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Association of Common Variants in the Glucocerebrosidase Gene with High Susceptibility to Parkinson's Disease among Chinese

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Abstract

The genetic variants in glucocerebrosidase (*GBA*) gene have been previously examined as potential susceptibility factors for Parkinson's disease (PD). Although of great interest, possible role of *GBA* gene in PD has not been well investigated in eastern Chinese population. To explore this association, we conducted a genetic screen of three common *GBA* variants (p.L444P, p.N370S, and p.R120W) in a case-control cohort comprised of 638 subjects of Chinese ethnicity. In order to provide a more precise estimate of this association, a meta-analysis was performed. We found that the *GBA* p.L444P allele was significantly more frequent ($P = 0.001$) in the PD patients (6/195 = 3.08%) than in the controls (0/443). The p.L444P mutation, but not p.N370S and p.R120W, was found to be associated with PD. Combined analysis including all previously published ancestral Chinese data yielded a highly significant association between the *GBA* gene and an increased risk for PD (OR = 8.13, 95% CI, 4.43-14.92, $P < 0.00001$). Our study suggests that the *GBA* gene may be a susceptibility gene for PD in the Chinese population. Efforts to elucidate in detail this interesting and biologically plausible genetic association are warranted.

Key Words: Parkinson's disease, glucocerebrosidase, genetic association studies, risk factors, meta-analysis

Introduction

Parkinson's disease (PD) is an insidious and progressive neurodegenerative disorder characterized by various combinations of motor (rest tremor, bradykinesia, rigidity and postural instability) and nonmotor (hyposmia, autonomic dysfunction, sleep disorders, cognitive impairment and depression) symptoms (10, 23). Different clinical phenotypes are distinguished by predominant motor features: a tremor-dominant type (TDT), an akinetic-rigid type (ART), and a mixed type (MT) (26). The pathogenesis of idiopathic PD remains unclear, but genetic factors are thought to confer a risk. Several loci, *SNCA*, *LRRK2*, and *MAPT*, have typically been identified as susceptibility genes for not only familial, but also common sporadic forms of PD (8, 15, 17).

The recent demonstration of glucocerebrosidase (*GBA*) gene as a causative PD gene has provided considerable insights into the pathophysiology of PD

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(34). Relatively few researches failed to reach statistical significance (32, 38). Most studies detected a markedly higher frequency of *GBA* mutations among patients with PD than in matched controls (34). The mutation rate varied between 10.63% and 31.31% among PD patients of Ashkenazi Jewish origin (1, 6, 14, 27) and between 2.74% and 9.36% in non-Ashkenazi Jewish patients (3, 7, 9, 11, 22, 24, 25, 29-31, 33, 35-37, 41, 45).

The heterozygotes for p.L444P and p.N370S were suggested to be the most common variants among non-Ashkenazi Jewish patients (39, 40). These findings have been supported by studies in different ethnic Chinese groups of patients with PD from Taiwan (20, 42, 45), Middle southern China (36), Western China (19, 28) and Singapore (37). However, the possible role of the third most common *GBA* variant (p.R120W) (34, 40) has not been investigated in the mainland Chinese population. The lack of similar findings in eastern Chinese population further emphasizes the importance of testing whether these three *GBA* variants are genetically associated with PD-risk in our specific ethnic and geographic origin of the subjects. Here we have attempted to examine this association by performing a case-control study and a meta-analysis.

Materials and Methods

Subjects

The study included 195 unrelated ethnic Han Chinese (56.41% men) with a diagnosis of idiopathic PD based on UK brain bank criteria (21). All individuals were consecutively recruited from the Second Affiliated Hospital of Wenzhou Medical College. Initial symptoms, age at onset (AAO), drug records, and family history were collected by a structured interview of the patient or their guardians. Examinations were performed by experienced neurologists that incorporated the Hoehn and Yahr staging system (18) and the Unified Parkinson's Disease Rating Scale (UPDRS) (12). Patients with known evidence of secondary causes, or patients who reported first- or second-degree relatives with Parkinsonism were excluded. Our age- and gender-matched control group consisted of 443 non-PD volunteers (58.24% men). The age at collection was ranged from 33 to 86 years, with an average age at enrollment of 64.68 (± 10.98) and 64.52 (± 11.60) years for cases and controls. All of them underwent the same evaluation as cases. All participants signed a local ethics committee-approved informed consent before entering the study.

