	-	24.8:D	1	1	1			
	Chr1:155205548	9	c.1312G > T*	p.D438Y	Missense	-	29.3:D	1	1	1			
Total								307 ^a	53	189 ^b	44	118 ^c	9

^aIn total cases; five of them carried more than one variant. ^bIn EOPD cases, four of them carried two variants. ^cIn LOPD cases, one carried three variants. * First reported in our study, gnomAD_EAS, East Asian population from gnomAD genome.

PD Parkinson's disease, EOPD early-onset PD, LOPD late-onset PD, AA amino acid.

CADD Combined Annotation Dependent Depletion, D Damaging, T Tolerable.

Table 2. Comparison of clinical features in N-PD, GBA1-PD and L483P-PD.

Clinical features	N-PD (N = 3733)	GBA1-PD (N = 301)		L483P-PD (N = 95)	
		Values	P1	Values	P2
Age (years)	59.56 ± 10.98	55.30 ± 9.79	<0.001	52.32 ± 8.76	<0.001
Age at onset (years)	54.15 ± 11.01	50.00 ± 9.64	<0.001	46.84 ± 8.38	<0.001
Disease duration (years)	5.37 ± 4.39	5.24 ± 4.49	0.404	5.48 ± 5.11	0.12
Sex (male)	1979 (53.01%)	168 (55.81%)	0.528	56 (58.95%)	0.374
Family history	469 (12.56%)	48 (15.95%)	0.144	13 (13.68%)	0.927
UPDRS-I score	2.45 ± 2.04	2.83 ± 2.26	<0.001	2.72 ± 2.41	0.023
UPDRS-II score	11.89 ± 6.52	12.17 ± 6.69	0.001	12.11 ± 6.41	0.012
UPDRS-III score	27.22 ± 14.86	26.60 ± 14.39	0.175	27.41 ± 14.71	0.101
Tremor score	3.76 ± 3.64	2.75 ± 3.10	<0.001	2.65 ± 3.06	0.008
Rigidity score	5.49 ± 4.15	5.93 ± 4.37	0.012	6.49 ± 4.40	0.026
Bradykinesia score	10.07 ± 6.36	9.99 ± 5.97	0.186	10.13 ± 6.09	0.171
Postural instability score	4.16 ± 3.03	4.07 ± 2.91	0.226	4.06 ± 2.95	0.203
Hoehn and Yahr Scale	1.98 ± 0.76	2.12 ± 0.76	<0.001	2.16 ± 0.75	<0.001
Hoehn and Yahr stage			0.001		<0.001
1–1.5	1161 (31.09%)	78 (25.91%)		22 (23.16%)	
2–2.5	1602 (42.92%)	148 (49.17%)		47 (49.47%)	
3–5	970 (25.99%)	75 (24.92%)		26 (27.37%)	
Motor subtype					
TD	950 (25.44%)	45 (14.86%)		16 (16.84%)	
Indeterminate	642 (17.20%)	46 (15.20%)	0.071	15 (15.79%)	0.344
PIGD	2141 (57.36%)	210 (69.93%)	<0.001	64 (67.37%)	0.003
MMSE score	26.35 ± 3.90	26.19 ± 4.01	0.004	26.27 ± 4.51	0.03
PDSS score	115.53 ± 28.06	115.93 ± 26.16	0.354	116.14 ± 27.54	0.495
ESS score	7.78 ± 6.30	7.68 ± 6.37	0.27	7.62 ± 6.66	0.368
EDS	1109 (34.22%)	87 (33.72%)	0.244	28 (36.36%)	0.119
RBDQ total score	16.11 ± 16.57	20.50 ± 18.99	<0.001	19.96 ± 19.57	0.002
pRBD	1163 (32.48%)	117 (42.31%)	<0.001	30 (38.96%)	0.016
HAMD score	5.81 ± 5.51	6.37 ± 5.69	0.048	6.13 ± 6.59	0.432
Depression	980 (30.39%)	97 (37.89%)	0.006	23 (30.26%)	0.93
HRS score	19.55 ± 6.54	18.19 ± 6.44	<0.001	18.58 ± 6.25	0.029
Olfactory dysfunction	1330 (40.95%)	146 (54.48%)	<0.001	44 (51.76%)	0.002
PDQ39 score	29.50 ± 25.69	30.28 ± 25.59	0.068	26.23 ± 23.68	0.791
Constipation	1207 (38.32%)	111 (44.22%)	<0.001	30 (37.04%)	0.096
Dyskinesia	433 (11.59%)	49 (16.04%)	0.053	17 (18.09%)	0.324
Freezing gait	903 (24.18%)	86 (28.67%)	0.048	27 (28.42%)	0.119
LEDD (mg)	402.59 ± 285.39	439.48 ± 230.76	0.036	476.08 ± 252.23	0.027

