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Research article

SLC6A3 rs28363170 and rs3836790 variants in Han Chinese patients with sporadic Parkinson's disease

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HIGHLIGHTS

- The association of two *SLC6A3* variants with Parkinson's disease was studied.
- 521 Han Chinese patients with sporadic Parkinson's disease were genotyped.
- Rs28363170 and rs3836790 are not related to Chinese Parkinson's disease.

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ABSTRACT

Parkinson's disease (PD; OMIM 168600) is the second most common neurodegenerative disorder characterized by the loss of dopamine-producing neurons in the substantia nigra and other brainstem nuclei. Recently, two variants (rs28363170 and rs3836790) in the solute carrier family 6 member 3 gene (*SLC6A3*) were identified to be significantly associated with PD patients in French population. The purpose of our study was to explore whether these two variants are associated with sporadic PD in Han Chinese population. We designed a case-control comparison study in 521 Han Chinese patients with sporadic PD and 502 age, gender and ethnicity matched normal controls from Mainland China. There is no statistically significant difference in either genotypic or allelic distribution between disease group and normal controls in our cohort for the two variants (all $P > 0.05$). In addition, we did not identify any related haplotype that would either increase the risk for PD or play a protective role against PD. Our data suggest that variants rs28363170 and rs3836790 are not associated with sporadic PD in Han Chinese population.

1. Introduction

Parkinson's disease (PD; OMIM 168600) is the second most common neurodegenerative disorder characterized by the loss of dopamine-producing neurons in the substantia nigra and other brainstem nuclei, resulting in motor symptoms, including asymmetric resting tremor, bradykinesia, rigidity, postural instability [1], and other non-motor symptoms, such as depression, autonomic dysfunction, sleep disorders, cognitive impairment, olfactory and other sensory abnormalities, etc. [2]. The exact pathogenetic mechanisms underlying the selective degeneration of dopamine-producing cells in PD are still not fully understood [3]. Multi-factors including genetic abnormalities, environmental and epigenetic factors play significant roles in the potential causes of PD [4]. Genetic research in PD has been extremely prolific over the past two decades, and more than 23 loci and 18 pathogenic genes involved in monogenic inherited PD have been reported [5–8]. However, mutations in these genes only explain a small proportion of PD patients. Approximately 90% of PD cases are sporadic, and non-genetic factors play a role, possibly through interacting with susceptibility genes [9]. Recently, two variants, rs28363170 and rs3836790, in the solute carrier family 6 member 3 gene (*SLC6A3*, OMIM 126455) were found to be significantly associated with PD patients in French population, and rs3836790 was significantly associated with the number of freezing of gait episodes, the number of steps and the completion time [10]. To evaluate whether these two variants in the *SLC6A3* gene are associated with sporadic PD in Han Chinese population, we performed a case-control comparison study in 521

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Table 1
Primers for the variants of the *SLC6A3* gene.

SNP	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)
rs28363170	GGTGTAGGGAACGGCCTGAGAG	CTTCCTGGACACGGCTCAAGG
rs3836790	GCACAAATGAGTGTTCGTGCATGTG	AGCAGGAGGGGCTTCCAGGC

SNP: single nucleotide polymorphism.

sporadic PD patients and 502 normal controls of Han Chinese population.

