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ORIGINAL ARTICLE

Mutations in the glucocerebrosidase gene are responsible for Chinese patients with Parkinson's disease

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Pathological mutations in the glucocerebrosidase gene (*GBA*) have been suggested to be associated with Parkinson's disease (PD) in various ethnic populations. Most studies on Chinese PD patients have only screened the N370S and L444P mutations in the *GBA* gene. To investigate the *GBA* mutations in Chinese population, we performed complete sequencing of the *GBA* gene in 184 Chinese PD patients and 130 Chinese control individuals. As a result, we identified three novel and nine reported *GBA* mutations. The novel mutations include 5-bp deletion (c.334_338delCAGAA), L264I and L314V and the nine reported *GBA* mutations are R163Q, F213I, E326K, S364S, F347L, V375L, L444P, RecNciI and Q497R. The novel 5-bp deletion (CAGAA) produces a short truncated *GBA* protein of 142 amino acids, which loses major function domains of the 536 amino acids. Our data also reveals that the frequency of *GBA* mutations within this Chinese PD cohort was 8.7%, which is significantly higher than 1.54% observed in the Chinese control cohort ($\chi^2 = 7.22$, $P = 0.0072$; odds ratio (OR) = 6.095, 95% confidence interval of OR = 1.546–24.030). The most common L444P mutation accounts 2.74%, which confer more genetic risk for PD in this Chinese population. In conclusion, novel and known *GBA* mutations were identified and were found to be associated to PD in this Chinese population.

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INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease, affecting 1–2% of the population over the age of 60. Most of the PD cases are sporadic and the rest are familial cases, which are caused by genetic mutations in PD-related genes. Over the last two decades, molecular studies have identified the genes of α-synuclein (*SNCA*), *Parkin (PARK2), DJ-1 (PARK7), PINK1, LRRK2, ATP13A2, VPS35* and *EIF4G1* as being disease causing, whereas *TAU(MAPT), NURR1(NR4A2), PARL* and *GBA* have been deemed as being susceptibility genes for PD.[1–5] Mutations in the *GBA* gene (MIM 606463) were originally reported in patients with Gaucher's disease. Subsequently, *GBA* mutations were also identified in patients with Gaucher's disease and Parkinsonism.[6]

There has been an increasing evidence of the association between the *GBA* gene and PD in different ethnic populations and that the common *GBA* mutations in PD patients were reported to be N370S, L444P, RecNciI and D409H in the American, British, French, Greek, Brazilian populations. The L444P and N370S were reported to account for 60–70% of the mutant alleles in some PD patients.[7–9] Several studies performed screening for *GBA* L444P and N370S mutations in Chinese PD cases and controls and indicated that *GBA* L444P mutation is associated with PD in some Chinese populations.[10–12] However, no studies have performed complete sequencing of the *GBA* gene for Chinese PD patients and controls. To characterize the *GBA* mutations and their association with PD in Chinese populations, we sequenced all exons of the *GBA* gene for PD patients and control individuals in a Chinese population.

SUBJECTS AND METHODS

Patients and control individual data

A total of 184 PD patients and 130 control individuals were recruited from two research hospitals in Eastern China. They include 74 patients from the Affiliated Liaocheng Hospital of Taishan Medical University (Liaocheng, Shandong, China) and 110 patients from Affiliated Reijing Hospital of Shanghai Jiaotong University (Shanghai, China). All these 184 PD patients are sporadic cases. The

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diagnosis of PD and the exclusion criteria for secondary PD or another neurological disease were made on the criteria published by Hughs and Savitt.[11,13] The age- and gender-matched 130 controls were collected from the same hospitals of PD cases. This study was approved by the Ethics Review Committees of Affiliated Liaocheng Hospital of Taishan Medical University and Affiliated Reijing Hospital of Shanghai Jiaotong University. Informed consents were obtained from all the PD patients and control individuals. Physical examinations were performed for all patients by two neurologists. The average age of the patients at onset of the PD was 60.89 years. The earliest age at onset was 36 years of age, whereas the latest age at onset occurred at 79 years of age in this Chinese population. The ratio of the men versus women in this PD cohort is 1.51.