Values are expressed as mean ± standard deviation, or number (%).

PD Parkinson's disease, N-PD patients without GBA1 variants, GBA1-PD patients with GBA1 variants, L483P-PD patients with p.L483P variant. UPDRS Unified Parkinson's disease Rating Scale, TD tremor-dominant, PIGD postural instability and gait difficulty, MMSE Mini-Mental State Examination, PDSS Parkinson's Disease Sleep Scale, ESS Epworth Sleepiness Scale, EDS excessive daytime sleepiness, RBDQ Rapid-eye-movement Sleep Behavior Disorder Questionnaire, pRBD probable rapid-eye-movement sleep behavior disorder, HAMD Hamilton Depression Scale, HRS Hyposmia Rating Scale, PDQ-39 Parkinson Disease Quality of Life Questionnaire-39 item version, LEDD levodopa equivalent daily dose. The scores of UPDRS items 20 and 21 added up to the tremor score. The score for item 22 was the rigidity score. The scores for items 23 to 26 added up to the bradykinesia score. The scores for items 27–30 added up to the postural instability score. 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.

5 years, shorter than those reported with differences in cognitive function. Whether the decline in cognitive function is faster in PD patients with GBA1 variants requires further conclusions through prospective studies.

Our study also analyzed the relationship between GBA1 variants and phenotypes by comprehensively assessing the clinical manifestations in patients with PD. Consistent with previous reports, we found that PD patients with GBA1 variants were likelier

to develop olfactory dysfunction. In addition, we found that depression was likelier to occur in PD patients with GBA1 variants. Previous studies have reported that PD patients with GBA1 variants have olfactory disturbances and depression at the same time²⁹ This observation could be attributed to the olfactory pathway affecting the serotonin circuit in the body, affecting the hippocampus, amygdala, and other emotional centers³⁰. Regarding sleep, we found that PD patients with GBA1 variants were

