Original Research

Thyrotroph embryonic factor polymorphism predicts faster progression of Parkinson's disease in a longitudinal study

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The thyrotroph embryonic factor gene is a circadian clock-controlled gene. The rs738499 polymorphism of this gene has been suggested to be associated with depression and sleep disturbance in Parkinson's disease in previous cross-sectional studies. We aimed to investigate whether this single nucleotide polymorphism is associated with the progression rates of various motor and non-motor symptoms in patients with Parkinson's disease. We recruited 186 patients with Parkinson's disease for a longitudinal study. Motor and non-motor symptoms were assessed at baseline and follow-up, and 170 Parkinson's disease patients completed the clinical evaluation twice with an average follow-up period of 3.3 $\pm$ 1.1 years. A stepwise linear regression model was used to validate factors associated with Parkinson's disease symptoms' annual progression rates. Faster annual worsening rates of sleep quality and Hoehn-Yahr stage were found in carriers with the homozygous dominant (TT). After adjustment for related clinical factors, the rs738499 polymorphism showed a contribution of 3.1% to the annual decline rate on the Parkinson's Disease Sleep Scale score and a contribution of 5.5% to the annual increase rate of the Hoehn-Yahr stage. Additionally, anxiety and axial symptoms predicted the progression of sleep disturbances and motor staging. The TT genotype of rs738499 might be a potential predictor of rapid deterioration in sleep quality and Hoehn-Yahr stage in patients with Parkinson's disease and may advance the understanding of the genetic contributions to Parkinson's disease.

Keywords

Parkinson's disease; Sleep; Thyrotroph embryonic factor; Circadian genes; Longitudinal study

1. Introduction

Parkinson's disease (PD) is characterized by progressive loss of dopaminergic neurons in the substantia nigra and Lewy bodies' formation in the midbrain. These pathological changes result in motor symptoms. Braak's staging theory [1] and the presence of multiple non-motor symptoms reflect the complex and multifaceted nature of PD [2]. Non-motor symptoms are often resistant to L-DOPA treatment and will inevitably become a significant factor affecting patients' function and quality of life in advanced PD stages. Therefore, further exploration of other pathophysiological mechanisms involved in these symptoms holds promise for other effective therapeutic strategies. During the last decade, increasing attention has been paid to the link between circadian rhythms and PD. It has been recognized that the circadian rhythm is disrupted in patients with PD [3]. First, circadian function biomarkers, such as body temperature, blood pressure, and hormone secretion, are dysregulated in PD [4, 5]. Second, patients in advanced stages lose normal motor activity, and actigraphy studies in patients with PD reported flattened diurnal motor activity patterns [6]. Third, patients receiving repeated L-DOPA administration often report a lower response to L-DOPA in the evening [7]. Fourth, common non-motor symptoms of PD, such as sleep-wake disturbances, depression, and visual symptoms, are modulated by circadian rhythms [8, 9].

A large sample cohort study of community-dwelling older men without PD reported that reduced circadian rhythmicity at baseline is associated with an increased risk of developing PD during an 11-year follow-up, suggesting that circadian disruption rhythms might be prodromal to PD [10]. Evidence indicates that the electrical activity of neurons within the master pacemaker of the circadian system, the suprachiasmatic nuclei (SCN), is disrupted at the onset of motor symptoms [11]. Therefore, circadian dysfunction may be a key cause of non-motor symptoms, especially for pre-motor symptoms. Sleep disturbances are common non-motor symptoms reported by 64% of patients with PD [12], including insomnia, rapid eye movement sleep behavior disorder, restless legs syndrome, sleep-disordered breathing, and excessive daytime sleepiness. Patients often demonstrate sleep alterations during the prodromal phase of PD [13]. It has been hypothesized that circadian and sleep changes may trigger neurodegeneration in the earliest phases of the disease [14]. Therefore, it is important to understand and improve sleep disturbances and circadian rhythm in patients with PD.