Genotyping

Genomic DNA was isolated from peripheral blood using standard protocols according to the manufacturer's instruction. The molecular-genetic investigation was based on the method of polymerase chain reaction followed by restriction fragment length polymorphism analysis (PCR-RFLP). To avoid amplifying and screening the highly conserved pseudogene, a large fragment of the *GBA* functional gene was amplified on the first stage. On the second stage, amplification of the region encompassing the p.L444P, p.N370S or p.R120W variant was performed using the fragment already obtained as template DNA. The PCR primers used for amplifying the exon 5-7, exon 8-10 (25), and the p.L444P (1), p.N370S (19) variants have been described previously. A fragment encompassing the p.R120W allele was amplified using the forward primer 5'-TGATAAGCAGAGTCCC ATAC-3' and the reverse primer 5'-GAGAAGCA CCCAGAGTTG-3' (annealing temperature 59°C, extension time 30 second). Then, the PCR products were digested with appropriate restriction endonucleases. The primers and restriction endonucleases used for detection of the mutations were described in Table 1. To distinguish the wild-type from the mutant allele, the sizes of the resulting fragments were resolved by agarose gel electrophoresis. Later, ambiguous or positive results and randomly selected samples were confirmed by direct sequencing of the purified PCR products (Fig. 1).

Statistical Analyses

Statistical analyses were performed using SPSS 13.0 software. Data are presented as mean (±SD) for continuous variables. Variant frequencies were compared using a two-tailed Fisher exact test. Differences in clinical characteristics were compared by means of the two-tailed Student's *t*-test. The level of statistical significance was set at $P < 0.05$. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to test the association between the *GBA* variants and PD.

Meta-Analysis

We performed a meta-analysis to assess the possible role of the *GBA* gene variants in PD in Chinese population. A systematic literature search of PubMed, Google Scholar, EMBASE, CINAHL, Web of Science, and the Cochrane Database was conducted for the years 1990 to July 2011. A search strategy utilizing the Medical Subject Headings (MeSH) and text keywords *GBA*, glucocerebrosidase, *GBA* mutation, glucocerebrosidase variant in combination with Parkinson's disease, and PD were used. Reference lists from retrieved articles were also examined to identify additional relevant studies. We included studies that previously

Table 1. Primers and restriction enzymes used for genotyping assay of *GBA* variants

Variant	Exon	Primer Sequence (from 5' to 3')	Enzyme	Fragment (bp)
L444P (T>C)	10	first PCR (exon 8-10): F:TGTGTGCAAGGTCCAGGATCAG R:ACCACCTAGAGGGGAAAGTG Nested PCR: F:GGAGGACCCAATTGGGTGCGT R:ACGCTGTCTTCAGCCCACTTC	 Nci I	1,682 638/102,536
N370S (A>G)	9	first PCR (exon 8-10) Nested PCR: F:TTGTCTCTTTGCCTTTGTCCTTACCCTC R:TTGGGTCCTCCTTCGGGGTT	 Xho I	1,682 97/26,71
R120W (C>T)	5	first PCR (exon 5-7): F:GACCTCAAATGATATACCTG R:AGTTTGGGAGCCAGTCATTT Nested PCR: F:TGATAAGCAGAGTCCCATAC R:GAGAAGCACCCAGAGTTG	 Nci I	2,049 87,302/389

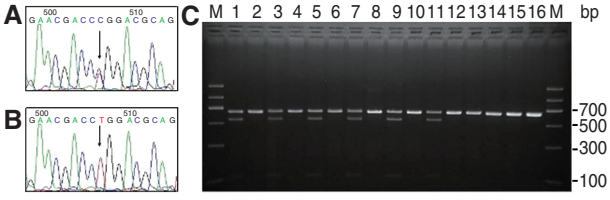

Fig. 1. Analysis of the p.L444P mutation in the *GBA* gene. A: Fluorescent chromatographs of the p.L444P mutation. The arrow points to the heterozygous substitution. B: Fluorescent chromatographs of normal sequence. C: PCR Analysis of the p.L444P mutation in patients with PD. When the mutation is present (lanes 1, 3, 5, 7, 9, and 11), the enzyme (*Nci* I) digests a 638-bp PCR product, producing two fragments of 536 and 102 bp. The wild-type PCR product remains uncut. Lane M denotes a marker.

sequenced the *GBA* gene variants in PD cases and controls of Chinese ancestry. We excluded the studies if they did not have a case-control study design, and if they did not report an adequate statistical analysis. We used RevMan 5.2 software to analyze data.