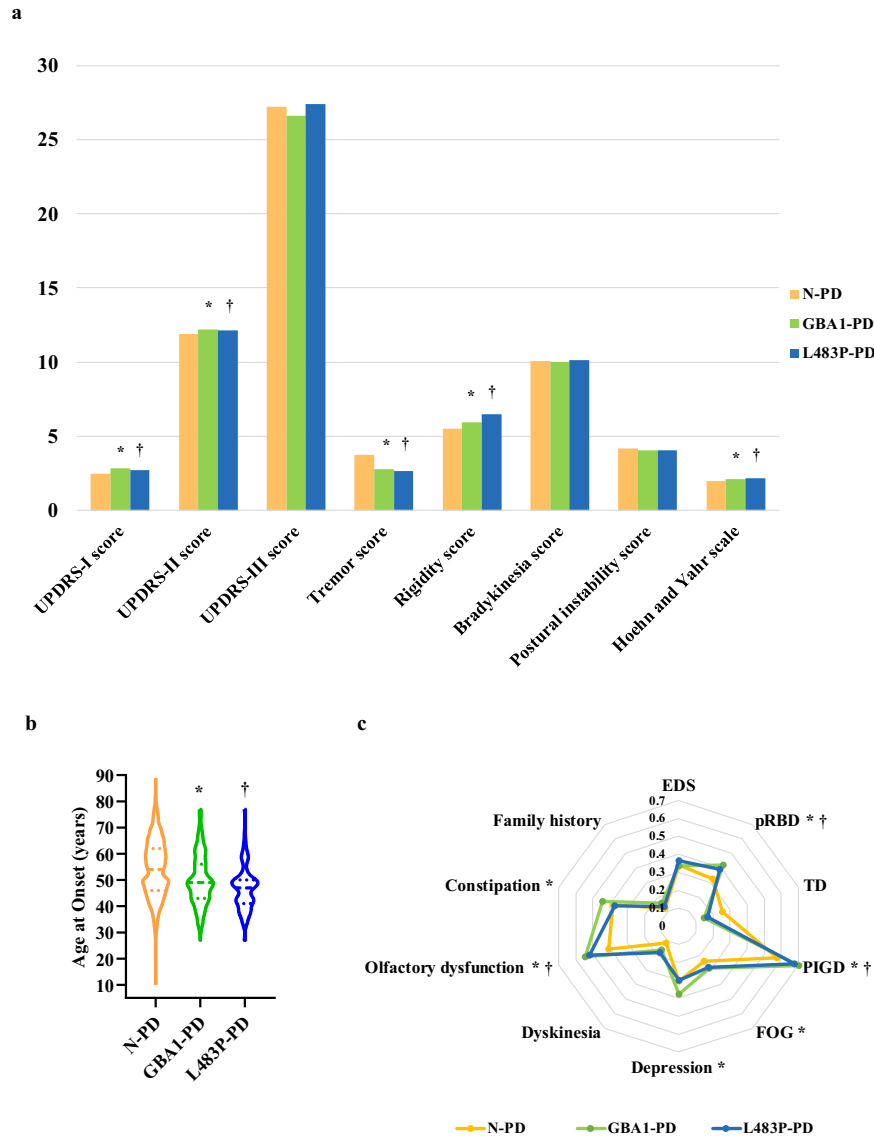


Fig. 3 Clinical characteristics of PD with *GBA1* variants. **a** Mean score of motor symptoms in N-PD, GBA1-PD, and L483P-PD. **b** AAO of N-PD, GBA1-PD, and L483P-PD. **c** Frequency (%) of family history and motor and non-motor symptoms in N-PD, GBA1-PD, and L483P-PD. * Significantly different between N-PD and GBA1-PD groups. † Significantly different between N-PD and L483P-PD groups. PD Parkinson's disease; N-PD patients without *GBA1* variant; GBA1-PD patients with *GBA1* variants; L483P-PD patients with *GBA1* p.L483P variant; AAO age at onset; UPDRS Unified Parkinson's Disease Rating Scale; EDS excessive daytime sleepiness; pRBD probable rapid-eye-movement sleep behavior disorder; TD tremor-dominant; PIGD postural instability and gait difficulty; FOG Freezing of Gait.

likelier to develop pRBD, which is consistent with the findings of previous studies of patients carrying *GBA1* variants with a significantly higher risk of RBD^{7,11}. However, no significant difference was found between excessive daytime sleepiness and overall sleep quality. Regarding autonomic function, PD patients with *GBA1* variants were more prone to constipation. Lastly, we found that PD patients with *GBA1* variants were likelier to have freezing of gait, indicating that *GBA1* variants may be an important risk factor affecting the occurrence of freezing of gait in PD patients.

In addition, we also explored the role of different types of *GBA1* variants including Severe-PD and Mild-PD in PD risk and the clinical characteristics. We found that patients with severe variants had a higher OR (16.67) compared to patients with mild *GBA1* variants (6.22), which is consistent with the findings of a large meta-analysis demonstrating that patients with mild variants have a lower OR (2.2) compared to patients with severe variants (10.3)¹³.

Furthermore, we found that patients with severe variants had an earlier average AAO and a higher LEDD relative to those with mild *GBA1* variants, which is in agreement with previously reported studies^{7,13,14}. Previous studies have reported that Severe-PD, compared to Mild-PD, presented with worse motor and non-motor manifestations of PD, including more severe cognitive dysfunction, hyposmia, depression, and a higher frequency RBD⁷. However, our study did not yield significant differences between the two groups in relation to these clinical characteristics. Although the findings did not achieve statistical significance, it's worth noting that the frequency of pRBD was conspicuously higher in Severe-PD compared to Mild-PD. The potential necessity for larger cohorts to effectively detect such differences is a consideration.

