SHORT REPORT

Glucocerebrosidase gene mutation is a risk factor for early onset of Parkinson disease among Taiwanese

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Background: Mutations in the glucocerebrosidase (*GBA*) gene have recently been identified as contributing to the development of Parkinson disease (PD) in Ashkenazi Jews.
Methods: To investigate whether this finding can be confirmed in a Taiwanese population, we conducted a case control study in a cohort of 518 PD patients and 339 controls for the three common *GBA* mutations in Taiwan, L444P, Rec*Nci*I and R120W, using PCR restriction enzyme assay and DNA sequencing.
Results: Heterozygous *GBA* mutations were detected in 16 PD patients (3.1%) and four controls (1.2%). Although this difference was not statistically significant (p = 0.0703), the average age at disease onset of the 16 PD patients (50.6 (12.3) years) was significantly younger than that of the total patient group (63.8 (10.5) years; p = 0.0007) and the non-carrier patient group (64.2 (10.2) years; p = 0.0005). After stratification by age, the frequency of mutation carriers was significantly higher for the early onset PD (EOPD, age at onset ⩽50 years) group than for age matched controls (12.9% vs 1.8%; p = 0.0335) and there was a trend towards an increased risk of the mutation carrier with EOPD (odds ratio 8.30; 95% CI 1.45 to 156.53). Clinically, all 16 patients carrying a *GBA* mutation presented with a typical parkinsonian phenotype and experienced a good or excellent response to levodopa.
Conclusions: Mutations of the *GBA* gene may be associated with the development of EOPD in Taiwan.

Parkinson disease (PD) is the second most common neurodegenerative disease, characterised by resting tremor, bradykinesia, rigidity and postural instability. These symptoms result predominantly from selective loss of dopaminergic neurons in the substantia nigra pars compacta and subsequent depletion of dopamine in their projections. Pathologically, PD is defined by the presence of Lewy bodies, intracellular neuronal inclusions in the substantia nigra pars compacta and other brain sites.[1] The pathogenesis of PD remains unclear. An interaction between environmental factors and genetic predisposition is thought to contribute to disease development.[2] Causal mutations in the genes for *α-synuclein*, *parkin*, *DJ-1*, *PTEN induced kinase 1* and *leucine-rich repeat kinase-2* have been identified.[3] However, mutations of these genes do not account for the occurrence of PD in all patients. Identifying novel PD genetic risk factors is important to understand its pathogenesis.

Gaucher disease (GD) is a recessively inherited glycolipid storage disorder caused by deficiency of the lysosomal enzyme glucocerebrosidase (GBA).[4] Clinically, GD is characterised by vast phenotypic heterogeneity and is classified into three types based on the severity of associated neurological symptoms.[5] Recent studies have reported genetic association between GD and PD.[6 7] The molecular pathogenic mechanism causing PD in *GBA* mutation carriers remains unclear.

The gene encoding GBA has been localised at chromosome 1q21, and there is a highly homologous pseudogene (*GBAP*) sequence located 16 kb downstream.[8] More than 200 mutations, including point mutations, deletions and rearrangements, have been identified in this gene (http://www.hgmd.cf.ac.uk/ac/gene.php?gene = GBA). Mutation analysis of GD patients in Taiwan has reported three common mutations, L444P, Rec*Nci*I (the recombination allele between the *GBA* and *GBAP* genes) and R120W, that account for 87% of Gaucher chromosomes.[9] R120W was defined as a mild mutation associated with non-neuropathic GD in Taiwanese.[9]

To investigate the possible association between the *GBA* gene mutations and PD in Taiwan, we examined the frequency of these three *GBA* mutations to determine whether the *GBA* mutations are genetically associated with PD, using a case control study design.

METHODS

Subjects

In total, 518 unrelated patients with sporadic PD (45.2% woman), mean age at onset 63.8 (10.5) years (range 27–85), were enrolled. All patients were examined clinically and observed longitudinally by two neurologists (YRW and CMC) at outpatient clinics at Chang-Gung Memorial Hospital Linkuo. Mean age of 339 unrelated healthy adult volunteers (47.5% female) was 60.3 (8.9) years (range 34–89). These control subjects were matched for age, gender, ethnic origin and area of residence. Data on family history, demographic characteristics, clinical data and neurological examination were completed for each patient. A clinical diagnosis of PD was based on published diagnostic criteria.[10] Patients with a known genetic cause of parkinsonism were excluded. All subjects gave consent to the study and the protocol was approved by the hospital's internal ethics and scientific boards.