Results

Present Case-Control Study

We identified 6 PD cases that were heterozygous for the p.L444P allele (c.1448T > C, p.L483P). We did not find the *GBA* gene p.N370S (c.1226A > G, p.N409S) and p.R120W (c.475C > T, p.R159W) variants among all the cases. None of these variants were detected in the controls. The p.L444P mutation encountered in cases at a rate of 3.08% was significantly more frequent than in controls (0/443) (P = 0.001). The rate of *GBA* mutations was higher in subsets of patients with ART (3/57) and MT (3/61) than in a subset of patients with TDT (0/77).

The following initial symptoms were reported in the entire cohort of PD patients: tremor (57.44%), bradykinesia (24.62%), gait disorder or postural instability (16.92%), and rigidity (13.85%). The frequency of patients reporting tremor as a first symptom was more than threefold higher among noncarriers (58.73%) compared with *GBA* mutation carriers (16.67%), but this did not reach a level of significance (P = 0.085). Clinical manifestations of patients in heterozygous state were summarized in Table 2. All of them had a good response to L-dopa or other dopaminergic agents.

Meta-Analysis

We identified 9 relevant studies concerning the *GBA* gene mutation and PD in Chinese (19, 20, 28, 34, 36, 37, 42, 43, 45). One study was excluded because it did not have a case-control study design (43). One study was excluded because the frequency of each mutation was not reported separately (34). Thus, seven articles fulfilled our inclusion criteria were included in the meta-analysis. We extracted the following data from each study: lead author, year of publication, population studied, sample size of the cohort, screened variants and frequency of variants (Table 3). These studies together with the present

Table 2. Clinical features of patients with *GBA* variants

Subject	Gender	AAO (years)	Initial Symptoms	Clinical Manifestations	subtype
1	F	56	B, G	B, G, R, depression	ART
2	M	63	G	B, G, R, depression	ART
3	M	54	R	Parkinsonism	MT
4	M	64	T	B, R, T	MT
5	M	63	B	B, R	ART
6	F	72	G	Parkinsonism	MT

AAO, age at onset; ART, akinetic-rigid type; B, bradykinesia; F, female; G, gait disturbances; M, male MT, mixed type; R, rigidity; T, Tremor.

Table 3. Characteristics of the studies included in the meta-analysis

Study	Population	Screened variants	Sample size		Carrier frequency (%)							
					any GBA variant		L444P		N370S		R120W	
			case	ctrl	case	ctrl	case	ctrl	case	ctrl	case	ctrl
Current Study	Eastern China	L444P, N370S, R120W	195	443	3.08	0	3.08	0	0	0	0	0
Ziegler *et al*. 2007 (45)	Taiwan	whole GBA scanning	92	92	4.35	1.09	1.09	0	0	0	0	0
Wu *et al*. 2007 (41)	Taiwan	L444P, RecNciI, R120W	518	339	3.09	1.18	2.51	0.59	-	-	0.19	0
Huang *et al*. 2011 (20)	Taiwan	whole GBA scanning	976	780	3.72	0.26	2.79	0.14	0	0	0	0
Tan *et al*. 2007 (37)	Singapore	L444P, N370S	331	347	2.42	0	2.42	0	0	0	-	-
Mao *et al*. 2010 (28)	Western China	L444P	616	411	3.25	0.24	3.25	0.24	-	-	-	-
Sun *et al*. 2010 (36)	Middlesouthern China	L444P, F213I, R353W, N370S	402	413	2.74	0	2.74	0	0	0	-	-
Hu *et al*. 2010 (19)	Western China	N370S	328	300	1.83	0.67	-	-	1.83	0.67	-	-

study involved a total of 6574 Chinese individuals. The *GBA* gene variants were identified at a markedly higher frequency among PD patients than among controls ($Z = 6.77$, $P < 0.00001$, OR = 8.13, 95% CI, 4.43-14.92, Fig. 2). Subset analysis revealed a strong association between the *GBA* p.L444P variant and PD risk ($Z = 6.04$, $P < 0.00001$, OR = 12.80, 95% CI, 5.60-29.25).