Nonetheless, our study has several limitations. Firstly, we did not carry out functional verification of novel variants. Additionally, we did not measure GCase activity, which has been linked to PD.

Moreover, our sequencing approach was not uniform. Due to limited funding and technology constraints, we initially focused on genetic information of early-onset PD (AAO <50 years old) and PD with a family history, sequenced via WES. Subsequently, in the second stage of the project, we directed our attention to the genetic information of idiopathic PD patients with late-onset (AAO > 50) and sequenced them using WGS, driven by advancements in sequencing technology. To further our understanding, we have initiated the Chinese Parkinson's Disease with *GBA1* Variants Registry (CPD-GBAR) study, a multicenter, nationwide PD cohort study (the clinicaltrials.gov identifier is NCT03523065) based on the PD-MDCNC. This study aims to explore disease progression, genetic modifying factors, and more in patients with *GBA1* variants.

In conclusion, our study not only expanded the spectrum of *GBA1* variants by identifying 8 novel *GBA1* variants but also underscored the relatively high prevalence of PD patients carrying *GBA1* variants within the Chinese population. Notably, the p.L483P variant emerged as the most frequent risk factor. Additionally, for the first time, we revealed that p.S310G and p.G241R variants contributed to an increased risk of PD. Furthermore, our findings offered insights into the clinical spectrum of *GBA1* variation in Chinese population and furnish valuable *GBA1* genotype-phenotype observations. Patients with *GBA1* variants, compared to those without *GBA1* variants, exhibited an earlier age at onset, and higher risk of pRBD, olfactory dysfunction, depression, and autonomic dysfunction.

METHODS

Participants

Participants were recruited between October 2006 and August 2021 at the Xiangya Hospital Central South University, as well as other sites affiliated with the Parkinson's Disease and Movement Disorders Multicenter Database and Collaborative Network in China (PD-MDCNC, <http://pd-mdcnc.com>). All the PD patients received diagnoses from experienced neurologists, adhering to either the UK Brain Bank Clinical Diagnostic Criteria for PD or the 2015 International Parkinson and Movement Disorder Society Clinical Diagnostic Criteria for PD. Neurological disease-free control participants consisted of community volunteers and spouses of the patients. Each participant provided informed consent prior to their involvement, and this study was approved by the Ethics Committee of Xiangya Hospital of Central South University. Notably, all participants were self-reported Chinese Han ethnic.

Clinical assessment

Demographic and clinical data were collected, including age, sex, family history, disease duration, and motor and non-motor manifestations. The Unified Parkinson's Disease Rating Scale (UPDRS)³¹ and the Hoehn and Yahr (H-Y) scale³² were used to evaluate motor severity. Patients were assessed during "OFF" medication conditions. UPDRS items for TD and PIGD designations were used to calculate mean TD and PIGD scores. Following the original classification methods, the ratio of the mean UPDRS tremor scores (8 items) to the mean UPDRS PIGD scores (5 items) was used to define TD subtype (ratio ≥ 1.5), PIGD subtype (ratio ≤ 1), and indeterminate subtype (ratio > 1.0 and < 1.5)³³. The 17-item Hamilton Depression Rating Scale (HAM-D-17)³⁴ was used to evaluate depression, and a score of it < 7 points suggests no depression. Sleep status was evaluated using the REM Sleep Behavior Disorder Questionnaire-Hong Kong (RBDQ-HK)³⁵, Epworth Sleepiness Scale (ESS)³⁶ and Parkinson's Disease Sleep Scale (PDSS)³⁷. A score of ESS ≥ 10 points represents excessive daytime sleepiness, while a factor 2 score of RBDQ-HK ≥ 7 or a total score ≥ 18 classifies pRBD. The olfactory function was

