Supplementary Material

CLOCK rs1801260 Polymorphism is Associated with Susceptibility to Parkinson's Disease in a Chinese Population

Fan Lou, Ming Li, Yan Ren, Xiao-Guang Luo, Na Liu, Xiaohong Li

Methods

Participants

In this case-control study from May 2010 to October 2015, 646 patients were recruited from the Parkinsonism Clinic and inpatients from Department of Neurology, the First Affiliated Hospital of China Medical University, Shenyang, China. All the patients were diagnosed according to the standards of the UK PD Brain Bank Criteria[1]. The 352 healthy controls were from the Physical Examination Center of the First Affiliated Hospital of China Medical University. All participants were Han Chinese from northeastern provinces of China; they were not relatives and their ages ranged from 36 to 88 years. The main general clinical data of patients with PD and healthy controls are shown in Table S1. There was no significant difference in gender (P = 0.221), age (P = 0.744), or years of education (P = 0.593) between the case-group and the control-group. All patients and controls underwent the Mini Mental State Examination, and those who scored <24 (<20 for individuals with <6 years of education) were excluded. Besides, patients with parkinsonism were excluded. People on day and night shift work were also excluded. Depression was assessed by a score in the 17-item Hamilton Rating Scale for Depression of ≥8. All participants signed informed consent, and the study was approved by the Ethics Committee of the First Affiliated Hospital of China Medical University.

DNA Extraction and Genotyping

Genomic DNA was extracted from leukocytes using the phenol-chloroform extraction method. DNA purity was assessed by spectrophotometer (Thermo Fisher Scientific, Wilmington, DE), and DNA samples were stored at −20 °C. Genotyping was determined using the Kompetitive Allele-Specific PCR (KASP) assay [2]. Each amplification reaction contained 50 ng/μl template DNA, KASP 2× Master Mix standard ROX (LGC Genomics, London, UK) and KASP-by-Design

assay mix (LGC Genomics). The primers (including forward primers marked by FAM, forward primers marked by HEX, and reverse primers) for allele-specific amplification of the rs1801260 and rs2304672 SNPs are shown in Table S2. The PCR thermo-cycling conditions for all primers were 15 min at 94 °C followed by 10 cycles for 20 s, 61 °C for 60 s followed by 26 cycles of 94 °C for 20 s, and 55 °C for 60 s. After amplification, the results from PCR plates were read with a Spectramax M5 FRET-capable plate reader (LGC Genomics). Finally, data were analyzed using Klustercaller software (LGC Genomics) to identify SNP genotypes.

Statistical Analysis

Continuous variables are presented as mean ± SD and categorical variables as percentages. Comparisons of continuous variables were evaluated by Student's t test. Differences between categorical variables were evaluated by the χ^2 test. Hardy-Weinberg analysis was used to check the frequencies of all genotypes with the χ^2 test [3]. Comparisons of the frequencies of alleles and genotypes between groups were analyzed using the χ^2 test. The associations between the two SNPs and susceptibility to PD were estimated by computing the odds ratios (OR) and 95 % confidence intervals (CI) from binary logistic regression analyses after adjusting for confounding risk factors, as indicated in Table S3. The statistical analyses were done using SPSS 20.0 (IBM, Armonk, NY) with the limit of significance set at $P < 0.05$ (two-tailed).

Table S1. Demographic features of participants

	Case (n = 646)	Control (n = 352)	P value
Age	62.21 ±9.83	62.99 ±9.52	0.221
Sex			
Male (%)	316 (48.92)	176 (50.00)	0.744
Female (%)	330 (51.08)	176 (50.00)	
Education (years)	9.59±3.59	9.47±3.69	0.593
Family history (%)	38 (5.88)	3 (0.85)	0.000[a]
Toxic exposure (%)	59 (9.13)	11 (3.12)	0.000[a]
Diabetes mellitus (%)	97 (0.15)	38 (10.80)	0.063
Hypertension (%)	239 (37.00)	141 (40.06)	0.905

Depression (%)	193 (29.88)	18 (5.11)	0.000[a]

Figures in parentheses indicate 95% confidence intervals. [a]$P < 0.01$

Table S2. PD risk factors and rs1801260 in logistic regression analysis

	P value	OR value	95%CI
Age	0.021[a]	1.996	1.289–2.789
Family history	0.046[a]	0.894	0.764–2.367
Toxic exposure	0.011[b]	2.017	1.379–3.689
CC+TC *vs* TT	0.039[a]	1.554	1.074–3.787
Depression	0.019[b]	0.987	0.148–2.021

Figures in parentheses indicate 95% confidence intervals. [a]$P < 0.05$, [b]$P < 0.01$.

Table S3. Primer sequences of SNPs in CLOCK and PER2 genes

SNPs	Primer sequences (Forward-FAM\Forward-HEX\Reverse)
rs1801260	5’GAAGGTGACCAAGTTCATGCTAAAACACTGTCAGAACTGGCTG 3’
	5’ GAAGGTCGGAGTCAACGGATTCCTAAAACACTGTCAGAACTGGCTA 3’
	5’ CCAGCCAGCAGGAGGTGATCAT 3’
rs2304672	5’ GAAGGTGACCAAGTTCATGCTCCTCTGTTTGCCAGCTTCGTTC 3’
	5’ GAAGGTCGGAGTCAACGGATTCCTCTGTTTGCCAGCTTCGTTG 3’
	5’ GCGGAAATTCCGCGTATCCATTCAT 3’

References

[1] Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992, 55: 181–184.

[2] He C, Holme J, Anthony J. SNP genotyping: the KASP assay. Methods Mol Biol 2014, 1145:

75–86.

[3] Rodriguez S, Gaunt TR, Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am J Epidemiol 2009, 169: 505–514.