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SHORT COMMUNICATION

LRRK2 P755L variant in sporadic Parkinson's disease

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Abstract Parkinson's disease (PD) is a neurodegenerative disorder of unknown etiology with probable involvement of genetic-environmental factors. The majority of PD cases (approximately 90–95%) are sporadic, while familial cases account for approximately 5–10% of PD. In a recent report, a heterozygous *LRRK2* P755L mutation within *LRRK2* exon 19 was found in 2% of Chinese sporadic PD patients and in 0% of normal controls or Caucasians, suggesting that the mutation is disease-associated with ethnic specificity. To further evaluate the role of *LRRK2* P755L variant in sporadic PD, we performed direct sequencing of *LRRK2* exon 19 in 501 Japanese sporadic PD patients (male 249, female 252, aged 28–92 years, mean 65.0 years) and 583 controls of the Japanese general population as an extended association study. In this group, we found six patients (6/501 = 1.2%) and eight controls of the general population (8/583 = 1.6%) with a heterozygous P755L variant ($P = 0.80$, $\chi^2 = 0.064$). No other variants were found in exon 19. Together with previous reports, our extended case-controlled study of large sample size suggests that *LRRK2* P755L is a non-disease-associated polymorphism in PD patients.

Keywords Parkinson's disease · Genetics · *PARK8* · *Leucine-rich repeat kinase 2 (LRRK2)* · Polymorphism · Association study · Japanese · Ethnic background

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Introduction

Parkinson's disease (PD, OMIM #168600) is the second most common neurodegenerative disorder next to Alzheimer's disease. The clinical features are characterized by levodopa-responsive parkinsonism, such as rigidity, resting tremor, bradykinesia, and postural instability. Although the cause of PD remains unclear, genetic-environmental interaction is suggested for the development of the disease. One of the autosomal-dominant forms of PD, *PARK8*, was originally mapped from a Japanese Sagamihara family (Funayama et al. 2002) and *LRRK2* (*PARK8*; *leucine-rich repeat kinase 2,* OMIM *609007) was identified as the causative gene for *PARK8*-linked PD (Paisán-Ruíz et al. 2004; Zimprich et al. 2004). Among *LRRK2* mutations, the most common *LRRK2* G2019S mutation in North Africans and Ashkenazi Jews has shown ethnic differences among Caucasian, Japanese, and Chinese (Nichols et al. 2005; Gilks et al. 2005; Lesage et al. 2006; Tomiyama et al.

2006; Tan et al. 2005). On the other hand, *LRRK2* G2385R variant has recently been found the most common genetic risk factor among Chinese and Japanese, but not Caucasians (Di Fonzo et al. 2006; Funayama et al. 2007; Tan et al. 2007; Farrer et al. 2007). Moreover, in a recent report (Wu et al. 2006), a heterozygous *LRRK2* p.P755L (c.2264c > *t*, rs34410987) mutation within *LRRK2* exon 19, corresponding to a predicted ankyrin-repeat-like domain of LRRK2, was found in 2% (12/598) of Chinese sporadic PD and 0% (0/765) of Chinese normal controls, suggesting its association with the disease. However, *LRRK2* P755L was reported as a polymorphism (3% of 92 normal controls) in the dbSNP database of Taiwanese. Thus, to determine the frequency and the role of *LRRK2* P755L in Asian PD, we screened for *LRRK2* exon 19 in Japanese sporadic PD patients.

Subjects and methods

The nucleotide sequences of *LRRK2* exon 19 were determined by direct sequencing in 501 sporadic Japanese PD patients and 583 controls of the Japanese general population (Table 1). All blood samples and clinical information were obtained by the attending neurologists after obtaining informed consent from their patients. The study was approved by the ethics review committees of Juntendo and Osaka Universities. Diagnosis of PD was made by the attending neurologists based on the presence of parkinsonism and good response to anti-PD treatment. Controls of the Japanese general population were evaluated by neurologists to ensure none of them had PD. DNA was prepared using standard methods. They were amplified by polymerase chain reaction (PCR) of exon 19 and sequenced using BigDye Terminator Chemistry and ABI310 and 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences of the primers, conditions of PCR, and conditions of sequencing were based on a previous report (Zimprich et al. 2004).

Results

We found 6 patients (6/501 = 1.2%) and 8 controls of the Japanese general population (8/583 = 1.6%) with a heterozygous P755L variant ($P = 0.80$, odds ratio = 1.15, 95% CI: 0.40–3.32, $\chi^2 = 0.064$) in *LRRK2* exon 19 (Table 2). No other variants were found in exon 19.