DNA isolation, PCR amplification and direct sequencing of the *GBA* gene

Genomic DNA was extracted from the venous blood of the patients and unaffected control individuals using the QiAamp Spin Column protocols of the QIAGEN kit (China-QIAGEN, Shanghai, China). Human *GBA* gene is located on chromosome 1q21 and includes 11 exons. In order to sequence only the *GBA* gene and not the highly similar *GBA* pseudogene (*GBAP*), we used three pairs of primers to specifically amplify three regions containing all of the exons in the *GBA* gene, with fragment 1 spanning exons 1–5, fragment 2 spanning exons 5–7 and fragment 3 spanning exons 8–11.[14] DNA sequencing was performed on ABI 3130xl genetic analyzer using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem, Foster City, CA, USA). The primer sequences for PCR amplification and DNA sequencing are available on ordering.

Mutation confirmation and functional prediction of the identified mutations

Single-nucleotide polymorphism (SNP) is generally defined as a common single-nucleotide variant that occurs in a significant proportion (>1%) of a large population. Those variants that were not detected in 100 controls and the SNP database (dbSNP137) should be deemed as being mutations. In this study, even though 130 normal controls were sequenced, the identified *GBA* sequence variants in PD patients, which results in nonsynonymous amino-acid change or those occurring in splice sites were screened in additional 100 normal control individuals with matching ethnic backgrounds to further confirm whether the variants are mutations or not. For the potential damage of the identified mutations to GBA protein, we used polyphen2 (http://genetics.bwh.harvard.edu/pph2) program to perform the functional prediction of the identified mutations.

Statistical analyses

The *GBA* mutation frequencies in PD patients and control individuals from Chinese population were statistically analyzed by using the χ^2-test (SPSS statistics software 19.1, IBM Corporation, Armonk, NY, USA).

RESULTS

Mutations in the *GBA* gene identified in PD patients and control individuals

After sequencing all 11 exons of the *GBA* gene in 184 PD patients, we identified 12 different mutations including 3 novel and 9 known mutations among 16 of the 184 PD patients of Chinese origin. We used NM_000157 as the *GBA* reference gene to compare the *GBA* sequencing data. The three novel identified mutations in PD patients are 5-bp deletion of c.334_338delCAGAA, L264I and L314V. We also detected the following nine known *GBA* mutations, R163Q, F213I, E326K, F347L, S364S, V375L, L444P, RecNciI (L444P+ A456P+ V460V) and Q497R within our PD patients. But we only found two mutations of F347L and A456P in the 130 control individuals we sequenced (Table 1 and Figure 1).

The most common *GBA* mutations in these Chinese PD patients are RecNciI including L444P, A456P and V460V (three patients), L444P (two patients) and L264I (two patients).

We compared the *GBA* mutation frequencies between PD patients and control individuals to determine whether *GBA* mutations are more frequent in PD patients than in control individuals. The *GBA* mutation frequency of 8.7% (16/184) in these Chinese PD patients is significantly >1.54% (2/130) of the control individuals ($\chi^2 = 7.22$, $P = 0.0072$; odds ratio = 6.095, 95% confidence interval of odds ratio = 1.546–24.030; Table 1). Our study indicated that Chinese PD patients have 10 times more risk to have *GBA* mutation than normal controls, and *GBA* mutations are associated with PD, suggesting that the *GBA* gene is the most frequent genetic risk factor for PD patients in this Chinese population.

Table 1 Identified mutations in Chinese cohorts of PD patients and control individuals

					PD patients (184)		*Controls (130)*	
GBA mutation	*Mutation status*	*cDNA*	*Protein*	*Exon*	N	*Frequency (%)*	N	*Frequency (%)*
del5 (CAGAA)	Novel	c.334-338 del5	Truncated	4	1	0.54	0	0.00
R163Q	Known	c.605G>A	p.Arg163Gln	6	1	0.54	0	0.00
F213I	Known	c.754T>A	p.Phe213Ile	6	1	0.54	0	0.00
L264I	Novel	c.907C>A	p.Leu264Ile	7	2	1.09	0	0.00
L314V	Novel	c.1057C>G	p.Leu314Val	8	1	0.54	0	0.00
E326K	Known	c.1093G>A	p.Glu326Lys	8	1	0.54	0	0.00
F347L	Known	c.1156T>C	p.Phe347Leu	8	1	0.54	1	0.77
S364S	Known	c.1209C>T	p.Ser364Ser	8	1	0.54	0	0.00
V375L	Known	c.1240G>C	p.Val375Leu	9	1	0.54	0	0.00
L444P	Known	c.1448T>C	p.Leu444Pro	10	2	1.09	0	0.00
RecNciI(L444P-A456P-V460V)	Known	c.1448T>C	p.Leu444Pro	10	3	1.63	0	0.00
		c.1483G>C	p.Ala456Pro		0	0.00	0	0.00
		c.1497G>C	p.Val460Val		0	0.00	0	0.00
A456P	Known	c.1483G>C	p.Ala456Pro	10	0	0.00	1	0.77
Q497R	Known	c.1607A>G	p.Gln497Arg	11	1	0.54	0	0.00
Total					16	8.70	2	1.54

