CLOCK 3111T/C Variant Correlates with Motor Fluctuation and Sleep Disorders in Chinese Patients with Parkinson’s Disease

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Background. The clock genes controlling biological rhythm play an important role in the pathophysiology of aging. The purpose of this study was to determine whether there is an association between a variant of the circadian locomotor output cycles kaput (CLOCK) gene and circadian dysfunction of Parkinson’s disease (PD).

Methods. Six hundred and forty-six cases of Parkinson’s disease from consecutive outpatients and inpatients ward from our hospital were included in this study. Kompetitive allele-specific PCR was used to determine the frequency distribution of genotypes and alleles. The examinations for the PD group were assessed in person in order to evaluate motor symptoms, cognitive function, sleep, and depression, including the Unified Parkinson’s Disease Rating Scale (UPDRS), Mini-Mental State Examination (MMSE), Pittsburgh Sleep Quality Index (PSQI), and 17-item Hamilton Rating Scale for Depression (HAMD-17).

Results. Motor fluctuation ($P < 0.001$) and sleep disorders ($P = 0.007$) were significantly different between the two groups. These correlations persisted after adjusting for confounding risk factors by further binary logistic regression analysis, suggesting that the CLOCK 3111T/C variant was associated with motor fluctuation (OR = 1.080, $P < 0.001$) and a subjective sleep disorder (OR = 1.130, $P = 0.037$).

Conclusion. The CLOCK 3111T/C variant can be an independent risk factor for motor fluctuation and sleep disorder in Parkinson’s disease.

1. Background

Parkinson’s disease (PD) is a degenerative neurological disorder characterized by the loss of dopaminergic neurons in the substantia nigra. It is generally believed that the interaction between multiple genes and environmental factors contributes to an extremely complicated mechanism of PD [1]. Symptoms of circadian dysfunction are the typical symptoms of neurodegenerative diseases, such as Alzheimer’s disease (AD) and PD [2–4]. Impaired circadian function in PD is implicated not only in impaired sleep and alertness but also in autonomic, behavior, mood, and fluctuating motor manifestations [5]. The daily life of patients with PD can be beset by circadian dysfunction, which may be more severe than motor symptoms alone and can become a heavy burden on the family.

The biological rhythm of the human body is regulated by the circadian system. Many physical activities are controlled by the circadian clock, such as the sleep-awake cycle, blood pressure, heart rate, respiration, temperature, visceral activity, immunity, neuroendocrine activity, cell division, and even DNA function. Recently, the clock genes that regulate the circadian rhythm in both the central and peripheral oscillators, located in the suprachiasmatic nucleus, including CLOCK (circadian locomotor output cycles kaput), BMAL1, Period (Per), and Cryptochrome (Cry) genes, have aroused the interest of many researchers [6]. Among those genes, CLOCK is the key gene that regulates the circadian rhythm in the brain and periphery, which can modulate the dopamine synthesis [7]. The 3111T/C is located in the 3′- untranslated region of the CLOCK gene, with a T→C transversion, which has previously been found to be associated with psychobehavioral control, hormone secretion, mood, and sleep [8, 9]. Recently, polymorphisms of some related clock genes have been reported to be associated with the circadian rhythm disturbances of PD [10, 11]. In a previous study, a significant association with CLOCK 3111T/C
in the dominant model in PD was observed, when compared to that of the controls. This suggests that the circadian rhythm gene can be considered to promote the pathophysiology of PD [12]. However, current knowledge regarding the relationship between circadian dysfunction in PD and the CLOCK gene variant is scarce.

Studies on the relationship between a single-nucleotide polymorphism of clock genes and circadian dysfunctions of PD may provide more insight into the genetics of motor and nonmotor symptoms of PD. Therefore, in the present study, we examined the association between a polymorphism of the CLOCK gene with symptoms of circadian dysfunction in PD in a northeastern Chinese population using a retrospective case study.

2. Methods

2.1. Subjects. The present study included 646 patients with PD who were from an unrelated Chinese population in the northeastern provinces of China. The patients were recruited from the movement disorder clinic and inpatient ward of the Department of Neurology, the First Affiliated Hospital of China Medical University, Shenyang, China, from May 2010 to October 2015. The diagnosis of PD was made based on the UK PD Brain Bank Criteria by movement disorder neurologists [13]. Ages of the all participants ranged from 36 to 88 years. All patients underwent the Mini-Mental State Examination (MMSE). We excluded those whose score fell above the limit of significance set at 0.05 (two-tailed).

