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Glucocerebrosidase mutations in Thai patients with Parkinson's disease

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ABSTRACT

Background: *GBA* mutations are an important risk factor in developing Parkinson's disease (PD) worldwide. The study aimed to determine the frequency and clinical characteristics of *GBA* mutations in a Thai PD cohort of 480 patients and 395 control subjects.

Methods: Direct sequencing of *GBA* was performed in all early-onset PD patients (EOPD: $n = 108$) and 100 PD patients with age at onset over 50 years (AAO > 50y-PD). The study subsequently screened all identified mutations in the remaining AAO > 50y-PD patients and all control subjects. Predictive factors associated with risk of developing PD were analyzed. Comparisons of clinical characteristics of PD patients with and without *GBA* mutations were also carried out.

Results: Heterozygous *GBA* mutations were identified in 24 patients (5%) and 2 controls (0.5%). Seven identified *GBA* point mutations comprised p.L444P, p.N386K, p.P428S, IVS2+1G > A, IVS9+3G > C, IVS10-9_10GT > AG and c.1309delG, of which five mutations were novel. Multiple logistic regression analysis revealed that *GBA* mutations were more frequent in EOPD than AAO > 50y-PD groups (OR = 4.64, $P < 0.022$). Patients with *GBA* mutations had mean age at onset (43.1 ± 10.2, mean ± standard deviation) earlier than patients without *GBA* mutations (54.4 ± 13.9, $P = 0.002$). The patients with *GBA* mutations also had a more rapid progressive course, in which they were more likely to have higher Hoehn and Yahr staging (OR = 4.20, $P = 0.006$) and slightly lower means of Schwab-England ADL score [74.1 ± 17.1 vs. 81.0 ± 18.08 (OR = 0.98, 95%CI = 0.96–1.01, $P = 0.162$)].

Conclusion: *GBA* mutations are an important risk of PD in the Thai population. Patients having the mutations are likely to have early onset and may exhibit more rapid motor progression.

1. Introduction

Glucocerebrosidase (GBA) is an important lysosomal enzyme responsible for hydrolyzing the β-glucosidic linkage of glucosylceramide in the plasma membrane into glucosyl and ceramide. Deficiency of the enzyme activity causes the most common inherited lysosomal storage disease, Gaucher's disease. Human *GBA* gene is located on chromosome 1q21. Importantly, 16 kb downstream to the *GBA* functional gene locates *GBA* pseudogene, in which about 96% of exons share homologous sequence to the functional *GBA* gene [1]. To date, hundreds of mutations including several complex recombinant alleles consisting of two or more mutations have been described in association with Gaucher's disease. Thus, frequent recombination events between the functional *GBA* gene and the highly homologous *GBA* pseudogene may explain the high number of recombinant alleles, and mutations identified in Gaucher's disease [2].

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Parkinsonism has been increasingly observed as one of the neurological manifestation in neuropathic Gaucher's disease [3]. A study on brain samples of patients clinically or pathologically diagnosed with Parkinson's disease (PD) demonstrated that *GBA* mutations were more frequent in PD patients than in the general population [4]. The association between the heterozygous *GBA* mutations and PD was then further supported by a study on the Ashkenazi cohort showing that PD patients had significantly higher odds for carrying the common *GBA* mutations than patients having Alzheimer's disease and healthy subjects [5]. Subsequent studies in various ethnicities including Chinese, Caucasians of multiple origins, and Japanese have consistently confirmed the correlation especially in individuals with early onset [6–10]. *GBA* mutations such as L444P, N370S, R120W and IVS2+1G > A were generally common among multiple ethnicities [8]. However, each population appeared to have its own unique and rare mutations [8,10,11]. Overall, the heterozygous *GBA* mutations were identified as risk factors of PD in about 3–9% of the patients. With the exception of the PD patients of Ashkenazi Jewish origin, in which the common N370S mutation is very prevalent, frequency of the PD patients having the heterozygous *GBA* mutations was much greater (up to 31%) [5].

There is increasing evidence of the importance of *GBA* mutations as one of the major risk factors of PD in various ethnicities, and no epidemiological or association data regarding *GBA* mutations and PD have been described to date in the Thai population. Furthermore, recognition of the specific *GBA* genotypes in association with PD in each specific population may be essential in order to diagnose PD cases at the very early stage of the disease, which in turn is one of the key factors for a successful neuroprotective therapy in the future. Therefore, we conducted the study in order to identify the frequency and the role of *GBA* mutations as the risk factors of developing PD, and their correlations with phenotypes in Thais.

2. Materials and methods

2.1. Patients and control subjects

All consecutive PD cases in the Neurology Clinic at the Ramathibodi Hospital and the Bhumibol Adulyadej Hospital were recruited during May 2008–October 2013. Other collaborating hospitals and institutes enrolled patients in only a one-year period. The research protocol was approved by the ethics committees from all collaborating hospitals. All participants provided both verbal and written informed consent prior to the enrollments. PD was diagnosed by using UK Parkinson's disease Brain Bank Criteria except allowed patients to have family history of PD [12]. Early-onset Parkinson's disease (EOPD) was defined as patients with age at onset ≤50 years [13]. Thus, the patients having age at onset over 50 years (AAO > 50y) would be categorized as AAO > 50y-PD group. Clinical information comprehensively obtained from the patients.

The majority of the Thai population is Thai and Chinese in origin (~90% of the population), and none of the studied participants were ethnically other than Thai or Chinese. Most Chinese migrated to Thailand during the Chinese Civil War and World War II. Regarding the ethnic origins of the participants, they were therefore determined by asking about their pedigrees up to their grandparents.