Abbreviations: cDNA, complementary DNA; GBA, glucocerebrosidase gene; PD, Parkinson's disease.

Novel *GBA* mutations

We identified three novel *GBA* mutations in 184 PD patients (Table 1 and Figure 1). One mutation, c.907C>A, changes the amino acid of leucine to ileucine (L264I) in exon 7 and was found in two Chinese patients. Another novel mutation of c.1057C>G was found in exon 8 of the coding region in one Chinese patient. The mutation of c.1057C>G changes the amino acid of leucine to valine (L314V). For these three novel *GBA* mutations of 5-bp deletion, L264I, L314V, R163Q and F347L (we thought these two mutations are novel, but found they are known mutations later), we sequenced additional 100 normal control individuals without finding anyone having one of these five mutations. Thus, for each specific mutation of 5-bp deletion, L264I, L314V, R163Q and F347L, we totally sequenced 230 control individuals, but we only sequenced 130 control individuals for the whole gene. Importantly, we identified a novel mutation of 5-bp deletion (CAGAA) of exon 4 in one Chinese patient. This 5-bp deletion produces a frame-shift mutation leading to a truncated protein of 142 amino acids (Figure 2). After the 5-bp deletion was identified in the patient, we had tried to get the fresh blood and skin fibroblast from this patient to perform RT–PCR and western blotting analysis, unfortunately the patient passed away because of another disease. In order to know whether the identifed mutations have some

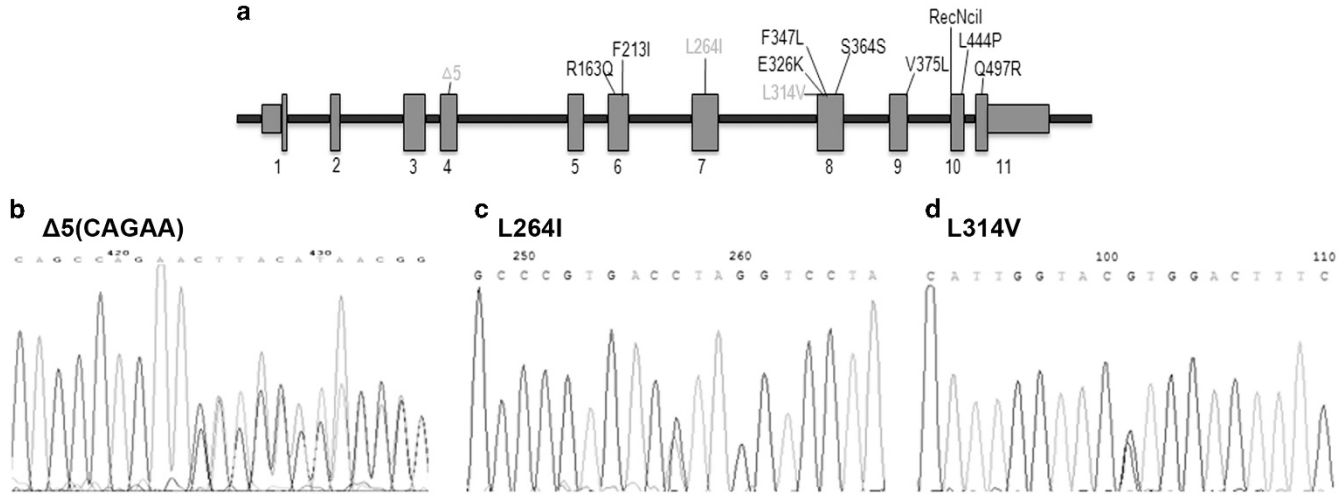

Figure 1 The identified glucocerebrosidase (*GBA*) mutations in Parkinson's disease (PD) patients from a Chinese population. (**a**) The exonic structure of *GBA* with 18 identified mutations, including 3 novel mutations and 9 known mutations. (**b**) Novel Δ 5 represents 5-bp deletion of exon 4 in patient S25. (**c**) Novel L264I mutation in patients of L10 and L44. (**d**) Novel L314V mutation in patient S184. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

