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SHORT COMMUNICATION

PLA2G6 variant in Parkinson's disease

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PLA2G6 **was reported recently as the causative gene for** ***PARK14*****-linked autosomal recessive early-onset dystonia-parkinsonism. In a recent study in Singapore, heterozygous** ***PLA2G6*** **p.P806R (c.2417C>G) mutation in exon 17 was reported to be a possible Parkinson's disease (PD)-related mutation. To determine the significance of the** ***PLA2G6*** **mutation, we conducted an association study by performing direct sequencing of** ***PLA2G6*** **exon 17 in 379 Japanese sporadic PD patients and 310 controls in the Japanese general population. In this group, we found 12 patients (12/379=3.16%) and 10 controls (10/310=3.23%) with a heterozygous p.P806R mutation (*P*=0.96, χ^2=0.0019). Therefore, our large case–controlled study suggests that** ***PLA2G6*** **p.P806R is not a disease-associated polymorphism in PD. Moreover, we performed direct sequencing of all exons and exon-intron boundaries of** ***PLA2G6*** **in 116 Japanese patients with sporadic PD. Two single heterozygous variants (p.R301C or p.D331N) were found (both frequencies: 1/379 patients vs 0/310 controls) and the roles of their variants were unclear. Finally, combined with the previous report, our findings emphasize that** ***PLA2G6*** **mutations are unlikely to be the major causes or risk factors of PD at least in Asian populations. However, further large studies in various populations are needed because patients with** ***PLA2G6*** **mutations can show heterogeneous clinical features.**
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Parkinson's disease (PD, OMIM no. 168600) is the second most common neurodegenerative disorder next to Alzheimer's disease. Although the cause remains unclear, PD is thought to be a heterogeneous disease caused by the interaction of multiple genetic factors and environmental factors associated with aging. Indeed, case–control studies identified some genetic risk factors for PD, such as *SNCA*,[1–4] *LRRK2*[3–8] and *GBA* variants.[9–11] To elucidate the exact etiology of PD, identifying the effect of each of the multiple factors and their combined effects is important.

Recently, *PLA2G6* was reported to be the causative gene for *PARK14* in patients with autosomal recessive early-onset dystonia-parkinsonism.[12] *PLA2G6* is also the causative gene for infantile neuroaxonal dystrophy, neurodegeneration associated with brain iron accumulation and Karak syndrome.[13–15] Some patients with neurodegeneration associated with brain iron accumulation show very early-onset and rapid psychomotor regression, early cerebellar signs, pyramidal signs and visual disturbances. Patients with *PLA2G6* mutations frequently exhibit brain iron accumulation, which is a feature of neurodegeneration associated with brain iron accumulation. In our recent study, we revealed two novel compound heterozygous *PLA2G6* mutations in Japanese patients who had levodopa-responsive parkinsonism with or without brain iron accumulation.[16] Although there are few *PLA2G6* mutation analyses in parkinsonism so far, its role in parkinsonism or PD and the mechanism of neurodegeneration and iron accumulation have not been clarified.

Very recently, Tan *et al.*[17] in Singapore reported the results of *PLA2G6* analysis in 96 PD patients with young-age onset/dystonia. One of the 96 patients, who had a novel heterozygous p.P806R (c.2417C>G) mutation in exon 17, had typical features of late-onset PD with levodopa responsiveness and dystonic spasms. Although they could not conduct a segregation analysis, this mutation was not found in 100 healthy controls. Their result emphasized the potential role of this mutation and the *PLA2G6* mutation as pathogenic mutations or risk factors for PD in Chinese or other races. To confirm this intriguing finding of *PLA2G6*, we conducted an extended mutation analysis and association study in Japanese patients with sporadic PD and normal controls.

The study was approved by the Institutional Review Board of Juntendo University, and all subjects provided an informed consent. We collected blood samples from each participant and extracted genomic DNA by using standard methods. Sequences of the primers/probes and conditions of PCR/sequencing are available upon request to the corresponding author or the first author. We directly sequenced the exon 17 of *PLA2G6* from 379 Japanese patients with sporadic PD and 310 normal Japanese subjects as controls (Table 1).

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Table 1 Profile of analyzed subjects and allele frequency of *PLA2G6* p.P806R (c.2417C>G) in Japanese patients with sporadic Parkinson's disease (SPD) and control subjects from the general population

	Japanese SPD			*Japanese control from the general population*		
	All analyzed subjects	*Heterozygous (C/G)*	*Wild type (C/C)*	*All analyzed subjects*	*Heterozygous (C/G)*	*Wild type (C/C)*
Number of patients (F:M)	379 (198:181)	12 (3:9)	367 (195:172)	310 (188:122)	10 (6:4)	300 (182:118)
Age at sampling[a] (range, *n*=number)	60.2 ± 14.0 (12–92, *n*=378)	58.6 ± 22.4 (12–87, *n*=12)	60.3 ± 13.7 (12–92, *n*=366)	58.5 ± 13.2 (23–98, *n*=294)	60.7 ± 12.8 (35–81, *n*=10)	58.5 ± 13.2 (23–98, *n*=284)
Age at onset[a] (range, *n*=number)	52.7 ± 14.3 (7–88, *n*=375)	52.7 ± 22.3 (11–83, *n*=12)	52.7 ± 14.0 (7–88, *n*=365)			
Disease duration[a] (range, *n*=number)	7.4 ± 5.6 (0–40, *n*=375)	5.9 ± 5.3 (1–18, *n*=12)	7.5 ± 5.6 (0–40, *n*=363)			
Allele frequency (%)	1.58			1.61		

No homozygous *PLA2G6* p.P806R mutation was found in this study. Genotypes of the patients and controls were concordant with Hardy–Weinberg equilibrium.
[a]Data are mean ± s.d.