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Even though there are reciprocal interactions between the dopaminergic system and circadian rhythms, the underlying mechanisms of circadian dysfunction in PD remain elusive. Circadian rhythms are regulated by circadian gene networks consisting of a series of clock genes (such as *CLOCK*, *BMAL1*, *BMAL2*, *PER1*, PER*2*, *PER3*, *CRY1*, and *CRY2*) and clock-controlled genes (such as *TEF*, *DEC1*, *DEC2*, and *TIMELESS*) [15]. Several studies have explored desynchronization at the molecular level. A study examining the expression profile of several principal circadian genes in peripheral leukocytes in whole blood for a 12-hour nighttime period found that the mRNA expression of *BMAL1* and *BMAL2* was significantly reduced in patients with PD in the evening. Furthermore, the level of *BMAL2* correlated with motor severity and sleep quality in the morning [16, 17]. Cai and his colleagues [18] discovered that genetic polymorphisms in *ARNTL* and *PER1* were significantly associated with PD risk. These reports suggest that circadian rhythm disruption is a result and a cause of the degeneration process. In this case, it would be expected to occur early in the disease's course (or even preceding disease onset) and exacerbate disease progression. There has been no longitudinal study focusing on the effect of circadian genes on the progression of PD.

The thyrotroph embryonic factor (*TEF*) gene is one of the downstream genes in the circadian gene network and expresses a broad range of cells and tissues. It encodes a thyrotroph embryonic factor, together with albumin D box-binding protein (DBP), human hepatic leukemia factor (HLF) are three members of the proline and acidic amino-acid-rich basic leucine zipper (PAR bZip) transcription factor family. Different members of the subfamily can readily form heterodimers and share DNA-binding and transcriptional regulatory properties. The thyrotroph embryonic factor can bind to proline and acidic amino-acid-rich response elements (PARRE) in promoters and elevate the transcription levels of light-induced circadian genes *NR1D1*, *NR1D2*, *CRY1a*, *CRY2b*, and *PER2* [19]. The *TEF* rs738499 polymorphism has been suggested to be associated with depression and sleep disturbance in PD in previous cross-sectional studies [20, 21]. The present study was conducted longitudinally in a cohort of patients with PD to investigate whether *TEF* is associated with worsening PD patients' clinical symptoms with an average follow-up period of 3.3 years.

2. Materials and methods

2.1 Study subjects

For this longitudinal study, the patients should satisfy the following criteria: (1) younger than 80 years, (2) Hoehn-Yahr stage (H-Y stage) $\leq$ 3.0, and (3) voluntary signing of informed consent. Exclusion criteria included other neurodegenerative disorders, psychiatric disorders, and severe physical illnesses. A total of 186 patients were included during the enrollment period between January 2008 and December 2011. The Ethics Committee approved the study of Nanjing Medical University.

2.2 Scale assessment

To assess disease progression, all patients underwent clinical evaluation at baseline and during each follow-up to confirm the diagnosis and assess disease progression. Motor disability was evaluated using the Unified Parkinson's Disease Rating Scale [22] containing Part-III (UPDRS-III), Part-II - Activities of Daily Living (ADL), and Part-V-H-Y stage. We calculated four composite motor scores from UPDRS-III [23]: tremor (items 20 and 21), rigidity (item 22), akinesia (items 19, 23-26, and 31), and axial symptoms (items 18 and 27-30). The non-motor symptoms were assessed using the Mini-Mental State Examination (MMSE) [24], 24-item Hamilton Rating Scale for Depression (HAMD) [25], 14-item Hamilton rating scale for anxiety (HAMA) [26], Parkinson's Disease Sleep Scale (PDSS) [27], and Parkinson's Disease Non-Motor Symptom Quest (NMSQ) [28]. All these assessments were carried out during an 'on' period. Dopaminergic medication and levodopa equivalent daily doses (LEDD) [29] were recorded at every visit. We successfully re-examined 170 patients during their follow-up visits between June 2012 and October 2014. The remaining 16 patients dropped out before the follow-up (four patients withdrew, six could not be contacted, two were too ill, and four patients were deceased).

