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Increased incidence of Parkinsonism among Chinese with β-glucosidase mutation in central Taiwan

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ABSTRACT

Background: Parkinson's disease (PD) is characterized by progressive neuronal cell loss and decline in movement. Recently, much attention has been focused on the dopaminergic neurotoxicity, which causes neurogeneration in the nervous system, thus implicating dysfunction in predisposition to PD pathogenesis. β-glucosidase (GBA), the enzyme deficient in Gaucher's disease, has been cited as linked with Parkinson's disease. We investigated whether GBA mutation is associated with PD patients in middle Taiwan.

Methods: GBA mutation was detected by polymerase chain reaction and sequencing in 148 patients with PD and 120 normal controls.

Results: The results revealed a significant difference between PD patients and normal controls in GBA mutation and a statistic correlation between GBA mutation L444P and PD formation. It could be concluded that patients who carry the T/C of GBA mutation, L444P, have a higher risk of developing PD in Chinese patients in middle Taiwan.

1. Introduction

Parkinson's disease (PD) is characterized by the progressive loss of dopaminergic neurons and the presence of intracellular Lewy body inclusions within the *substantia nigra*. The presence of age-associated cytopathogenic process is required for a definite diagnosis. Clinically, PD patients exhibit at least one of resting tremor, muscle rigidity, bradykinesia, and postural instability, which are features of neurodegenerative disorder [1,2]. Patients with familial PD provide a good insight for examination of candidate genes; however, the incidence of PD pathogenesis is still too complex to unify the common mechanisms of etiologies. In particular, the major causes of the disease occur in a sporadic manner, possibly suggesting

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that the susceptibility of PD is involved in a wide spectrum of genetic and environmental factors.

Current theories of PD pathogenesis are based on available data from familiar histories of the disease. Mutations in five causal genes—α-synuclein, parkin, DJ-1, PINK1, and leucine-rich repeat kinase 2 (LRRK2) [3–9]—have been identified as molecular events increasing risk of disease. Evidence for the association of the PD candidate gene *LRRK2*, which encodes the protein kinase dardarin and is located within *PARK8* locus on chromosome 12p11.2-q13.1 [4,5], is growing. Genetic evidence of its linkage to hereditary late-onset PD was originally detected in a Japanese family [4,10]. Then, as currently identified from a rare familial pedigree of PD, finding several *LRRK2* gene mutations revealed it as causing autosomal-dominant familiar Parkinsonism [4,5].

The gene encoding β-glucosidase (also known as glucocerebrosidase; GBA1) has been localized to chromosome 1q21 [11]; a highly homologous pseudogene sequence exists at 16 kb downstream [11,12]. More than 200 mutations, point mutations, deletions, insertions, splicing aberrations, and various rearrangements have been identified within the *GBA* gene [13–15]. Environmental factors are known to play a role in PD pathogenesis [16,17]. This case–control association study explored a hypothesis of common coding variation within *GBA* gene also tending toward susceptibility of sporadic PD among Chinese in central Taiwan. In the search for variants, we undertook the potentially functional mutation for frequent analysis in sporadic forms of PD cases and ethnically-matched controls among the Taiwanese population.

2. Patients and methods

2.1. Patients

A standard clinical examination was performed on every participant, and PD diagnosis was confirmed according to published neurological criteria [1,2,18]. The cohort enrolled 148 PD cases and 120 healthy controls from the same population in central Taiwan. Patients were of Taiwanese ethnicity, with sporadic PD based on pedigree assay, as each member in the absence of family history was observed.

2.2. PCR amplification

PCR amplification of the DNA fragments covering mutation L444P of GBA was conducted from genomic DNA of patients and controls; PCR amplified the DNA fragments of GBA according to Tsai et al [9]. Briefly, PCR reaction was performed under the conditions: PCR carried out in total volume of 50 μL, containing 50 ng genomic DNA, 1× Taq polymerase buffer, 5 pmol of each primer, and 0.25 U of‘ AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA, USA). Amplification conditions were: (1) denaturation at 95°C for 4 minutes; (2) 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 45 seconds; and (3) final elongation at 72°C for 10 minutes. Preventive contamination entailed PCR reaction mixture without DNA (negative control) in each run of amplification.

2.3. DNA sequencing

All PCR fragments were purified by QIAEX II (Qiaqgen, Hilden, Germany) and directly sequenced to identify mutations. The direct sequencing was achieved by an ABI Prism 3100 DNA sequencer (PE Applied Biosystems) with the BigDye Dideoxy Terminator Sequencing Kit (PE Applied Biosystems).

2.4. Statistical analysis

The parameters were compared using Fisher's exact test, with $p < 0.05$ statistically significant. SPSS software (version 10.0; SPSS Inc., Chicago, IL, USA) was used to performed calculations.

3. Results

The L444P mutation was screened by PCR and sequencing. The screening of L444P mutation showed all patients carrying PD hetero- or homozygote genotype in their *GBA* gene. Table 1 plots genotype distribution and allelic frequencies for L444P mutation in patients and controls. Significant differences arose between patients and controls ($p < 0.001$) in L444P genotype distribution, with T/C homozygote distribution in patients (4.51%) higher than in controls (0%). Also, allelic frequency of L444P mutation showed statistical significance, distinguishing PD patients from controls ($p = 0.048$; Fisher's exact test).

4. Discussion

This study analyzes the *GBA* gene in PD among the Chinese population of central Taiwan. Data indicate GBA mutation L444P is associated with PD. Since significance is dominant, this association is not likely to stem from a chance event. Earlier study showed L444P mutation as second most common Gaucher's disease (GD) mutation among non-Jewish patients, accounting for 37% of total mutations surveyed [19,20]. In our research, L444P accounts for 4.51% of PD cases. A cause for the linkage of mutant *GBA* with Parkinsonism remains unclear; further studies must identify the pathological mechanism. Recently, the mutation in *GBA* was noted in relation to

Table 1 – Distribution of genotypes among Parkinson's disease (PD) patients and healthy controls.

	PD patients	Normal controls	*p*
	n (%)	*n* (%)	
Genotype			
T/T	148 (95.48)	120 (100)	<0.001
T/C	7 (4.51)	0 (0)	
C/C	0 (0)	0 (0)	
Allelic frequency			
Allele T	303 (97.74)	240 (100)	0.048
Allele C	7 (2.26)	0 (0)	

p values calculated by Fisher's exact test.

synucleinopathies. Some studies pay attention to this association [21,22]; others find a correlation between Parkinsonism and the *GBA* mutation, the gene encoding the enzyme deficient in autosomal, recessively-inherited lysosomal storage, also known as GD. Rare GD cases developing Parkinsonism have been identified [23,24]. Neuropathological evaluation of several such patients showed intraneuronal inclusions with α-synuclein in brain regions related to GD [24,25]. Additional studies focusing on diverse cohorts with Parkinsonism indicate higher frequency of specific *GBA* mutations. Increasing *GBA* mutations in PD, identification of mutations in the spectrum of synucleinopathy [26], and higher frequency of Parkinsonism among GD patients [25] led us to research this correlation further, via the genetic approach. Our analysis lends compelling evidence of the *GBA* gene L444P mutation contributing significant association, thereby implicating it as one possible genetic risk factor for sporadic PD and accounting for higher prevalence of disease in allele frequencies. These data may extend the survey of GBA to other populations for sporadic form of PD. Detailed study of molecular mechanisms must explore the role of GBA and related drug development in PD.

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