MEFSSPSREECPKPLSRVSIMAGSLTGLLLLQAVSWASGARPCIPKSFGYSSVVCVCNAT 60
MEFSSPSREECPKPLSRVSIMAGSLTGLLLLQAVSWASGARPCIPKSFGYSSVVCVCNAT 60

YCDSFDPPTFPALGTFSRYESTRSGRRMELSMGPIQANHTGTGLLLTLQPE 111
YCDSFDPPTFPALGTFSRYESTRSGRRMELSMGPIQANHTGTGLLLTLQPE 111

VPESEGIWR GHDRCCCSQH PCPVTPCPKFAT* 142

QKFQKVKGF GGAMTDAAAL NILALSPPAQ NLLLKSYFSE EGIGYNIIRV PMASCDFSIR 170

TYTYADTPDDFQLHNFSLPE EDTKLKIPLI HRALQLAQRP VSLLASPWTS PTWLKTNGAV 230

NGKGSLKGQP GDIYHQTWAR YFVKFLDAYA EHKLQFWAVT AENEPSAGLL SGYPFQCLGF 290

TPEHQRDFIARDLGPTLANS THHNVRLLML DDQRLLLPHW AKVVLTDPEA AKYVHGIAVH 350

WYLDFLAPAKATLGETHRLF PNTMLFASEA CVGSKFWEQS VRLGSWDRGM QYSHSIITNL 410

LYHVVGWTDWNLALNPEGGP NWVRNFVDSP IIVDITKDTF YKQPMFYHLG HFSKFIPEGS 470

QRVGLVASQKNDLDAVALMH PDGSAVVVVL NRSSKDVPLT IKDPAVGFLE TISPGYSIHT 530

YLWRRQ* 536

Figure 2 The amino-acid sequence alignment of the truncated glucocerebrosidase (*GBA*) protein produced by the 5-bp deletion of the *GBA* gene and the wild-type *GBA* protein. The amino-acid sequence alignment of the truncated *GBA* protein produced by the 5-bp deletion of the *GBA* gene and the wild-type *GBA* protein. This 5-bp deletion produces a frame-shift mutation leading to a truncated *GBA* protein of 142 amino acids, highlighted in red. The wild-type *GBA* protein of 536 amino acids is in black. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

pathological effects on the function of GBA protein, we used polyphen2 program (http://genetics.bwh.harvard.edu/pph2) to predict the protein damage of these novel and known mutations. As a result, we found that novel L264I mutation, known V375L and L444P mutations have possible damaging effects on GBA protein, whereas novel L314V and other known mutations are benign to GBA protein as shown in Table 2.

As novel mutation of L264I (c.907C>A) was identified in two Chinese PD patients (L10 and L44), we performed haplotype analysis to know whether or not this mutation is from the same founder mutation using single-nucleotide polymorphism flanking this mutation. As a result, one PD patient has haplotype of CACC/GAAT (the third letter is C>A mutation), whereas another patient has haplotype of CGCT/GGAT. No common haplotype was shared in these two patients, excluding this L264I mutation coming from the same founder.

Table 2 Predicted protein damage of the novel and known mutations identified in Chinese PD patients

GBA Mutation[a]	*Score*	*Sensitivity*	*Specificity*	*Effects*
R163Q	0.012	0.96	0.78	Benign
F213I	0.117	0.93	0.86	Benign
L264I	0.613	0.87	0.91	Possibly damaging
L314V	0	1	0	Benign
E326K	0.015	0.96	0.79	Benign
F347L	0.002	0.99	0.3	Benign
V375L	0.963	0.78	0.95	Probably damaging
L444P	0.938	0.8	0.94	Possibly damaging
A456P	0.037	0.94	0.82	Benign
Q497R	0.001	0.99	0.15	Benign

Abbreviation: GBA, glucocerebrosidase.
[a]The protein effects of 5-bp deletion producing shorted protein of 42 AA and S364S could not be predicted by polyphen2 program.