2.3 Genotyping

DNA samples were extracted from citric acid-anticoagulated peripheral blood specimens using DNA extraction kits (Tiangen Biotech, Beijing, P. R. China) according to the manufacturer's recommendations. DNA samples were randomly distributed into 96-well plates and sent to CapitalBio Corporation (Beijing, China) for genotyping. Laboratory personnel was blinded to the subject status and our study objective. The iPLEX Gold SNP genotyping kit, software, and experimental equipment used by the laboratory were provided by the MassARRAY platform (Sequenom, San Diego, CA). In brief, an oligonucleotide primer annealed upstream to the single nucleotide polymorphism (SNP) site was genotyped to accomplish a locus-specific primer extension reaction. A 1 μL DNA sample (20-50 ng/μL) was then used for a locus-specific polymerase chain reaction. After amplification, residual nucleotides in the product were dephosphorylated with shrimp alkaline phosphatase enzyme before the iPLEX Gold primer single-base extension. After the extension, products were desalted with SpectroCLEAN resin. Subsequently, 10 nL of the desalted product was spotted onto a 384-format SpectroCHIP with the MassARRAY Nanodispenser. The mass determination was performed using a MALDI-TOF mass spectrometer. MassARRAY Typer 4.0 software was used for data acquisition. SNP genotypes were called after cluster analysis using the default setting. Genotype calls were further reviewed manually to correct any uncertain calls due to clustering artifacts. All 170 subjects included in the final follow-up study were successfully genotyped, with a call rate $\geq$ 90%.

2.4 Statistical analyses

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Version 18.0, IBM). The Hardy-Weinberg equilibrium of SNPs was calculated with the SHEsis program [30]. Baseline information between different genotypes (TT vs. TG + GG) was compared using the Mann-Whitney U test for continuous data and a chi-square test for categorical data. Changes between assessments at baseline and follow-up were compared using the Wilcoxon test. Each PD symptom's progression was defined by the annual change in the scale score and described by median (min-max).

Meanwhile, the annual progression rates of clinical symptoms were compared between genotypes using the Mann-Whitney U test. The correlations between genotypes, the baseline clinical variables, and the annual progress ratio of each scale were analyzed by Spearman's rank correlation and variables with $P < 0.2$ were included in the stepwise linear regression model to explore the risk factors affecting the annual progression rate of the PDSS score and H-Y stage adjusted by sex and gender. The criterion for statistical significance was set at $P < 0.05$.

3. Results

The distribution of genotypes was fitted with the Hardy-Weinberg equilibrium in the PD sample. The mean age of patients with PD was 58.7 $\pm$ 11.3 years, 58.8% were men. The mean duration at baseline was 4.5 $\pm$ 3.5 years and the mean follow-up period was 3.3 $\pm$ 1.1 years. The demographics and clinical characteristics at baseline showed no significant difference between genotypes (TT vs. TG + GG). All clinical symptoms worsened significantly during the follow-up period (Table 1). A faster annual decline rate of the PDSS score ($P = 0.019$) and progression rate of the H-Y stage ($P = 0.016$) was found in carriers of the TT genotypes of *TEF* rs738499 (Table 2).

Spearman's rank correlation showed that the annual decline rate of the PDSS score was associated with the PDSS score at baseline, the annual progression rates of HAMA and NMSQ during follow-up, and the TT genotype of *TEF* rs738499. The H-Y stage's progression rate was associated with the H-Y stage, tremor score, rigidity score at baseline, and the annual progression rates of HAMD, HAMA, NMSQ, ADL, MMSE, tremor, rigidity, akinesia, axial symptoms, and the TT genotype of *TEF* rs738499. Table 3 displays all related factors with $P < 0.2$.

Stepwise linear regression showed that the progression rate of anxiety, the initial severity of sleep disturbance and axial symptoms, and the TT genotype of *TEF* rs738499 were independent risk factors affecting the decline rate of PDSS score. These four predictors together could explain the 40.5% variation in the decline rate of PDSS. *TEF* rs738499 alone accounted for 3.1% of the variance ($P = 0.030$) (Table 4). Meanwhile, stepwise linear regression revealed that the progression rate of axial symptoms, the TT genotype of *TEF* rs738499, and the initial severity of anxiety were independent risk factors affecting the H-Y progression rate stage. These three predictors together could explain the 49.1% variation in the progression rate of the H-Y stage. *TEF* rs738499 alone accounted for 8.3% variance ($P = 0.021$) (Table 5).