evaluated using the Hyposmia Rating Scale (HRS)^{38,39}, and a score of it ≤ 22 indicates hyposmia. The Mini-Mental State Examination (MMSE)⁴⁰ was used to evaluate cognitive function. The Functional Constipation Diagnostic Criteria Rome III (ROME III)⁴¹ and the Scale for Outcomes in Parkinson's disease for Autonomic Symptoms (SCOPA-AUT)⁴² were used to evaluate constipation status. Dyskinesia and freezing gait were evaluated using the Dyskinesia Screening Scale⁴³ and the Freezing Gait (FOG) Scale⁴⁴, respectively. The 39-item Parkinson's disease questionnaire (PDQ-39)⁴⁵ was used to evaluate quality of life⁴⁶.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes following standard procedures. Variants within PD patients with age at onset (AAO) of 50 years or younger and those with a family history of PD and control participants without neurological disease were identified through WES. Meanwhile, variants within sporadic late-onset PD cases (AAO > 50) and matched healthy control were identified through WGS. The data generation and quality control procedures for the WES and WGS data have been detailed previously⁴⁷. Briefly, the sequencing data were first processed using a bioinformatics pipeline for WES and WGS sequencing data (BWA-GATK-ANNOVAR)⁴⁸, and subsequently, the PLINK software was used to perform a series of quality control procedures for individuals and variant⁴⁹. Similar to the quality control standards used in our earlier study⁴⁷, the high-quality variants were extracted: allele depth (AD) ≥ 5 , total depth (DP) ≥ 10 , genotype quality (GQ) ≥ 20 , and missingness rate $< 5\%$ for variants from the WES cohort, whereas AD ≥ 2 , DP ≥ 5 , GQ ≥ 15 for SNPs, GQ ≥ 30 for indels, and missingness rate $< 5\%$ for variants from the WGS cohort. High-quality variants are located in the *GBA1* transcript region and 2 bp of the boundary region between exons and introns, relative to transcript NM_000157 (chr1: 155204243–155211040; hg19). Of note, patients with pathogenic/likely pathogenic variants of PD-causing genes (*SNCA*, *PRKN*, *UCHL1*, *PINK1*, *DJ1*, *LRKK2*, *ATP13A2*, *GIGYF2*, *HTRA2*, *PLA2G6*, *FBXO7*, *VPS35*, *EIF4G1*, *DNAJC6*, *SYNJ1*, *TMEM230*, *CHCHD2*, *VPS13C*, *RIC3*, *DNAJC13*, *LRP10*, *RAB39B*, *POLG*, *DAGLB*) from the WES cohort were excluded from this study, as described in our previous study^{3,18}.

Variant verification

We have performed validation experiments of *GBA1* variants using a Sanger sequencing method. For the variants, primer design was performed using the Primer 3.0 online primer design database. Given the presence of a pseudogene with high homology to *GBA1* gene, we deliberately selected fragments for primer design located exclusively within the *GBA1* gene, effectively avoiding any overlap with the pseudogene. Related primers were shown in Supplementary Table 6 and the TaKaRa Premix Ex Taq™ DNA Polymerase Hot Start Version (Takara Bio RR030A) was used to amplify different exons of *GBA1* gene. The cycling conditions for amplification were as follows: initial denaturation at 95 °C for 5 min, 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min. Lastly, samples were held at 4 °C.

Specifically, three exons (exon 1, exon 3 and exon 5) were amplified using previously described primers⁵⁰. *GBA1* was amplified in a large fragment: a 2972 bp fragment encompassing exons 1–5 using previously described primers and a unique 64 °C to 54 °C touch-down PCR program. PCR products were sequenced with internal primers, adjacent to coding exons and exon-intron boundaries. Related primers were shown in Supplementary Table 6.

Classification of GBA1 Variants

The *GBA1* variants were classified into four different types based on their deduced and observed phenotypic effects on GD or PD: severe variants (known to cause GD type II or III), mild variants (known to cause GD type I), risk variants (variants that are associated with risk for PD but do not cause GD), and unknown variants (reported variants of unknown significance or unreported missense variants⁶).