Analysis of *GBA* mutations

Mutation screening was performed by PCR amplification using two primer pairs separately. The PCR products were digested with restriction enzyme *Nci*I and the resulting fragments were separated by agarose gel electrophoresis (table 1). The L444P (1448 T>C), Rec*Nci*I (1448 T>C, 1483 G>C, 1497 G>C) and R120W (475 C>T) mutations were confirmed by direct sequencing of PCR products from a second amplification. Mutation frequencies in patients and controls were compared using a two tailed Fisher exact test. Statistical significance was set at $p<0.05$. The difference in age at onset between carriers and non-carriers or total patients was tested using the two tailed Student's t test. Odds ratios with 95% confidence

Abbreviations: EOPD, early onset Parkinson disease; GBA, glucocerebrosidase; GD, Gaucher disease; PD, Parkinson disease

Table 1 PCR conditions and restriction enzyme detection of glucocerebrosidase mutations

Mutation	Anneal (℃)/ $MgCl_2$ (mM)	Enzyme (fragment bp)
R120W (C>T)		
F: GCAGAGTCCCATACTCTCCT	56/1.25	*Nci*I: CCGGG
R: TGGGTGACAGAGAGAGAGACT		(454, 83/537)
L444P (T>C)		
F: GGAGGACCCAATTGGGTGCGT	58/1.0	*Nci*I: CCCGG
R: ACGCTGTCTTCAGCCCACTTC		(638/536, 102)

intervals (95% CI) were calculated to test for the association between the *GBA* mutation and PD.

RESULTS

The distributions and association of *GBA* mutations in patients and controls are shown in table 2. In total, 16 patients (3.1%) carried a heterozygous mutant *GBA* allele: 13 had L444P, two had Rec*Nci*I and one had R120W. In addition, four control subjects (1.2%) had a heterozygous mutant *GBA* allele: two had L444P and two had Rec*Nci*I (table 3). The difference in percentages of mutation frequencies in patients (3.1%) and controls (1.2%) was not statistically significant ($p = 0.0814$). However, the average age at disease onset of the 16 PD patients (50.6 (12.3) years) was significantly younger than that of the total patient group (63.8 (10.5); $p = 0.0007$) and the non-carrier patient group (64.2 (10.2); $p = 0.0005$). Following stratification by age, the frequency of mutation carriers was significantly higher for the early onset PD (EOPD) group (age at onset $\leqslant 50$ years) than age matched controls (12.9% vs 1.8%; $p = 0.0335$). There was a trend towards an increased PD risk for the mutation carrier (odds ratio 8.30, 95% CI 1.45 to 156.53; $p = 0.0496$) (table 2).

Table 3 presents the demographic and clinical characteristics of the 20 carriers. Clinically, all 16 patients carrying a *GBA* mutation presented with a typical parkinsonian phenotype and experienced a good or excellent response to levodopa. Marked fluctuations and dyskinesias were seen in two EOPD patients (H80 and H86). Three patients (H86, H213 and H351) experienced an excellent response to subthalamic nucleus deep-brain stimulation. During disease progression, two patients (H1405 and H1421) experienced depression and one (H507) developed dementia after having the disease for >10 years.

DISCUSSION

We examined the possible role of *GBA* mutations in PD in an ethnically homogeneous Taiwanese population. Although PD patients (3.1%) had a higher frequency of the three *GBA* mutations compared with controls (1.2%), the difference was not significant. This finding is similar to, and more relevant than, the study reported by Toft *et al*[11] in which 2.3% of Norwegian patients with PD carried N370S or L444P mutation compared with 1.7% of controls ($p = 0.58$). Our results differ from those of increased susceptibility to PD in *GBA* mutation carriers in an Ashkenazi Jewish population in which 31.3% of PD patients carried 1 of 6 examined *GBA* mutations,[6] and from those in Caucasians of Canadian origin in which 5.7% of PD patients carried 1 of 7 examined *GBA* mutations.[7] Notably, following stratification by age, we observed a higher frequency of the *GBA* mutations in EOPD patients (12.9%) compared with controls aged <50 years (1.8%) ($p = 0.0335$). Average age at onset of the carrier PD group (50.6 (12.3) years) was significantly younger than that of non-carriers (64.2 (10.2) years; $p = 0.0005$). This finding is compatible with that reported by Sato *et al*[7] where a marginally significant association of *GBA* mutations with PD was demonstrated, 70% of all PD patients were aged <50 years at onset and all *GBA* mutation carriers presented with asymmetrical early onset (range 31–52 years). Combined with a trend towards an increased risk of having the mutation carrier with EOPD (odds ratio 8.30, 95% CI 1.45 to 156.53), our results demonstrate that the *GBA* gene mutation is a risk factor for EOPD in Taiwanese subjects.