4. Discussion

The present research indicates that the TT genotype in *TEF* rs738499 correlated with a faster deterioration rate of sleep quality and H-Y stage of PD patients. Moreover, patients with more severe sleep disturbance and axial symptoms at baseline and faster worsening rate of anxiety during the follow-up period presented a faster deterioration rate of sleep quality, and patients with more severe anxiety at baseline and faster worsening rate of the axial symptoms during the follow-up period experienced a quicker progression rate of the H-Y stage.

The sleep-wake cycle is under the dual control of homeostatic and circadian processes. Homeostatic processes are responsible for the sleep rebound following sleep deprivation, and the circadian processes determine the timing of the sleep period via the circadian clock in the SCN. However, little is known about the circadian system's molecular mechanism in patients' sleep problems with PD. Several neurotransmitters with circadian rhythmicity, such as dopamine, melatonin, 5-hydroxytryptamine (5-HT), glutamate, and gamma-aminobutyric acid (GABA), have been reported to be implicated in the regulation of sleep-wake behavior [31–33]. David *et al.* [34] found that reduced circulating melatonin levels were associated with reduced slow-wave sleep and reduced rapid eye movement (REM) sleep.

Furthermore, a lower melatonin onset concentration was associated with increased sleep latency in patients with PD. *TEF* is involved in maintaining neurotransmitter homeostasis (such as 5-HT and dopamine) [35]. We speculate that the association of *TEF* with the rapid deterioration of sleep quality and H-Y stage might be related to its above-described function, but further studies are needed to provide further evidence.

Our results show that anxiety and axial symptoms were closely related to worse sleep quality and motor staging. The etiology of sleep disturbances in PD is multifactorial and previous studies have shown that anxiety symptoms are a risk factor for sleep quality and motor complications [36, 37]. Axial nocturnal hypokinesia in PD manifests fewer turning-over episodes, turns with smaller degrees, less velocity, and acceleration. Patients with severe nocturnal hypokinesia usually need to take a higher total and bedtime levodopa equivalent dose, and drinking more water at night could result in frequent nocturia that compels the patients to get out of bed, resulting in lower sleep quality [38, 39]. The onset of falls is a warning symptom of H-Y stage aggravation. Spanish researchers [40] employed an objective technology for rigidity assessment. They reported that axial rigidity was related to the risk of falls in patients with PD. Axial

Table 1. Baseline characteristics were compared by genotype and the comparison between baseline and follow-up data of all subjects.

Characteristic	TT	TG + GG		Overall PD		
	(n = 102)	(n = 68)	*P*-value	(n = 170)		*P*-value
	Baseline	Baseline		Baseline	Follow-up	
Gender, % male	59.8	57.4	0.750	58.8	–	–
Age at onset, years	59.3 ± 10.5	57.7 ± 12.3	0.211	58.7 ± 11.3	–	–
Age at interview, years	69.2 ± 9.9	68.2 ± 11.7	0.375	68.8 ± 10.6	–	–
Duration, years	4.4 ± 3.2	4.8 ± 4.1	0.709	4.5 ± 3.5	–	–
Years of follow-up	3.3 ± 1.1	3.3 ± 1.0	0.827	3.3 ± 1.1	–	–
Years of education	10.4 ± 3.9	10.9 ± 3.9	0.634	10.6 ± 3.9	–	–
LEDD, mg	405.4 ± 176.2	391.1 ± 218.9	0.410	399.4 ± 194.5	530.7 ± 230.8	0.000
H-Y stage	2.0 (1.0-4.0)	2.0 (1.0-3.0)	0.144	2.0 (1.0-4.0)	2.5 (1.0-4.0)	0.000
UPDRS-III	20.7 ± 11.0	22.9 ± 12.0	0.262	21.6 ± 11.5	21.6 ± 11.6	0.000
Tremor	3.9 ± 3.6	4.5 ± 3.2	0.128	4.2 ± 3.4	5.0 ± 4.1	0.001
Rigidity	3.5 ± 3.4	3.8 ± 3.1	0.381	4.2 ± 3.4	6.2 ± 4.1	0.000
Bradykinesia	8.0 ± 5.1	9.0 ± 5.9	0.282	4.2 ± 3.4	11.4 ± 6.5	0.000
Axial symptoms	4.1 ± 2.8	4.0 ± 2.7	0.778	4.2 ± 3.4	5.8 ± 3.7	0.000
ADL	11.0 ± 5.5	12.1 ± 6.2	0.326	11.5 ± 5.8	14.0 ± 6.6	0.000
MMSE	28.4 ± 2.0	28.5 ± 2.1	0.583	28.4 ± 2.0	26.7 ± 3.7	0.000
HAMD	12.1 ± 9.9	11.7 ± 8.0	0.794	11.9 ± 9.2	14.2 ± 9.5	0.001
HAMA	9.3 ± 7.9	9.9 ± 6.5	0.237	9.6 ± 7.4	11.1 ± 7.5	0.020
PDSS	121.3 ± 20.0	117.6 ± 20.2	0.154	119.8 ± 20.1	114.3 ± 24.2	0.001
NMSQ	10.3 ± 5.3	9.5 ± 4.8	0.449	10.0 ± 5.1	12.6 ± 5.5	0.000