Discussion

The purpose of the present study was to clarify the role of an ethnic-specific variant in the causative gene for PD. Although PD is considered a heterogeneous disease with genetic-environmental interaction, some cases certainly exhibit a Mendelian-inherited disease or are associated with strong genetic and ethnic background. Indeed, the reported frequency of *LRRK2* G2385R was higher in Asian sporadic PD patients than in controls (Di Fonzo et al. 2006; Funayama et al. 2007; Tan et al. 2007), although this is not the case in Caucasians. Moreover, Wu et al. (2006) in Nanjing, China, recently reported that a heterozygous *LRRK2* P755L mutation was found in 2% (12/598) of Chinese sporadic PD and 0% (0/765) of normal controls, whereas none (0/463) of the Caucasian PD patients had this mutation (Deng et al. 2007), suggesting ethnic differences, like *LRRK2* G2385R. However, our results of large case-controlled study in Japanese revealed that *LRRK2* P755L is a non-disease associated polymorphism. Consistent with our data, this variant was present at similar frequency in Taiwanese PD patients (7/578 = 0.99%) and Taiwanese normal controls (10/339 = 0.97%) (Di Fonzo et al. 2006). Furthermore, the latest report in the Chinese population in Singapore showed the absence of segregation and association of P755L with PD (case 4/204 = 2.0%, control 6/235 = 2.6%, $P = 0.76$) (Tan et al. 2008). These findings might be based on ethnic or native differences in human migration history or human genetics.

We reported previously that the most common *LRRK2* G2019S mutation in Mendelian-inherited and sporadic PD

Table 1 Profile of analyzed samples in this study

Parameter	Patients	Controls of general population
Total sample, *n* (%)	501 (100)	583 (100)
Male, *n* (%)	249 (49.7)	312 (53.5)
Female, *n* (%)	252 (50.3)	271 (46.5)
Age at sampling (years)[a]	65.0 ± 9.6 (28–92)	45.0 ± 17.0 (21–98)
Male[a]	64.3 ± 10.2 (28–92)	43.6 ± 15.0 (22–92)
Female[a]	65.4 ± 9.9 (28–92)	46.8 ± 19.0 (21–98)
Age at onset (years)[a]	58.0 ± 10.5 (20–88)	
Male[a]	57.7 ± 10.9 (20–88)	
Female[a]	58.3 ± 10.1 (25–82)	

[a] Data are mean ± SD (range)

Table 2 Allele frequency of *LRRK2* c. 2264C > T (p. P755L) in Japanese patients with Parkinson's disease and controls of general population

	Genotype, *n* (%)			Allele, *n* (%)			
	C/C	C/T	T/T	C	T	χ^{2a}	OR (95% CI)
Patients ($n = 501$)	495 (98.8)	6 (1.2)	0 (0)	996 (99.4)	6 (0.6)	0.06	1.15 (0.40–3.32)
Controls of general population ($n = 583$)	575 (98.6)	8 (1.4)	0 (0)	1,158 (99.3)	8 (0.7)		

[a] Compared with the control

OR odds ratio, *CI* confidence interval

was rare in Asians compared to North Africans or Caucasians (Tomiyama et al. 2006). *LRRK2* variants are reported to spread worldwide with some ethnic differences among each variant, such as R1441G, R1441C, R1441H (exon 31, ROC domain), G2019S, I2020T (exon 41, MAPKKK domain), and G2385R (exon 48, WD40 domain) (Mata et al. 2005). Since *LRRK2* consists of as many as 51 exons, it is important to decide which exon(s) of this gene should be screened first for efficient analysis of mutation in patients with various ethnic backgrounds. In this regard, *LRRK2* exon 41 and 31 are reasonable to be screened first; however, exon 19 is not likely a candidate exon for causative mutation screening in PD. In addition, although MAPKKK and ROC domain are reported to be associated with kinase activity of LRRK2 (Paisán-Ruíz et al. 2004; Zimprich et al. 2004; Smith et al. 2006), the existence and the role of the predicted ankyrin repeat-like domain in LRRK2 have not been established yet.

So far, *LRRK2* P755L as well as G2385R variants have been found in only Chinese, Taiwanese, and Japanese (Asians) with similar frequencies in some Asians, but have not been found in Caucasians. Thus, these variants could occur independently in very ancient Asians with a single founder effect (Farrer et al. 2007). Although the HapMap project has been very successful, the presence of ethnic differences among *LRRK2* variants such as G2019S, R1441G, G2385R, and P755L suggest that further establishment of ethnic-specific or native-specific data is essential for more accurate SNP analyses and genome-wide association studies.

Conclusion

Our extended association study in Japanese with large sample size suggests that *LRRK2* P755L is a non-disease-associated polymorphism in PD patients.

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