Mutation frequency of L444P and N370S

Jewish PD patients were reported to have a high frequency of L444P and N370S accounting for 70% of PD cases among those with *GBA* mutation.[7,8] Other ethnic PD patients were also found to have high frequency of L444P mutation.[15,16] In 184 Chinese PD patients, we found two patients with the L444P mutation and three patients with the RecNciI mutation (L444P+ A456P+ V460V), but none of the Chinese PD patients had the N370S mutation. The mutation frequency of L444P in these Chinese PD patients was 2.72% (5/184). There were no mutations of N370S, L444P and RecNciI found in 130 control individuals. Overall, L444P represents 31.2% (5/16) of the *GBA* mutations in this Chinese PD cohort, which is similar to that of PD patients in other ethnic groups except for those of Jewish ancestry.[17]

Genotype–phenotype analyses of PD patients with *GBA* mutations

We analyzed the phenotype characteristics of PD patients with *GBA* mutation and found that most of the PD patients with *GBA* mutations are L-DOPA responsive with motor fluctuations (Table 3). A previous study has suggested PD patients with *GBA* mutations had an earlier age of onset.[17] We found that Chinese patients with *GBA* mutations had an age at onset of 55.9 years, whereas patients without *GBA* mutations had an age at onset of 61.6 years. However, no significant difference was observed between the age at onset of patients with *GBA* mutations and without *GBA* mutations ($t=0.412$, $P=0.522$), probably because the samples with *GBA* mutations are too small. The patients with the RecNciI mutation were also found to have more severe clinical symptoms of tremor and slow movement. We found that most of the PD populations have *GBA* mutation frequencies in the range of 4–10% except for the Jewish patients who have a higher frequency of 13–20%.[7,8,18,19]

DISCUSSION

Recent studies have reported mutations in *GBA* for PD from different ethnic origins and revealed that *GBA* mutations have conferred more

Table 3 Clinical phenotype characteristics of Chinese PD patients with GBA mutations

Patient number	*Mutations*	*Clinical diagnosis*	*Sex*	*Age at onset (years)*	*L-DOPA responsiveness*	*Family history Number of patients*	*First symptom*	*Cognitive symptoms*
L10	L264I	PD definite	Female	60	Yes	No	Stiffness in the right arm	No
L14	E326K	Parkinsonism	Male	67	Yes	No	Tremor in the legs	No
L15	S364S	Parkinsonism	Male	49	Yes	No	Tremor in the left leg	No
L17	RecNciI	PD definite	Female	38	Yes	No	Stiffness in the left arm	No
L33	F347L	PD definite	Male	58	Yes	No	Slow movement in the right arm	No
L43	RecNciI	PD definite	Female	54	Yes	No	Stiffness in the legs	Yes
L44	L264I	PD definite	Male	63	Yes	No	Cognitive impairment	Yes
L75	RecNciI	PD definite	Female	46	yes	No	Stiffness in the legs	No
S25	del5 (CAGAA)	PD definite	Male	49	Yes	No	Tremor on the left side	No
S47	L444P	PD definite	Male	56	Yes	No	Tremor in the arms	No
S50	V375L	PD definite	Male	59	Yes	No	Stiffness in the right larm	No
S51	L444P	PD definite	Male	63	Yes	No	Stiffness in the legs	No
S157	Q497R	PD definite	Female	65	Yes	No	Tremor in the right leg	Yes
S159	F213I	PD definite	Female	51	Yes	No	Slow movement in the left arm	No
S184	L314V	PD definite	Female	61	Yes	No	Tremor and stiffness in the legs	No
S195	R163Q	PD definite	Female	56	Yes	No	Tremor in the arms	No
Average				55.94				

Abbreviations: GBA, glucocerebrosidase; PD, Parkinson's disease.

risk to the development of PD than any of other PD-related genes such as *LRRK2* and *Parkin (PARK2)*. However, most of these studies focused on the specific *GBA* mutations without sequencing the full *GBA* gene. By complete sequencing analyses of the *GBA* gene, we found that PD patients have a significantly higher frequency of *GBA* mutations than their corresponding control individuals from a Chinese population. The *GBA* mutation frequency (8.7%) in this Chinese cohort of PD patients is comparable to the reported *GBA* mutation frequencies in PD patients with different ethnic backgrounds in other studies.[7,8,15] Our study provided further evidence supporting the idea that the overall frequency of *GBA* mutations within PD patients might not be affected by ethnic origin except for the Jewish population.[5]