2.2. Evaluation. We completed the following standard assessment measures when patients had not taken levodopa or other PD medications for at least 12 h (the “off state”):

(1) Hoehn and Yahr staging was used for determining the stage of the disease.
(2) Severity of Parkinsonism was evaluated using the Unified Parkinson’s Disease Rating Scale (UPDRS) II + III, and the tremor, postural, and gait instability scores were further calculated according to the report from Kang et al. [14].
(3) Depression was determined by a score ≥ 8 on the 17-item Hamilton Rating Scale for Depression (HAMD-17).
(4) The motor fluctuation of the PD group was determined by a score of > 1 on item 39 from the UPDRS IV [15].
(5) A subjective sleep disorder in the PD group was determined by a Pittsburgh Sleep Quality Index (PSQI) ≥ 7 [16].
(6) Orthostatic hypotension was determined by a decrease in the systolic blood pressure > 20 mmHg or a decrease in the diastolic pressure > 10 mmHg, accompanied by the symptoms of hypoperfusion, such as dizziness, within 3 minutes of moving into the upright position.
(7) Dyskinesia was determined using the total score of items 32 to 35 from the UPDRS IV.

2.3. DNA Extraction and Genotyping. Genomic DNA was extracted from leukocytes using the sodium dodecyl sulfate-protease K phenol-chloroform method. DNA purity was assessed by spectrophotometry, and DNA samples were stored at −20°C. Genotypes were determined using the Kompetitive allele-specific PCR (KASP) assay [17]. Each amplification reaction contained 50 ng/μl of template DNA, KASP 2x Master Mix standard ROX (LGC Genomics, UK), and KASP-by-Design assay mix (LG Genomics, UK). For the allele-specific amplification of CLOCK gene 3111T/C, the primers (including forward primers marked by FAM and HEX) were 5’ GAA GTG ACAA GTT CAT GTC TAA AC ACT GT CAG AACT GCT G3’ and 5’ GAA GTG CGG AGT CAA CGG AT TCT C TAA AC ACT GT CAG AACT GG CT A3’.

During PCR, the thermocycling conditions of all the primers were 15 min at 94°C, followed by 10 cycles of 94°C for 20 s and 61°C for 1 min (dropping −0.6°C per cycle to achieve a 55°C annealing temperature), followed by 26 cycles of 94°C for 20 s and 55°C for 1 min. After amplification, PCR plates were read with a SpectraMax M5 FRET capable plate reader (LGC Genomics, UK) using the recommended excitation and emission values. Data were subsequently analyzed using Clustal software (LGC Genomics, UK) to identify single-nucleotide polymorphism genotypes.

2.4. Statistical Analysis. The continuous variables are presented as mean ± SD and categorical variables as percentages. Differences of the continuous variables were evaluated by the Student’s t-test, depending on the shape of the distribution curves. Categorical variables were compared using the χ² test. All genotype frequencies were checked using the Hardy-Weinberg analysis in two groups through the χ² test [18]. Differences in the distributions of the alleles and genotypes between the cases and controls were analyzed using the χ² test. The association between the CLOCK gene polymorphisms and circadian dysfunctions of PD was estimated by computing the odds ratios (ORs) and 95% confidence intervals (CIs) from a binary logistic regression analysis using the backward-stepwise method. The statistical analyses were performed using SPSS version 20.0 (IBM, USA) with the limit of significance set at P < 0.05 (two-tailed).

3. Results

We identified the CLOCK T3111C variant in 124 of 646 patients with PD, including the TC and CC genotypes, in a northeastern Chinese population, and we found a significant difference between patients with PD and the healthy controls in the dominant model [12]. Therefore, in order to further explore the clinical characteristics of the patients with variant, we divided the patients into the following two
In the current study, we examined the association of a polymorphism of CLOCK 3111T/C with symptoms of circa
dian dysfunction in PD in a northeastern Chinese pop-
ulation using a retrospective case study. We found that
3111T/C variant carriers scored significantly higher than
noncarriers in motor fluctuation, a subitem of UPDRS.
(P = 0.044), were significantly associated with motor fluctuation in
the logistic regression. These results showed that a polymorphism
of CLOCK 3111T/C can be an independent risk factor for motor
fluctuation (OR = 1.080, 95% CI = 1.057–1.104, P < 0.001). Similar-
larly, as illustrated in Table 3, subjective sleep disorder was as-
dicated with a polymorphism of CLOCK 3111T/C (P = 0.037),
UPDRS II + III (P < 0.001), and depression (P < 0.001). This
showed that a polymorphism of CLOCK 3111T/C can be an
independent risk factor for the sleep disorder in Parkinson’s
disease (OR = 1.130, 95% CI = 1.007–1.2689, P = 0.037).