2. Material and methods

2.1. Subjects

A total of 521 Han Chinese patients with sporadic PD (male/female: 314/207; age: 65.82 ± 10.31 years; age at onset: 62.42 ± 7.83 years), and 502 gender, age and ethnicity matched normal controls (male/female: 300/202; age: 65.92 ± 10.52 years) from Mainland China were recruited in our case-control comparison study. The diagnosis of PD was established according to the common diagnostic criteria [11]. All controls were healthy without any neurological diseases. This study was conducted in conformity with the Declaration of Helsinki and approved by the Institutional Review Board of the Third Xiangya Hospital, Central South University. All participants or their legally authorized caregivers provided written informed consent. Some patients had previously been analyzed and were negative for causal mutations in several known genes potentially associated with PD: 24.95% (130/521) PD patients were screened and had no evidence of mutation in the vacuolar protein sorting 35 gene (*VPS35*) [12], 73.51% (383/521) were negative for any mutation in the S100 calcium binding protein B gene (*S100B*) [13], 65.07% (339/521) had no evidence of mutation in the F-box protein 48 gene (*FBXO48*) [14], 73.51% (383/521) were negative for any mutation in the RAB39B, member RAS oncogene family gene (*RAB39B*) [15], 58.73% (306/521) were negative for either p.A502V or p.R1205H mutations in the eukaryotic translation initiation factor 4-gamma 1 gene (*EIF4G1*) [16], 95.97% (500/521) were tested for variants rs10788972 and rs12046178 in the transcription elongation factor A (SII) N-terminal and central domain containing 2 gene (*TCEANC2*) [17], 98.27% (512/521) were tested for variants rs3212366, rs33932559, and rs34090186 in the melanocortin 1 receptor gene (*MC1R*) [18], 98.27% (512/521) were tested for variants rs75932628 and rs2234253 in the triggering receptor expressed on myeloid cells 2 gene (*TREM2*) [9], and 98.27% (512/521) were tested for variants rs1801131 and rs1801133 in the methylenetetrahydrofolate reductase gene (*MTHFR*) [5].

2.2. DNA preparation and SNP genotyping

Blood samples from all participants were collected, and standard phenol-chloroform extraction method was used to extract genomic DNA from leukocytes in peripheral blood [5]. Primers for polymerase chain reaction (PCR) amplification were designed using the Primer3 (http://primer3.ut.ee/) and the sequences of the primers are shown in Table 1. The cycling conditions for amplification were as follows: Taq polymerase activation at 96 °C for 5 min, followed by 35 cycles of degeneration at 94 °C for 60 s, annealing at 65 °C for 30 s, and extension at 72 °C for 60 s, and then an extra 5 min at 72 °C. Amplification products were resolved in 5% polyacrylamide gel electrophoresis was used to separate the amplification products. In addition, Sanger sequencing was performed in randomly selected samples on an ABI 3500 DNA sequencer (Applied Biosystems, Foster City, CA, USA) to check the reliability and accuracy of the method.

2.3. Statistical analysis

Pearson's χ^2 test was used to calculate the significance in differences of genotypic and allelic distributions in both PD patients and healthy controls. Statistical significance was defined at a value of P less than 0.05. The haplotype construction and association analysis, as well as the calculation of odds ratios (ORs) and 95% confidence intervals (CIs) were performed using SHEsis Online Version (http://analysis.bio-x.cn/SHEsisMain.htm). All the variants were tested for deviation from Hardy-Weinberg equilibrium.

3. Results

Distributions of the genotypes in the PD and control groups were in Hardy-Weinberg equilibrium. There is no statistically significant difference in either genotypic distribution or allelic distribution between the PD cohort and control cohort for the two variants (rs28363170: $\chi^2 = 0.585$, $P = 0.746$ for genotypic distribution; $\chi^2 = 0.410$, $P = 0.522$ for allelic distribution, and rs3836790: $\chi^2 = 0.879$, $P = 0.972$ for genotypic distribution; $\chi^2 = 0.809$, $P = 0.847$ for allelic distribution) (Table 2). Multivariable haplotype based

Table 2
Genotype and allele distribution of the *SLC6A3* gene (rs28363170 and rs3836790).

		Repeats	Patients (%)	Controls (%)	P value (χ^2)	OR (95% CI)
rs28363170	Genotype	10/10	451 (86.6)	442 (88.0)		
		10/9	64 (12.3)	54 (10.8)		
		9/9	6 (1.2)	6 (1.2)	0.746 (0.585)	
	Allele	10	966 (92.7)	938 (93.4)		
		9	76 (7.3)	66 (6.6)	0.522 (0.410)	1.118 (0.794–1.574)
rs3836790	Genotype	7/6	7 (1.3)	10 (2.0)		
		7/5	1 (0.2)	1 (0.2)		
		6/6	371 (71.2)	349 (69.5)		
		6/5	122 (23.4)	121 (24.1)		
		5/5	19 (3.6)	20 (4.0)		
		5/4	1 (0.2)	1 (0.2)	0.972 (0.879)	
	Allele	7	8 (0.8)	11 (1.1)		
		6	871 (83.6)	829 (82.6)		
		5	162 (15.5)	163 (16.2)		
		4	1 (0.1)	1 (0.1)	0.847 (0.809)	–

OR: odds ratio; CI: confidence interval.