Identification of genes that cause PD (*α-synuclein* and *parkin*) indicates that the underlying ubiquitin–proteasome pathway is involved in the pathogenesis of PD. Perturbation of normal α-synuclein function probably plays a role in onset or progression of sporadic PD. The lysosomal pathway is an alternative mechanism for regulation and degradation of proteins. As glucocerebroside accumulation interferes with the affinity of a functional form of α-synuclein for lipid surfaces,[12] a recent finding that elevated binding of α-synuclein to glucocerebroside containing glycosphingolipids in *GBA* mutation carriers supports this scenario.[13] Further evidence suggests that processing of α-synuclein occurs in lysosomes,[14] the site of glycosphingolipid metabolism. Mutations in GBA may lead to its misfolding and affect lysosomal targeting and proteosomal function, thereby interfering with α-synuclein aggregation clearance. A possible PD–GD connection would therefore involve a toxic gain of function related to improper α-synuclein degradation, aberrant α-synuclein accumulation or a combination of both processes.

Although mutations in the *GBA* gene are relevant to idiopathic PD in Ashkenazi Jews, no difference in age at disease onset between carriers and non-carrier patients was reported.[15] This is different from our observation that PD patients carrying the *GBA* mutations had an earlier age of onset of disease. These different findings may be explained by the fact that the modifier gene(s) contributing to the disease phenotype may differ in different populations, and that epistatic gene–gene interactions or specific multi-loci haplotype may be involved in determining age of disease onset. Moreover, other environmental factors may account for this diversity. Our observation that variations in the *GBA* gene may be a common

Table 2 Distributions and association of glucocerebrosidase mutations in patients and controls

	PD (518) (n (%))	Controls-1 (339) (n (%))	Odds ratio* (95% CI)	p Value
Non-carrier	502 (96.9)	335 (98.8)	1.00	
Carrier of GD	16 (3.1)	4 (1.2)	2.67 (0.97–9.38)	0.0814
	EOPD (62) (n (%))	**Controls-2 (57) (n (%))**	**Odds ratio* (95% CI)**	**p Value**
Non-carrier	54 (87.1)	56 (98.2)	1.00	
Carrier of GD	8 (12.9)	1 (1.8)	8.30 (1.45–156.53)	0.0496

EOPD, early onset Parkinson disease; GBA, glucocerebrosidase; GD, Gaucher disease; PD, Parkinson disease.
*Odds ratios of *GBA* mutation carriers were calculated by comparing the value to the non-carrier individuals.

Table 3 Clinical data for patients with Parkinson disease and glucocerebrosidase mutations

Study ID	Mutation	Age at onset* (y)	Disease duration (y)	Family history of PD	Sex	Additional information
Patients						
H44	L444P	60	7	N	F	L-dopa response (+), motor fluctuation (+)
H46	R120W	62	4	N	M	L-dopa response (+)
H80	Rec*Nci*I	34	8	N	F	L-dopa response (+), motor fluctuation (+)
H86	L444P	29	13	N	M	L-dopa response (+), motor fluctuation (+), STN-DBS implantation (+)
H213	L444P	57	19	Y	M	L-dopa response (+), motor fluctuation (+), STN-DBS implantation (+)
H351	L444P	57	11	N	F	L-dopa response (+), motor fluctuation (+), STN-DBS implantation (+)
H434	L444P	46	3	N	F	L-dopa response (+), motor fluctuation (+)
H507	L444P	64	12	N	F	L-dopa response (+), motor fluctuation (+), dementia (+)
H590	L444P	67	4	N	F	L-dopa response (+)
H707	L444P	55	5	N	M	L-dopa response (+)
H837	L444P	46	3	N	M	L-dopa response (+)
H867	L444P	64	2	N	M	L-dopa response (+)
H1279	L444P	30	8	N	M	L-dopa response (+), motor fluctuation (+)
H1405	Rec*Nci*I	49	4	N	F	L-dopa response (+), depression (+)
H1421	L444P	50	7	N	F	L-dopa response (+), depression (+)
H1500	L444P	40	7	N	M	L-dopa response (+), motor fluctuation (+)
Controls						
C769	L444P	76	–	N	F	–
C806	L444P	73	–	N	F	–
C877	Rec*Nci*I	57	–	N	F	–
C972	Rec*Nci*I	43	–	N	F	–

PD, Parkinson disease; STN-DBS, subthalamic nucleus deep brain stimulation.
*Age at study inclusion for control individuals.

susceptible factor for the development of EOPD may provide an example of the heterozygosity of a Mendelian disorder gene acting as a risk factor for a complex disease. A large series of clinically and genetically well defined studies is warranted to resolve the true frequency of *GBA* mutations in the general population and their contribution to PD, thereby strengthening the association observed in this study.

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