4. Discussion

PD is multifactorial with many features exhibiting diurnal
fluctuations, such as thermoregulation disorders, circadian
variation of blood pressure and heart rate, emotional dys-
regulation, and even reactions to anti-PD pharmacotherapy
that manifest as abnormalities in rhythm [3, 19–23]. Par-
ticularly, motor activity can vary throughout the day [24].
Recently, increasing studies show that these circadian
rhythm dysfunctions of neurodegenerative disorders are
associated with the abnormal regulation of clock genes [2, 4].

In the current study, we examined the association of a
polymorphism of CLOCK gene with symptoms of circa-
dian dysfunction in PD in a northeastern Chinese pop-
ulation using a retrospective case study. We found that
3111T/C variant carriers scored significantly higher than
noncarriers in motor fluctuation, a subitem of UPDRS.
Normal motor activity gradually increased during the

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### Table 1: Demographic data and clinical profiles of carriers and noncarriers.

<table>
<thead>
<tr>
<th></th>
<th>CLOCK 3111T/C carriers (TC + CC)</th>
<th>CLOCK 3111T/C noncarriers (TT)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62.35 ± 10.19</td>
<td>61.70 ± 10.13</td>
<td>0.515</td>
</tr>
<tr>
<td>Sex (%)</td>
<td>53 (42.7)</td>
<td>263 (50.4)</td>
<td>0.126</td>
</tr>
<tr>
<td>Onset age (years)</td>
<td>58.52 ± 10.33</td>
<td>58.02 ± 10.79</td>
<td>0.645</td>
</tr>
<tr>
<td>EOPD (%)</td>
<td>12 (9.7)</td>
<td>26 (5.0)</td>
<td>0.046&lt;</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>3.90 ± 1.38</td>
<td>3.74 ± 3.04</td>
<td>0.550</td>
</tr>
<tr>
<td>Family history (%)</td>
<td>7 (5.6)</td>
<td>31 (5.9)</td>
<td>0.987</td>
</tr>
<tr>
<td>Formal education (years)</td>
<td>9.47 ± 3.38</td>
<td>9.63 ± 3.65</td>
<td>0.657</td>
</tr>
<tr>
<td>Hoehn-Yahr stage</td>
<td>1.84 ± 0.78</td>
<td>1.74 ± 0.73</td>
<td>0.165</td>
</tr>
<tr>
<td>UPDRS II + III</td>
<td>41.81 ± 19.73</td>
<td>40.26 ± 19.04</td>
<td>0.417</td>
</tr>
<tr>
<td>Mean tremor score</td>
<td>4.27 ± 4.19</td>
<td>4.00 ± 3.79</td>
<td>0.476</td>
</tr>
<tr>
<td>Mean PIGD score</td>
<td>7.71 ± 5.26</td>
<td>8.02 ± 5.25</td>
<td>0.691</td>
</tr>
<tr>
<td>Motor fluctuation</td>
<td>1.93 ± 1.88</td>
<td>0.83 ± 1.41</td>
<td>0.000&lt;</td>
</tr>
<tr>
<td>Dyskinesia</td>
<td>0.33 ± 0.94</td>
<td>0.25 ± 1.03</td>
<td>0.453</td>
</tr>
<tr>
<td>L-dopa therapy initial dosage (mg/day)</td>
<td>148.7 ± 38.86</td>
<td>154.6 ± 41.12</td>
<td>0.215</td>
</tr>
<tr>
<td>L-dopa therapy duration (year)</td>
<td>3.22 ± 2.57</td>
<td>2.95 ± 2.80</td>
<td>0.071</td>
</tr>
<tr>
<td>Good response to levodopa</td>
<td>107 (86.3)</td>
<td>474 (90.8)</td>
<td>0.133</td>
</tr>
<tr>
<td>MMSE</td>
<td>25.23 ± 3.51</td>
<td>25.37 ± 3.32</td>
<td>0.682</td>
</tr>
<tr>
<td>Sleep disorder (%)</td>
<td>48 (38.7)</td>
<td>138 (26.4)</td>
<td>0.007&lt;</td>
</tr>
<tr>
<td>Depression</td>
<td>10.26 ± 3.67</td>
<td>8.88 ± 3.73</td>
<td>0.183</td>
</tr>
<tr>
<td>Orthostatic hypotension (%)</td>
<td>16 (12.9)</td>
<td>69 (13.2)</td>
<td>0.926</td>
</tr>
</tbody>
</table>