The L444P mutation was reported to be the most frequent *GBA* mutation in PD populations occurring at 1.14%, 2.00% and 1.39% incidence among the Canadian, American non-Jewish and British patients, respectively.[15,17,20] Sun *et al.*[10] screened 402 Chinese PD patients and reported that the L444P allele frequency was 2.74% (11/402). Mao *et al.*[11] screened L444P mutation in 616 Chinese PD patients and found that the frequency of this L444P mutation is 3.2% (20/616), which is similar than 2.74% in our Chinese PD patients. Even though Ashkenazi Jewish and some European PD patients were reported to have the N370S allele frequencies of 15 and 46%,[7,8] the N370S mutation frequency is relatively lower in Asian PD patients.[9,21,22] We did not identify the N370S mutation in any of the 184 Chinese PD patients, further supporting that N370S mutation occurred rarely in Asian PD patients.

We only saw a few patients with cognitive impairment and dementia, and did not see an increased risk of developing cognitive impairment and dementia symptoms in patients with *GBA* mutations, a result which differs from others.[23,24] Even though the patients with *GBA* mutations were reported to have bradykinesia and rigidity as the earlier symptom,[25] the earlier symptoms of our PD patients with *GBA* mutations included tremors, rigidity and cognitive impairment and vary from one to another. We could not find any difference in the motor symptoms, cognitive impairment or disease progression between the *GBA* mutation carriers and non-*GBA* mutation carriers.

We have identified 3 novel *GBA* mutations in 184 Chinese PD patients, suggesting that the screening of *GBA* mutation in a larger cohort of Chinese PD patients would further help understand the role *GBA* has in the pathogenesis of PD. One of the novel *GBA* mutations (5-bp deletion) was identified in a Chinese PD patient. As this 5-bp deletion produces a short truncated *GBA* protein, potentially resulting in a more severe pathology, we further evaluated the clinical characteristics of the patient. This patient developed a tremor on the left side and slow movement as the first symptoms at the age of 49. He is L-DOPA-responsive but does not have other cognitive symptoms. The L264I mutation was found in two sporadic Chinese PD patients, but haplotype analysis showed that they do not share the same haplotype, suggesting that this mutation does not occur as linkage disequilibrium in this Chinese population.

GBA mutation in patients with Lewy body Dementia, Alzheimer's disease and multiple system atrophy[17,26,27] were ever examined to determine whether *GBA* mutations contributed to pathogenesis of other neurodegenerative diseases. As a result, *GBA* mutations were also identified in Lewy body Dementia and Alzheimer's disease patients, but not in the multiple system atrophy patients, suggesting that the *GBA* mutations could induce neurodegeneration in other neurological disorders through mechanisms similar to PD.[28]

The mechanism whereby *GBA* mutations lead to the death of dopaminergic neurons is unknown. In most patients with *GBA* mutations, β-glucosidase activity was not affected, suggesting that the *GBA* mutations may affect the dopamine neuron survival through other mechanisms. Some studies showed that mutant *GBA* affects dopamine neuron development and maturation by interfering with α-synuclein in the mitochondria and protein trafficking signaling of the dopamine neurons.[12] A recent study investigating the over-expression of the *GBA* mutants (L444P, N370, D409H, D409V, E235A and E340A) showed a significant increase in the levels of α-synuclein in cultured human and mouse neural cells.[29] Another study suggested that the *GBA* mutations could interfere with the endoplasm reticulum-associated degradation of the proteins to induce cell death.[30] The prediction analysis on functional effects of these novel and known mutations suggested that L264I, V375L and L444P are possibly damaging the GBA protein, whereas others may not have pathological effects on the GBA protein. To further validate the damaging effects of L264I, V375L and L444P mutations more studies are required to overexpress these mutations in cell cultures and in gene-targeted animal models to understand the molecular pathways these mutations are involved in. In conclusion, this study indicates that *GBA* mutations are the risk factor in Chinese PD patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Author contributions: Dr Fabin Han contributed to the research design, clinical diagnosis of PD patients, some experimental work and manuscript preparation; Dr Weidong Le contributed to the research design, clinical diagnosis of PD patients and manuscript corrections; Zhe Yu, Ting Wang, Dr Jun Xu, Wang Wang, Chao Chen, Lili Zheng, Li Pan, Fang Li, Lemuel Racacho contributed to the experimental work; Dr Bin Zhang, Dr Dianrong Gong, Dr Huaiqian Qu, Dr Guifang Wang, Dr Xueli Li and Dr Jun Xu contributed to clinical diagnosis of PD patients.

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