In order to avoid the control subjects developing PD after the enrollment, the study tried to recruit elderly participants as old as possible, and keep the number of controls similar to the patient group. So controls were recruited by enrolling participants aged ≥65 years old, who have no signs of parkinsonism. Nevertheless, two of the controls developed signs and symptoms of PD afterward (at the age of 71 and 73 years), and they were excluded from the study. Medical conditions of the control subjects included hypertension, type 2 diabetes mellitus, dyslipidemia, cerebrovascular disease, epilepsy, hemifacial spasm, polymyositis, trigeminal neuralgia, cancers of breast, and colon, atrial fibrillation, coronary artery disease, chronic kidney disease, asthma, degenerative bone and joint diseases, depression and no underlying disease.

2.2. Sequencing analysis

Genomic DNA was extracted from peripheral blood leukocytes by phenol-chloroform method or using QIAGEN DNA purification kit (QIAGEN, CA, USA). All 11 exons and exon-intron boundaries of *GBA* were sequencing on both strands in all EOPD patients ($n = 108$), and 100 patients of AAO > 50y-PD group by using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, CA, USA) as previously described methods in order to avoid amplification of *GBA* pseudogene [14]. The PCR products were then loaded on the 3730XL DNA Analyzer and analyzed with the Sequence Analysis software v3.0 (Applied Biosystems, CA, USA). Prediction of splice-site scores were analyzed by using NNSPLICE version 0.9 (http://www.fruitfly.org/seq_tools/splice.html) [15].

2.3. Screening for identified GBA mutations

The remaining samples of AAO > 50y-PD group ($n = 274$) and all of the control samples ($n = 396$) were subsequently screened for all putative mutations identified from the sequencing analyses. Appropriated PCR, mismatch-PCR and restriction fragment length polymorphisms (RFLPs) were designed to screen these seven mutations (supplement table). All samples, which RFLP results suggested harbored the mutations, would then be confirmed the existence of mutations by direct sequencing.

2.4. Statistical analysis

Data was described by means, standard deviations (SD), and frequencies (%) for continuous and categorical data, respectively. Characteristic features of the participants among EOPD, AAO > 50y-PD and control groups were compared using analysis of variances and chi-square tests for continuous and categorical data, respectively.

In order to evaluate the predictive factors for PD, analysis of all participants together, in which the methods of *GBA* genotyping were performed by different techniques, might result in some bias since individuals who were analyzed by only RFLPs might miss identifying some other unknown variants. Therefore a logistic regression model was applied to assess whether *GBA* mutations and other factors were associated with PD by comparisons between EOPD vs. AAO > 50y-PD groups (data from *GBA* sequencing), and AAO > 50y-PD vs control groups (data from RFLPs). Odds ratio (OR) along with 95% confidence interval (95%CI) for each factor was then estimated by exponential of coefficient. A goodness of fit of the logistic model was subsequently assessed using Hosmer–Lemeshow goodness of fit test.

Clinical characteristics of patients carrying *GBA* mutations and non-carriers were compared using unpaired *t* test and Fisher exact test, where it was appropriated. All analyses were performed using STATA version 13.0 (Stata Corp. TX, USA). *P* value <0.05 was considered as statistically significant.

3. Results

Demographic data of the patient cohort and the control subjects are shown in Table 1. We enrolled 108 (22.4%) Thai patients having EOPD, 374 (77.6%) patients having AAO > 50y and 396 control subjects. Family history of PD was more frequent in the patients with EOPD (14.8%), and AAO > 50y-PD (9.4%) than the control subjects (0.8%). Thai ethnicity was more common in PD than in the control group ($P = 0.009$). The presence of the heterozygous *GBA*

Table 1
Describes characteristics of subjects between Parkinson's disease groups.

Characteristics	EOPD (%)		AAO > 50y-PD (%)		Control (%)		*P* value
	$n = 108$		$n = 372$		$n = 395$		
Age, year, mean (SD)	42.0	(7.1)	64.8	(8.1)	71.7	(7.6)	<0.001[a]
Sex, no (%)							
Male	56	(51.9)	202	(54.3)	153	(38.7)	<0.001[a]
Female	52	(48.1)	170	(45.7)	242	(61.3)	
Ethnicity, *N* (%)							
Thai	80	(74.1)	253	(68.0)	231	(58.8)	
Chinese	13	(11.1)	50	(13.4)	57	(14.5)	0.007[a]
Thai-Chinese	15	(14.8)	69	(18.6)	105	(27.0)	
Family history, *N* (%)							
Yes	16	(14.9)	35	(9.4)	3	(0.8)	<0.001[a]
No	92	(85.1)	337	(90.6)	392	(99.2)	
Smoking, *N* (%)							
Yes	14	(13.0)	59	(15.9)	75	(19.0)	0.230
No	94	(87.0)	313	(84.1)	320	(80.0)	
GBA mutation							
Yes	14	(13.0)	10	(2.7)	2	(0.5)	<0.001[a]
No	94	(87.0)	362	(97.3)	393	(99.5)	

Abbreviations as follow: PD = Parkinson's disease; EOPD = early-onset Parkinson's disease; AAO > 50y-PD = Parkinson's disease with age at onset over 50 years; *GBA* = glucocerebrosidase gene; *N* = number.

[a] Parameters are statistically difference among different groups ($P < 0.05$).

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