The χ² square test was used to compare the categorical variables. Data are expressed as numbers with percentages in parentheses or as mean ± SE. UPDRS: Unified Parkinson’s Disease Rating Scale; MMSE: Mini-Mental State Examination; EOPD: early-onset PD; PIGD: postural instability and gait difficulty. *P < 0.05 and **P < 0.01.
daytime and reached the lowest level at night. However, the rhythm is modified in patients with PD, and motor fluctuation is very common [25]. Motor fluctuation is considered to be associated with a longer disease progression and higher levodopa daily dose [26]. In our research, there were no differences in the initial dosage of levodopa and the higher levodopa daily dose [26]. In our research, there were no differences in the initial dosage of levodopa and the levodopa therapy duration between the two groups (P > 0.05), which is consistent with the results from other studies [20, 27]. Therefore, we inferred that the main influencing factors of motor fluctuation could be attributed to the gene variant rather than the long-term effects of levodopa. In order to exclude the impact of related factors, we performed a logistical analysis. After adjusting for probable risk factors, such as age, sex, disease duration, severity of disease, and impact of levodopa therapy, motor fluctuation was still related to the 3111T/C variant and the risk of motor fluctuation of carriers was 1.077 times that of noncarriers, which shows that motor fluctuation may be associated with the variant of the CLOCK gene in the circadian system. Many studies found that fluctuations in motor activity may be caused by the circadian system. Boulamery found that rhythmic changes induced by lesions in unilateral 6-OHDA PD animal model, which has a loss of circadian rhythmicity, had reduced locomotor activity [28]. Other studies found that exposure to light or administration of the melatonin receptor antagonist facilitates recovery of motor function in chronic experimental models of PD [29, 30]. There is increasing evidence for the existence of interactions between the circadian rhythm and dopamine system and the modulation of dopamine synthesis by circadian genes. The CLOCK gene can regulate the transcription of tyrosine hydroxylase [31], which is the rate-limiting enzyme for dopamine synthesis. CLOCK can also affect the dopaminergic activity at the posttranscriptional level, which leads to the motor fluctuations [32]. Above all, our results indicate that patients with PD who are CLOCK 3111T/C variant carriers are more susceptible to the development of motor fluctuation than their wild-type counterparts.

Our study also found that since the 3111T/C variant carriers scored significantly higher than the noncarriers on the PQSI, carriers were associated with a higher risk of subjective sleep disorder when compared to that of the noncarriers. Numerous studies show that the polymorphism of CLOCK 3111T/C is associated with sleep disturbance in human beings, which could affect diurnal behavior pattern [33, 34]. Disturbed sleep-wake cycles are common nonmotor manifestations of PD, affecting up to 90% of the patients. It is suggested that the etiology of sleep disorders in PD depends on the adverse effects of antiparkinsonian medications and primary neurodegeneration of central sleep regulatory areas [35, 36]. We also found that patients with PD who have the CLOCK gene variant are more susceptible to the development of sleep disorders after adjusting for impact factors related to sleep, such as depression, severity of motor symptoms, age, sex, and disease duration. Therefore, we further confirmed that the polymorphism of CLOCK 3111T/C can be an independent risk factor for sleep disorders in PD. We also found that the proportion of patients with EOPD in the carrier group was significantly higher than that in the noncarrier group. This may reveal that the 3111T/C variant may play a role in lowering the onset age of PD and needs to be confirmed by further research.

In previous studies, different researchers have found that the polymorphism of CLOCK 3111T/C is associated with psychiatric disorders [37, 38]. Hua et al. [10] found that a polymorphism of Tef, which is a downstream regulatory gene of CLOCK, is associated with the depression of PD in Chinese population. Although in our study we found no statistical significant difference between the two groups in depression, a tendency could be found, which warrants further research. However, with regard to the other symptoms of circadian rhythm disorders, such as dyskinesia, orthostatic hypotension, and response to levodopa, no differences were found between the two groups. Different results may be attributed to the different loci of clock genes, sample size, different regions and nations, and different statistical methods.