Discussion

Elucidating the relationship between *GBA* mutations and PD risk in our population is valuable for the following reasons: First, it has been suggested that the frequencies of any specific variant may vary by race and geographic origin. Whether common *GBA* variants are genetically associated with PD-risk has not been well investigated in the patients from eastern China. Second, there are more than 1.72 million people with PD aged 55 years or older in China alone (44), accounting for almost one quarter of the world's PD patients.

This is the first study to assess the p.R120W allele as a susceptibility variant in PD patients of Han ethnicity in mainland China. A statistically significant association between *GBA* variants and increased PD risk in eastern Chinese population has been described for the first time. The p.L444P variant yielded a carrier frequency of 3.08% in patients, which was significantly higher than controls. The absence of the p.N370S and p.R120W variants from all the subjects challenges their influence on PD frequency at the population level. The *GBA* variants rate in our cohort is slightly lower than published data, but similar to previous studies from mainland China (28, 36). This discrepancy may be attributed to the different ethnic backgrounds of the examined populations.

The notion of a higher *GBA* gene variant rate in PD patients is also supported by our ethnicity-specific meta-analysis. Of note, a meta-analysis regarding similar issue has been previously reported by Sun *et al*. (36) However, only four studies were available (including 2,534 subjects) at that time. Since then, additional studies with larger sample sizes have been reported (19, 20, 28), which warrants for the current study a more precise estimation that greatly improves the reliability of our conclusions.

Clinical traits, such as AAO, motor symptoms, cognitive deficits and response to L-dopa treatment, were of no statistical difference between the patients harboring p.L444P variants and noncarriers. However, these results should be interpreted with caution. Our series may not be large enough to validate variants that contribute to complex manifestations and are likely to have the modest effects. Interestingly, we observed a lower frequency of tremor as initial symptoms among *GBA* mutation carriers. This consistent