Statistical analysis

SPSS version 26.0 (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.) was used to analyze the data. Analysis was adjusted for age of onset, disease duration, sex and LEDD, and multiple comparison using Bonferroni corrections ($p < 0.05$). Significance was determined for all analyses if alpha was < 0.05 (corrected). Chi-square tests and Fisher exact tests were used to analyze the influence of *GBA1* variants on the onset of PD. Because no control carried the p.G241R variant, we used the Haldane-Anscombe correction to calculate OR. Briefly, we added "0.5" to numbers in each cell of the 2x2 Table and then calculated the OR over these adjusted cell counts. Linear regression was used to compare demographic data with covariate adjustments. The connection between genetic status and clinical manifestations was evaluated through linear regressions, in which continuous scores correlated with genetic status. This analysis was adjusted for variables including age of onset, disease duration, sex, and LEDD. Meanwhile, the correlations between symptom status (H-Y stage, motor complications or non-motor symptoms) and genetic status were analyzed using logistic regression adjusting for age of onset, disease duration, sex and LEDD. Furthermore, an analysis of motor subtypes was conducted using multinomial logistic regression, using the tremor-dominant group as the reference group.

DATA AVAILABILITY

The summary data of *GBA1* variants can be accessed after an approved application to the Open Archive for Miscellaneous Data (OMIX) of National Genomic Data Center (NGDC). The accession code is OMIX004514. The clinical data used in this study are owned by PD-MDCNC (<http://pd-mdcnc.com>). There are no current sharing agreements, and data were held under a data use contract with PD-MDCNC.

CODE AVAILABILITY

The related codes and scripts used in this study don't present restrictions and are available upon request for information that is made and that can be provided by the corresponding author.

Received: 20 April 2023; Accepted: 16 August 2023;