We identified a heterozygous p.P806R mutation in 12 patients with PD and in 10 controls (χ^2=0.0019, P=0.96; odds ratio (genotype)=1.02, 95% confidence interval: 0.44–2.37, Table 1). We found no homozygous p.P806R mutations. The allele frequency was 1.58% in sporadic PD and 1.61% in controls. We also found heterozygous synonymous p.T787T (c.2355C>T) variant in two patients and one control. No other variants were found in exon 17. Moreover, we performed direct sequencing of all exons and exon–intron boundaries of *PLA2G6* in 116 Japanese patients with sporadic PD (males 60, females 56; age range, 12–92 years; mean age, 60.7 ± 18.1 years; mean disease duration, 6.3 ± 5.8 years). Among them, we found two novel single heterozygous non-synonymous variants (p.R301C, p.D331N). Both frequencies of the two variants were 1/379=0.26% in patients and 0/310=0% in Japanese normal controls. The roles of their rare variants found in Japanese patients with sporadic PD remained unclear (Table 2).

The reported clinical features of neurodegeneration associated with mutations in the *PLA2G6* gene (PLAN) are axonal dystrophy, dystonia, dementia, visual disturbances, cerebellar signs and brain atrophy with or without iron accumulation.[12–15,18,19] Showing clinical heterogeneity, patients with *PARK14*-linked parkinsonism have levodopa responsiveness, levodopa-induced dyskinesia and dementia with an older-age onset and a longer disease duration than those with infantile neuroaxonal dystrophy.[12,16] These studies have suggested that patients with *PLA2G6* mutation can show heterogeneous phenotype.

Although the precise function of *PLA2G6* in neurodegeneration and iron accumulation remains obscure, defective phospholipid metabolism is implicated in neurodegenerative diseases featuring brain iron dyshomeostasis.[14] *PLA2G6* is thought to be responsible for the development of autosomal recessive disorders through its loss of function; hence, the role of a single heterozygous *PLA2G6* mutation is intriguing. Indeed, two of the 10 infantile neuroaxonal dystrophy patients were previously reported to have one-allele mutations, suggesting that single heterozygous mutation in *PLA2G6* could be pathogenic.[19]

The aim of this study was to clarify the role of the *PLA2G6* mutation in PD. Although patients with *PLA2G6* mutations have been reported to show atypical parkinsonism, the heterozygous *PLA2G6* p.P806R mutation was found in late-onset PD patients with typical parkinsonism.[17] In our extended case–controlled study of a large sample size, no association of *PLA2G6* p.P806R was identified in Japanese PD patients and controls. Thus, our data suggest that *PLA2G6* p.P806R is a non-PD-associated polymorphism at least in Japanese PD patients. This result should help clinicians in genetic counseling for PD patients.

Furthermore, in the previous report, there were no other possible PD-associated variants in any of the 17 exons in the 96 PD patients.[17] Therefore, combined with the data from Singapore,[17] our findings emphasize that *PLA2G6* mutations are unlikely to be the major causes or risk factors of PD at least in Asian populations.

However, because there have been no adequate *PLA2G6* mutation analyses in parkinsonism, disease-associated variants in *PLA2G6* could exist in patients with atypical/typical parkinsonism, or PD in specific races. In parkinsonism-dystonia patients, *PLA2G6* mutations have thus far been reported in only certain populations, such as Indians, Pakistanis and Iranians.[12,15,19] In heterogeneous clinical setting of patients with *PLA2G6* mutations, the roles of *PLA2G6* should be clarified including the effect of heterozygous mutation. As brain iron accumulation is frequently observed in common diseases, such as PD and Alzheimer's disease, the role of *PLA2G6* in iron accumulation is elusive in neurodegenerative disorders.

Table 2 *PLA2G6* variants (excluding p.P806R) found in patients with sporadic PD and the allele frequency

				Frequency in this study		
Exon	*Position*	*Amino acid*	*Accession number*	*Patients (%)*	*Allele frequency in patients (%)*	*Controls (%)*
2	c.87G>A	p.V29V	rs2267369	18/116 (15.52)	8.19	
7	c.901C>T	p.R301C	(novel)	1/379 (0.26)	0.13	0/310 (0)
7	c.991G>A	p.D331N	(novel)	1/379 (0.26)	0.13	0/310 (0)

Abbreviation: PD, Parkinson's disease.

Thus, further large studies in various populations and functional studies for *PLA2G6* are needed in neurodegenerative disorders with or without brain iron accumulation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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