Values are expressed as mean ± SD. The H-Y stage is expressed by the median (min-max).

ADL, Activities of Daily Living; APR, annual progression rate; HAMA, 14-item Hamilton Rating Scale for Anxiety; HAMD, 24-item Hamilton rating scale for depression; H-Y stage, Hoehn-Yahr stage; LEDD, levodopa equivalent daily doses; MMSE, Mini-Mental State Examination; n, number of samples; NMSQ, Parkinson's Disease Non-Motor Symptom Questionnaire; PD, Parkinson's disease; PDSS, Parkinson's Disease Sleep Scale; UPDRS-III, Unified Parkinson's Disease Rating Scale Part-III.

Table 2. Comparison of the annual progression rates of clinical symptoms between the *TEF* rs738499 genotypes.

Annual Progression Rates	Overall PD	TT	TG + GG	*Z*	*P*-value
	(n = 170)	(n = 102)	(n = 68)		
LEDD, mg	26.1 (-151.6 ~ 332.1)	41.4 (-151.6 ~ 332.2)	0.0 (-89.7 ~ 183.8)	-1.940	0.053
H-Y stage	0.1 (-0.9 ~ 1.3)	0.2 (-0.1 ~ 1.3)	0.1 (-0.9 ~ 1.0)	-2.404	0.016
UPDRS-III	3.0 (-8.6 ~ 19.2)	3.0 (-3.5 ~ 18.8)	3.0 (-8.6 ~ 19.2)	-0.588	0.556
Tremor	0.0 (-4.7 ~ 5.2)	0.3 (-4.7 ~ 4.1)	0.0 (-3.2 ~ 5.2)	-1.399	0.162
Rigidity	0.7 (-2.6 ~ 4.9)	0.7 (-2.6 ~ 4.9)	0.8 (-2.5 ~ 4.9)	-0.263	0.793
Bradykinesia	0.8 (-4.8 ~ 8.5)	0.8 (-3.6 ~ 6.8)	0.8 (-4.8 ~ 8.5)	-0.773	0.439
Axial symptoms	0.4 (-2.8 ~ 6.5)	0.4 (-1.4 ~ 5.4)	0.3 (-2.8 ~ 6.5)	-0.489	0.625
ADL	1.1 (-3.9 ~ 8.0)	1.1 (-2.7 ~ 6.2)	1.1 (-3.9 ~ 8.10)	-0.982	0.326
MMSE	0.3 (-2.1 ~ 5.6)	0.4 (-2.1 ~ 5.6)	0.1 (-0.8 ~ 3.0)	-1.305	0.192
HAMD	0.5 (-14.4 ~ 28.9)	0.3 (-14.4 ~ 28.9)	1.4 (-7.0 ~ 9.2)	-0.438	0.661
HAMA	0.6 (-7.0 ~ 12.1)	0.6 (-3.6 ~ 9.4)	0.3 (-7.0 ~ 12.1)	-0.570	0.569
PDSS	1.6 (-36.1 ~ 57.1)	2.7 (-36.1 ~ 57.1)	0.4 (-32.0 ~ 15.7)	-2.346	0.019
NMSQ	0.9 (-6.0 ~ 10.8)	1.0 (-6.0 ~ 10.8)	0.6 (-4.0 ~ 5.3)	-0.388	0.698

Values are expressed as the median (min-max).