Published online: 01 September 2023

REFERENCES

- Bloem, B. R., Okun, M. S. & Klein, C. Parkinson's disease. *Lancet* **397**, 2284–2303 (2021).
- Guo, J. F. et al. Coding mutations in NUS1 contribute to Parkinson's disease. *Proc. Natl Acad. Sci. USA* **115**, 11567–11572 (2018).
- Zhao, Y. et al. The role of genetics in Parkinson's disease: a large cohort study in Chinese mainland population. *Brain* **143**, 2220–2234 (2020).
- Sidransky, E. et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N. Engl. J. Med.* **361**, 1651–1661 (2009).
- Migdalska-Richards, A. & Schapira, A. H. The relationship between glucocerebrosidase mutations and Parkinson disease. *J. Neurochem.* **139**, 77–90 (2016).
- Parlar, S. C., Grenn, F. P., Kim, J. J., Baluwendrat, C. & Gan-Or, Z. Classification of GBA1 variants in Parkinson's disease: the GBA1-PD browser. *Mov. Disord.* **38**, 489–495 (2023).
- Thaler, A. et al. Parkinson's disease phenotype is influenced by the severity of the mutations in the GBA gene. *Parkinsonism Relat. Disord.* **55**, 45–49 (2018).
- Creese, B. et al. Glucocerebrosidase mutations and neuropsychiatric phenotypes in Parkinson's disease and Lewy body dementias: review and meta-analyses. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **177**, 232–241 (2018).
- Maple-Groden, J. et al. Association of GBA genotype with motor and functional decline in patients with newly diagnosed Parkinson disease. *Neurology* **96**, e1036–e1044 (2021).
- Szwedo, A. A. et al. GBA and APOE impact cognitive decline in Parkinson's disease: a 10-year population-based study. *Mov. Disord.* **37**, 1016–1027 (2022).
- Huang, J., Cheng, Y., Li, C. & Shang, H. Genetic heterogeneity on sleep disorders in Parkinson's disease: a systematic review and meta-analysis. *Transl. Neurodegener.* **11**, 21 (2022).
- Menozi, E. & Schapira, A. H. V. Exploring the genotype-phenotype correlation in GBA-Parkinson disease: clinical aspects, biomarkers, and potential modifiers. *Front. Neurol.* **12**, 694764 (2021).
- Gan-Or, Z. et al. Differential effects of severe vs mild GBA mutations on Parkinson disease. *Neurology* **84**, 880–887 (2015).
- Gan-Or, Z. et al. Genotype-phenotype correlations between GBA mutations and Parkinson disease risk and onset. *Neurology* **70**, 2277–2283 (2008).
- Ren, J. et al. Prevalence and genotype-phenotype correlations of GBA-related Parkinson disease in a large Chinese cohort. *Eur. J. Neurol.* **29**, 1017–1024 (2022).
- Chen, Y. et al. Evaluating the role of SNCA, LRRK2, and GBA in Chinese patients with early-onset Parkinson's disease. *Mov. Disord.* **35**, 2046–2055 (2020).
- Sun, Y. M. et al. The genetic spectrum of a cohort of patients clinically diagnosed as Parkinson's disease in mainland China. *NPJ Parkinsons Dis.* **9**, 76 (2023).
- Liu, Z. et al. Deficiency in endocannabinoid synthase DAGLB contributes to early onset Parkinsonism and murine nigral dopaminergic neuron dysfunction. *Nat. Commun.* **13**, 3490 (2022).
- Jin, H. et al. A novel p.L216I mutation in the glucocerebrosidase gene is associated with Parkinson's disease in Han Chinese patients. *Neurosci. Lett* **674**, 66–69 (2018).
- Lim, J. L. et al. Glucocerebrosidase (GBA) gene variants in a multi-ethnic Asian cohort with Parkinson's disease: mutational spectrum and clinical features. *J. Neural Transm (Vienna)* **129**, 37–48 (2022).
- Straniero, L. et al. The SPID-GBA study: sex distribution, penetrance, incidence, and dementia in GBA-PD. *Neurol. Genet.* **6**, e523 (2020).
- Ruskey, J. A. et al. Increased yield of full GBA sequencing in Ashkenazi Jews with Parkinson's disease. *Eur. J. Med. Genet.* **62**, 65–69 (2019).
- Zhang, Y. et al. A meta-analysis of GBA-related clinical symptoms in Parkinson's disease. *Parkinsons Dis.* **2018**, 3136415 (2018).
- Cilia, R. et al. Survival and dementia in GBA-associated Parkinson's disease: the mutation matters. *Ann. Neurol.* **80**, 662–673 (2016).
- Nichols, W. C. et al. Mutations in GBA are associated with familial Parkinson disease susceptibility and age at onset. *Neurology* **72**, 310–316 (2009).
- Malek, N. et al. Features of GBA-associated Parkinson's disease at presentation in the UK Tracking Parkinson's study. *J. Neurol. Neurosurg. Psychiatry* **89**, 702–709 (2018).
- Davis, M. Y. et al. Association of GBA mutations and the E326K polymorphism with motor and cognitive progression in Parkinson disease. *JAMA Neurol.* **73**, 1217–1224 (2016).
- Leocadi, M. et al. Longitudinal clinical, cognitive, and neuroanatomical changes over 5 years in GBA-positive Parkinson's disease patients. *J. Neurol.* **269**, 1485–1500 (2022).
- Bansal, Y., Singh, R., Saroj, P., Sodhi, R. K. & Kuhad, A. Naringenin protects against oxido-inflammatory aberrations and altered tryptophan metabolism in olfactory bulbectomized-mice model of depression. *Toxicol Appl. Pharmacol.* **355**, 257–268 (2018).
- Uemura, N. et al. alpha-Synuclein spread from olfactory bulb causes hyposmia, anxiety, and memory loss in BAC-SNCA mice. *Mov Disord* **36**, 2036–2047 (2021).
- Fahn, S. E. R. UPDRS Unified Parkinson's disease rating scale. *Recent Dev. Parkinson's Dis.* **2**, 153–163 (1987).
- Hoehn, M. & Yahr, M. Parkinsonism: onset, progression and mortality. *Neurology* **17**, 427–442 (1967).
- Stebbins, G. T. et al. How to identify tremor dominant and postural instability/gait difficulty groups with the movement disorder society unified Parkinson's disease rating scale: comparison with the unified Parkinson's disease rating scale. *Mov. Disord.* **28**, 668–670 (2013).
- HAMILTON, M. A rating scale for depression. *J. Neurol. Neurosurg. Psychiatry* **23**, 56–62 (1960).
- Li, S. X. et al. Validation of a new REM sleep behavior disorder questionnaire (RBDQ-HK). *Sleep Med.* **11**, 43–48 (2010).
- Johns, M. W. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* **14**, 540–545 (1991).
- Chaudhuri, K. R. et al. The Parkinson's disease sleep scale: a new instrument for assessing sleep and nocturnal disability in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* **73**, 629–635 (2002).