Although the circadian timing controls the temporal patterning of molecular, cellular, and physiological processes throughout the body and the potential disruption of this timing system in PD would be expected to produce widespread symptoms [6], there is little known about the mechanism of the variant of clock genes leading to circadian

### Table 2: Binary logistic regression of association between motor fluctuation and CLOCK 3111T/C variant.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3111T/C variant</td>
<td>1.080</td>
<td>1.057–1.104</td>
<td>0.000a</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.645</td>
<td>0.202–2.067</td>
<td>0.461</td>
</tr>
<tr>
<td>Gender</td>
<td>0.962</td>
<td>0.649–1.424</td>
<td>0.845</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>1.656</td>
<td>0.508–5.395</td>
<td>0.403</td>
</tr>
<tr>
<td>Hoehn-Yahr stage</td>
<td>0.940</td>
<td>0.885–0.998</td>
<td>0.044b</td>
</tr>
<tr>
<td>UPDRS II + III</td>
<td>4.698</td>
<td>2.868–7.695</td>
<td>0.000a</td>
</tr>
<tr>
<td>L-dopa therapy duration (year)</td>
<td>0.884</td>
<td>0.885–0.998</td>
<td>0.440</td>
</tr>
<tr>
<td>L-dopa therapy initial dosage (mg/day)</td>
<td>1.179</td>
<td>0.961–1.558</td>
<td>0.115</td>
</tr>
<tr>
<td>Response to levodopa</td>
<td>0.884</td>
<td>0.780–1.002</td>
<td>0.053</td>
</tr>
<tr>
<td>Mean PIGD score</td>
<td>1.002</td>
<td>0.945–1.062</td>
<td>0.957</td>
</tr>
<tr>
<td>EOPD</td>
<td>0.911</td>
<td>0.662–1.252</td>
<td>0.565</td>
</tr>
</tbody>
</table>

UPDRS: Unified Parkinson’s Disease Rating Scale; MMSE: Mini-Mental State Examination; EOPD: early-onset PD. *P < 0.01 and bP < 0.05.

### Table 3: Binary logistic regression of association between sleep disorders and CLOCK 3111T/C variant.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3111T/C variant</td>
<td>1.310</td>
<td>1.007–1.268</td>
<td>0.037a</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.008</td>
<td>0.989–1.028</td>
<td>0.407</td>
</tr>
<tr>
<td>Gender</td>
<td>1.348</td>
<td>0.905–2.008</td>
<td>0.142</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>1.039</td>
<td>0.971–1.111</td>
<td>0.266</td>
</tr>
<tr>
<td>Hoehn-Yahr stage</td>
<td>1.098</td>
<td>0.795–1.516</td>
<td>0.571</td>
</tr>
<tr>
<td>UPDRS II + III</td>
<td>1.044</td>
<td>1.022–1.066</td>
<td>0.000b</td>
</tr>
<tr>
<td>Mean PIGD score</td>
<td>0.976</td>
<td>0.917–1.038</td>
<td>0.443</td>
</tr>
<tr>
<td>Depression</td>
<td>3.872</td>
<td>2.455–6.135</td>
<td>0.000b</td>
</tr>
<tr>
<td>Response to levodopa</td>
<td>1.044</td>
<td>0.986–1.106</td>
<td>0.141</td>
</tr>
</tbody>
</table>

UPDRS: Unified Parkinson’s Disease Rating Scale; MMSE: Mini-Mental State Examination. *P < 0.05 and bP < 0.01.
dysfunctions in PD. However, pharmacotherapeutics and behavior pattern can intervene with pathological changes caused by circadian rhythm dysfunction [39]. Daily evening exposure to supplemental bright light improved sleep quality and mood, as well as bradykinesia and rigidity [40].

In conclusion, our results showed that the 3111T/C variant of CLOCK gene was closely related to the occurrence of motor fluctuation and sleep disorders in a northeastern Chinese population. It raises the possibility that circadian dysfunctions experienced by patients with PD may not merely be a subsidiary of the motor symptoms but an integral part of circadian system related to the disease itself. Our study confirmed a correlation between the symptoms of the circadian dysfunctions and Parkinson’s disease from the view of the gene level. These findings suggest that circadian system may be a novel diagnostic and therapeutic target in motor fluctuation and sleep disorders with PD. However, multiple factors should be involved, such as genetic background, gene-environment interactions, and interaction of other clock genes. Therefore, more research is required to better understand the relationship between the polymorphism of clock genes and clinical profiles of PD.

5. Conclusion

CLOCK 3111T/C variant can be an independent risk factor for motor fluctuation and sleep disorder in Parkinson’s disease in Chinese population.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References


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