ADL, Activities of Daily Living; APR, annual progression rate; HAMA, 14-item Hamilton Rating Scale for Anxiety; HAMD, 24-item Hamilton rating scale for depression; H-Y stage, Hoehn-Yahr stage; LEDD, levodopa equivalent daily doses; MMSE, Mini-Mental State Examination; n, number of samples; NMSQ, Parkinson's Disease Non-Motor Symptom Questionnaire; PD, Parkinson's disease; PDSS, Parkinson's Disease Sleep Scale; UPDRS-III Unified, Parkinson's Disease Rating Scale Part-III; Z, Mann-Whitney U-test statistic.

Table 3. Factors associated with the annual decline rate of PDSS or with the annual progression rate of H-Y stage by Spearman's rank correlation ($P < 0.2$).

Variables	Annual Decline Rate of PDSS		Annual Progression Rate of H-Y stage	
	r	*P*-value	*R*	*P*-value
Years of follow-up	0.056	0.512	-0.107	0.120*
APR of LEDD	-0.045	0.666	0.129	0.195*
Baseline H-Y stage	-0.047	0.583	-0.567	0.000*
Baseline tremor	-0.030	0.738	-0.249	0.002*
APR of tremor	0.007	0.939	0.184	0.017*
Baseline rigidity	0.067	0.452	-0.293	0.000*
APR of baseline rigidity	0.024	0.776	0.258	0.001*
Baseline akinesia	0.016	0.860	-0.114	0.158*
APR of baseline akinesia	0.016	0.860	0.248	0.001*
Baseline axial symptoms	0.156	0.078*	-0.053	0.511
APR of axial symptoms	-0.094	0.298	0.386	0.000*
ADL	0.144	0.136*	-0.066	0.457
APR of ADL	0.097	0.328	0.264	0.005*
MMSE	-0.103	0.227	0.115	0.140*
APR of MMSE	0.070	0.433	0.275	0.001*
HAMD	-0.004	0.966	-0.060	0.467
APR of HAMD	0.153	0.093*	0.208	0.015*
HAMA	-0.131	0.144*	-0.145	0.079*
APR of HAMA	0.322	0.000*	0.197	0.029*
PDSS	0.363	0.000*	0.047	0.551
APR of NMSQ	0.245	0.004*	0.199	0.013*
TEF rs738499	-0.202	0.017*	-0.159	0.040*

ADL, Activities of Daily Living; APR, annual progression rate; HAMA, 14-item Hamilton Rating Scale for Anxiety; LEDD, levodopa equivalent daily doses; HAMD, 24-item Hamilton rating scale for depression; H-Y stage, Hoehn-Yahr stage; MMSE, Mini-Mental State Examination; NMSQ, Parkinson's Disease Non-Motor Symptom Questionnaire; PDSS, Parkinson's Disease Sleep Scale; *r*, Spearman rank correlation coefficient; *$P < 0.2$.

Table 4. The independent risk factors affecting the annual decline rate of the PDSS score resulting from the stepwise linear regression model.

Predictor	*r*	Adjusted r^2	r^2 Change	*Beta*	*t*	*P*-value	VIF
Annual progression rate of HAMA	0.445	0.189	0.198	0.361	4.366	0.000	1.069
Baseline PDSS	0.554	0.292	0.110	0.323	3.845	0.000	1.107
Baseline axial symptoms	0.632	0.380	0.092	0.301	3.762	0.000	1.003
TEF rs738499	0.656	0.405	0.031	-0.18	-2.210	0.030	1.038

Beta standardized regression coefficient stands for each variable's contribution to the dependent variable; HAMA, 14-item Hamilton rating scale for Anxiety; PDSS, Parkinson's Disease Sleep Scale; *r*, multiple correlation coefficient; r^2, determination coefficient; r^2 change stands for each variable's contribution to the model; *t*, *t*-test statistic for Beta; VIF, variance inflation factor, estimate whether the multicollinearity model exists or not.

Table 5. The independent risk factors affecting the annual progression rate of the H-Y stage resulting from the stepwise linear regression model.