38. Millar Vernetti, P., Perez Lloret, S., Rossi, M., Cerquetti, D. & Merello, M. Validation of a new scale to assess olfactory dysfunction in patients with Parkinson's disease. *Parkinsonism Relat. Disord.* **18**, 358–361 (2012).
39. Zhou, Y. et al. Olfactory dysfunction and its relationship with clinical features of Parkinson's disease. *Front. Neurol.* **11**, 526615 (2020).
40. Folstein, M., Folstein, S. & McHugh, P. "Mini-mental state": A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatric Res.* **12**, 189–198 (1975).
41. Drossman, D. A. & Dumitrascu, D. L. Rome III: New standard for functional gastrointestinal disorders. *J. Gastrointest Liver Dis.* **15**, 237–241 (2006).
42. Visser, M., Marinus, J., Stiggelbout, A. M. & Van Hilten, J. J. Assessment of autonomic dysfunction in Parkinson's disease: the SCOPA-AUT. *Mov. Disord.* **19**, 1306–1312 (2004).
43. Goetz, C. G. et al. Utility of an objective dyskinesia rating scale for Parkinson's disease: inter- and intrarater reliability assessment. *Mov. Disord.* **9**, 390–394 (1994).
44. Nieuwboer, A. et al. Reliability of the new freezing of gait questionnaire: agreement between patients with Parkinson's disease and their carers. *Gait Posture* **30**, 459–463 (2009).
45. Bushnell, D. M. & Martin, M. L. Quality of life and Parkinson's disease: translation and validation of the US Parkinson's Disease Questionnaire (PDQ-39). *Qual. Life Res.* **8**, 345–350 (1999).
46. Zhou, X. et al. The Chinese Parkinson's Disease Registry (CPDR): study design and baseline patient characteristics. *Mov. Disord.* **37**, 1335–1345 (2022).
47. Zhao, Y. W. et al. The association between lysosomal storage disorder genes and Parkinson's disease: a large cohort study in Chinese mainland population. *Front. Aging Neurosci.* **13**, 749109 (2021).
48. Yang, H. & Wang, K. Genomic variant annotation and prioritization with ANNOVAR and wANNOVAR. *Nat. Protoc.* **10**, 1556–1566 (2015).
49. Chang, C. C. et al. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* **4**, 7 (2015).
50. Stone, D. L. et al. Glucocerebrosidase gene mutations in patients with type 2 Gaucher disease. *Hum. Muta.t* **15**, 181–188 (2000).

ACKNOWLEDGEMENTS

The authors would like to thank the study participants. We thank Bioinformatics Center, Xiangya Hospital, Central South University and High Performance Computing Center of Central South University for assisting in data analysis. This study was supported by the Hunan Innovative Province Construction Project (Grant No. 2021SK1010, 2019SK2335), the National Key Research and Development Program of China (Grant No. 2016YFC306000), National Natural Science Foundation of China (Grant No. 82001359, No. 82071437, and U20A20355), the Natural Science Foundations of Hunan Province (Grant No. 2021JJ31115).

AUTHOR CONTRIBUTIONS

Z.L. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design were done by Z.L., H.W., B.T. and Y.Z. Acquisition, analysis, and interpretation of data were done by Y.Z., Y.W., J.W., Y.Z., H.P., Q.Z., X.Z., R.H., X.Z., Y.X., Z.Z., B.C., Q.S., Q.X., J.T., X.Y., J.L., L.S., H.J., J.G., B.T., H.W., and Z.L. Y.Z. and Z.L. drafted the original manuscript. All authors were involved in reviewing and editing the manuscript, and approved the manuscript for submission.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41531-023-00571-4>.

Correspondence and requests for materials should be addressed to Heng Wu or Zhenhua Liu.

Reprints and permission information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2023