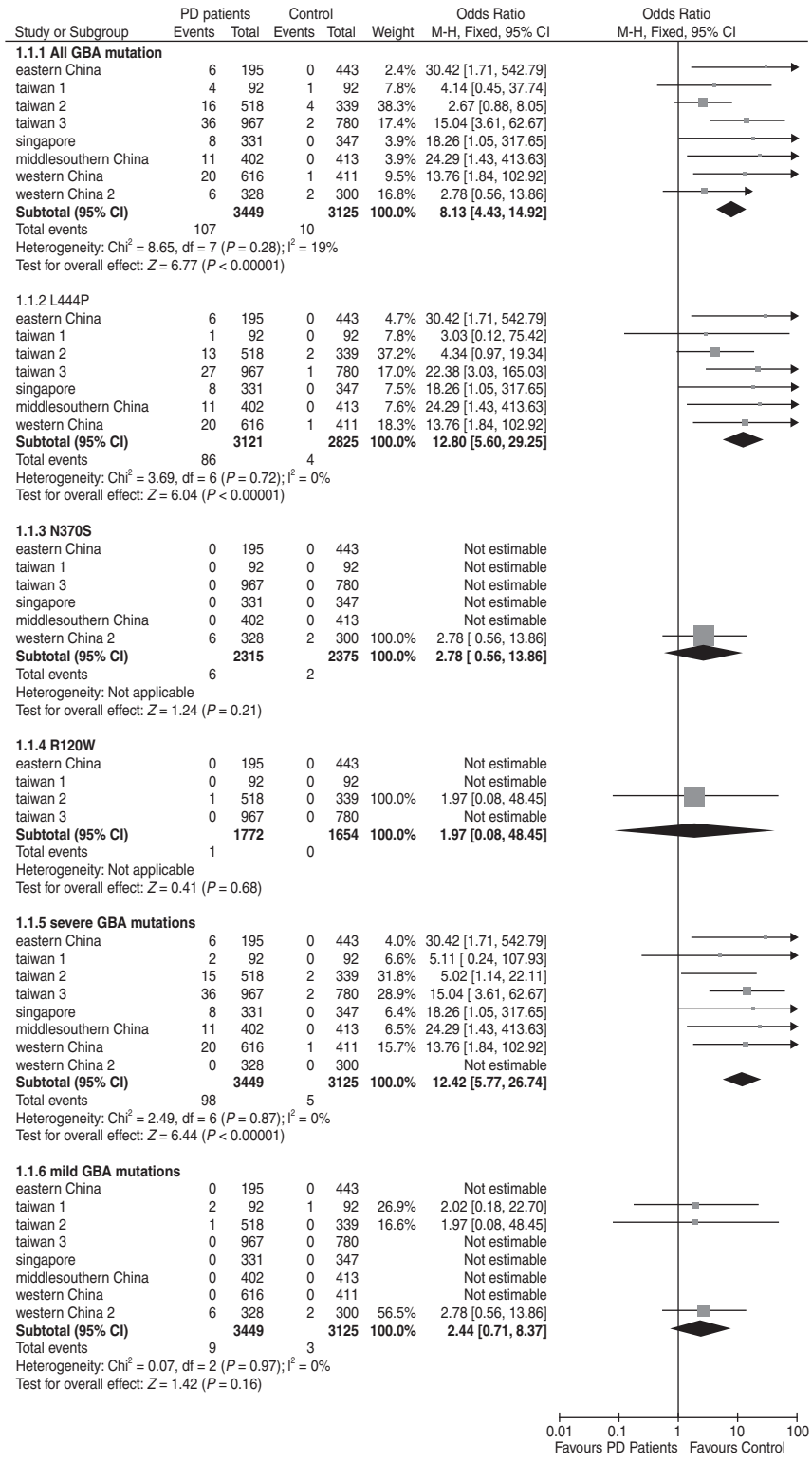

Fig. 2. Forest plots showing association between the *GBA* gene variants and PD in Chinese population. The *GBA* gene was detected at a significantly higher frequency among PD patients, when compared with the controls ($Z = 6.77$, $P < 0.00001$, OR = 8.13, 95% CI, 4.43-14.92). Subset analysis revealed a strong association between the *GBA* p.L444P variant and PD: $Z = 6.04$, $P < 0.00001$, OR = 12.80, 95% CI, 5.60-29.25. The difference between the frequency of the *GBA* gene p.N370S, p.R120W variants in PD patients and in control group was nonsignificant: ($Z = 1.24$, $P = 0.21$, OR = 2.78, 95% CI, 0.56-13.86; $Z = 0.41$, $P = 0.68$, OR = 1.97, 95% CI, 0.08-48.45, respectively). The OR for carriers of all severe *GBA* mutations in our meta-analysis was more than fourfold higher compared to the OR of mild *GBA* mutations carriers (OR = 12.42, 95% CI, 5.77-26.74 and OR = 2.44, 95% CI, 0.71-8.37).

with previously published results (13, 24, 34), indicating that *GBA* mutation may be clinically relevant with an effect of genetic alterations on the initial presentation of the disease.

GBA variants detected here are classified as severe (p.L444P) and mild (p.N370S and p.R120W), depending on their observed phenotypic implications in GD (2, 5). These variants are suggested to be pathogenic even in the heterozygous state. They are predicted to cause decreased catalytic activity or conformational changes in the β-glucocerebrosidase protein. The fact that we did not find the *GBA* gene p.N370S and p.R120W does not allow us to examine the possible clinical effects of carrying mild versus severe variants. The OR for carriers of all severe *GBA* mutations in our meta-analysis was more than fourfold higher compared to the OR of mild *GBA* mutations carriers (OR = 12.42, 95% CI, 5.77-26.74 and OR = 2.44, 95% CI, 0.71-8.37). However, the mild *GBA* mutations were rare in our population. Further study is needed to evaluate in detail this genotype-phenotype correlation.

Despite the limitation of a moderate sample size, our results combined with meta-analysis confirm that *GBA* mutation carriers have an increased risk of PD in Chinese populations. These findings add to the accumulating evidence that *GBA* gene plays an important role in the etiology of parkinsonism. Until now, there are five major postulated causal mechanisms underlying this association, including lipid dysregulation, lysosomal alteration, autophagic dysfunction, endoplasmic reticulum stress and interruptions to the ubiquitin-proteasome pathway (39). Although dopamine-replacement treatment ameliorates symptoms, no drug has yet been identified that definitively slows or stops the progression of PD. Researchers continue developing new treatments for PD (4, 16). These findings will reveal new treatment interventions that focus on the underlying molecular mechanisms. Clearly, comprehensive studies in various ethnicities are needed, before new therapeutic strategies can be offered to this group of patients.

In conclusion, our results suggest that variants in the *GBA* gene, even in heterozygous state, are risk factors for the development of PD as seen elsewhere in China.

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