Predictors	*r*	Adjusted r^2	r^2 Change	*Beta*	*t*	*P*-value	VIF
Annual progression rate of axial symptoms	0.619	0.371	0.383	0.651	6.557	0.000	1.008
TEF rs738499	0.682	0.444	0.083	0.33	3.279	0.002	1.035
Baseline HAMA	0.722	0.491	0.055	-0.239	-2.379	0.021	1.035

Beta the standardized regression coefficient stands for each variable's contribution to the dependent variable; HAMA, 14-item Hamilton rating scale for Anxiety; H-Y stage, Hoehn-Yahr stage; *r*, multiple correlation coefficient; r^2, determination coefficient; r^2 change stands for each variable's contribution to the model; *t*, *t*-test statistic for beta; VIF, variance inflation factor, estimate whether the multicollinearity model exists or not.

symptoms contribute to the postural instability and gait difficulty (PIGD) subtype of PD. The PROPARK study [41] reported that PIGD-dominant patients had more severe motor and non-motor symptoms, a more rapid disease progression, and a poorer prognosis PD compared to the tremor-dominant (TD) patients. Another circadian rhythm-related study [42] showed that agomelatine, acting as a melatonin receptor (MT1 and MT2) agonist and as a serotonin receptor (5-HT2b and 5-HT2c) antagonist, improves not only depressive symptoms in PD but also improves sleep problems and motor symptoms. This confirmed the intimate internal relations of these three clinical phenotypes. Therefore, much closer relationships between sleep and mood disorders and motor symptoms may have a specific impact on the genotypic association.

The longitudinal follow-up is the main strength of this study, and a comprehensive follow-up evaluation of multiple non-motor symptoms of PD was carried out. There are several limitations to this study. First, the sample size was small, and no control group to perform gene-disease association analysis was included. We genotyped the *TEF* rs738499 polymorphism in 480 PD patients and 500 controls in a previous study. We found that the T allele and TT genotype were significantly higher in patients with PD, but this result was not shown in that study [20]. Second, the participants were not assessed during the same time of the day or the same season for baseline, and follow-up visits as both factors will influence motor and non-motor scores. Therefore, case-control studies with a larger sample size and more strict control of various factors affecting circadian rhythm are needed to explore the role of circadian rhythm in different clinical stages of PD.

5. Conclusions

This longitudinal study aimed to determine the association between a circadian gene's polymorphism and PD symptoms progression. It also revealed that *TEF* might be considered a predictive marker of the progression of sleep disturbances and motor staging in PD. This advances our understanding of the genetic associations in PD and suggests circadian dysfunction may be involved in Parkinson's disease's pathophysiology.

Abbreviations

ADL, Activities of Daily Living; APR, annual progression rate; ARNTL, Aryl Hydrocarbon Receptor Nuclear Translocator Like (another name of BMAL1 gene); BMAL, brain and muscle ARNT-like protein; CLOCK, circadian locomotor output cycles kaput; CRY, cryptochrome; DEC, Differentiated embryonic chondrocyte; HAMA, 14-item Hamilton rating scale for Anxiety; HAMD, 24-item Hamilton rating scale for depression; H-Y, stage Hoehn-Yahr stage; LEDD, levodopa equivalent daily doses; MMSE, Mini-Mental State Examination; NMSQ, Parkinson's Disease Non-Motor Symptom Quest; NPAS2, neuronal PAS domain protein 2; NR1D1, Nuclear Receptor Subfamily 1 Group D Member 1; PD, Parkinson's disease; PDSS, Parkinson's Disease Seep Scale; PER, period; TEF, Thyrotroph embryonic factor; TIMELESS, Timeless Circadian Regulator; UPDRS-III, Unified Parkinson's Disease Rating Scale Part-III.

Author contributions

Study concept and design: Hua and Liu; Acquisition of data: Liu, Xu, Yu, Yao and Cui; Analysis and interpretation of data: Hua and Cui; Drafting of the manuscript: Hua; Critical revision of the manuscript for important intellectual content: Liu; Obtained funding: Liu; Administrative, technical, and material support: Chen.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Nanjing Medical University. Informed consent was obtained from each participant.

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Conflict of interest

The authors declare no competing interests.

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