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PERIOD3 (PER3) VNTR VARIANT ASSOCIATED WITH SEASONAL PATTERN AND FAMILY HISTORY IN BIPOLAR DISORDER

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SUMMARY

Background: *Dysregulation of circadian rhythms has been thought to be associated with psychiatric disorders such as bipolar disorder (BD) and depression. We aimed to evaluate the relationship between clinical specifiers of BD, mainly seasonal pattern (SP), and the variable number tandem repeat (VNTR) variant of the PERIOD3 (PER3) gene (rs57875989) in BD patients by comparing genotype distributions with healthy controls considering clinical parameters.*

Subjects and methods: *A sample of 98 BD patients and 97 healthy volunteers were included in the study. The Clinical Interview for DSM-IV Axis-I Disorders (SCID-I) was administered to all participants. The patients were evaluated with some scales (Sociodemographic and Clinical Data Form, The Young Mania Rating Scale (YMRS), the Hamilton Depression Rating Scale (HAM-D), and The Clinical Global Impression Scale (CGI)) in terms of clinical features and symptom severity. Blood samples were obtained from participants to isolate their DNA. PCR-RFLP was used to determine the PER3 gene variant.*

Results: *The PER3 genotype (4/4, 4/5, 5/5) distribution of BD was found to be significantly different from the control group. There was a statistically significant difference in the PER3 genotype distribution between BD patients with SP and BD patients without SP. Again, the PER3 allele (4, 5) distributions of BD patients with the SP were statistically different from the control group. The BD patients' PER3 genotype distributions with a family history of BD were significantly different from the BD patients without family history or control group.*

Conclusion: *It was found that the VNTR variant of the PER3 gene (rs57875989) may be associated with the SP and family history of BD as well as the BD itself. Further studies with the VNTR variant of the PER3 gene (rs57875989) in different ethnic populations are also required to determine these polymorphisms' exact role in BD.*

Key words: *bipolar disorder - PER3 – VNTR - seasonal pattern - family history*

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INTRODUCTION

Bipolar disorder (BD) causes some changes in circadian rhythms, and repetitive fluctuations of mood and sleep disruptions have suggested a potential dysfunction of the biological clock (Garbazza & Benedetti 2018). Furthermore, because BD is strongly familial with an estimated heritability of 60-85% (Smoller & Finn 2003), a growing interest in recognizing genetic risk factors has been supported by different researches studying the association between BD and some clock genes (Partonen 2016). Approximately one-fourth of patients with BD have depressive episodes with a seasonal pattern (SP) linked to more severe disease. Although BD's underlying genetic factors with SP remain mysterious, the clock genes seem to be potential candidates. It is critical to note that SP is thought to be heritable and, therefore, driven by genetic variants. Among genes, clock or melatonin associated genes are guessed as candidates (Geoffroy et al. 2015).

Among clock genes, PERIOD3 (PER3) shows a 54-nucleotide coding region repeating in 4 or 5 units, affecting sleep regulation and homeostatic replies to sleep deprivation in an average population (Sara Dallaspezia et al. 2016). BD is characterized by changes in the sleep-wake cycle. Because a variable number tandem repeat (VNTR) in the PER3 gene has been related to sleep disorders, PER3 gene variants might be crucial for the etiology and prognosis of BD (Karthikeyan et al. 2014). Since the association studies between the VNTR variant in the PER3 and BD are still debated, we choose in the present case-control study to investigate the impact of this variant on the susceptibility to BD. Thus, we hypothesized that the VNTR variant in the PER3 might be related to SP in BD. To our knowledge, this is the first study examining the relationship between the VNTR variant in the PER3 and clinical specifiers of BD in the Turkish population. This study aims to investigate the relationship between clinical specifiers of BD, especially SP and VNTR variant in the PER3 gene (rs57875989) in BD patients by comparing genotype distributions with healthy controls considering scale scores or clinical parameters.

SUBJECTS AND METHODS

Patient Selection

A sample of 98 patients with BD was followed consecutively in the Bakirkoy Mazhar Osman Mental Health and Neurology Training and Research Hospital outpa-

tient clinic for six months in 2018; additionally, 97 healthy volunteers have included in the study which was designed as a case-control study. The Clinical Research Ethics Committee of the Istanbul Faculty of Medicine approved the study. The patients of 18 to 65 years of age, of either gender, were literate, agreed on the participation in the study, diagnosed with a BD according to the SCID-I interview, had no other systemic/ neurological disease that may affect cognitive functions (dementia, epilepsy, Parkinson disease, head trauma accompanied by loss of consciousness) included in the study. We had excluded subjects who had mental retardation, neurodevelopmental disorders such as autism, a diagnosis of axis-1 disorder other than BD as a result of the SCID-I interview, a BD secondary to a general medical condition, dementia or brain damage.

Diagnostic Tools And Scales

The participants were informed in detail about the purpose, method, and procedures of the study, and written consent of all the participants was obtained. The interview was initiated by filling out data forms that included questions about clinical information such as sociodemographic characteristics, family history, disease history, and BD complaints. Afterward, the Structured Clinical Interview for DSM-IV Axis-I Disorders (SCID-I) (Çorapçıoğlu et al. 1999, First et al. 1997) was used to confirm the diagnosis according to DSM-IV-TR criteria, and the presence of any psychiatric diagnosis was decided as the basis for exclusion from the study in the healthy control group. To evaluate the severity and changes in manic or depressive conditions the Young Mania Rating Scale (YMRS) (Karadağ et al. 2001, Young et al. 1978) and the Hamilton Depression Rating Scale (HAM-D) (Akdemir et al. 1996, Hamilton 1960), to assess the severity of disease and response to treatment The Clinical Global Impression Scale (CGI) were administered to patients with BD (Guy 1976).

DNA Analyses

Blood samples were obtained from participants at the Istanbul Faculty of Medicine Laboratory of Medical Biology to isolate their DNA material. Functional 4 and 5 VNTR polymorphism in the coding region of the PER3 (rs57875989 4/5) (Geng et al. 2015) variant was genotyped by polymerase chain reaction (PCR) described previously and agarose gel electro electrophoresis. Polymerase chain reaction (PCR) was performed to amplify the exon 18 using the primers: upstream, 5'-CCTTGGTTGACCACAGGTAA-3' and downstream, 5'-CCACTACCTGATGCTGCTGA-3', and in 40 ml mixture containing 3.2 mL (2.5 mM) deoxyribonucleotide triphosphate, 3.2 mL (25 mM) Mg+2, 0.4 mL (5 U/mL) Taq polymerase, 4 mL 10_ buffer; 25.4 mL H_2O, 1 mL of each primer (10 mM) and 1.8 mL (50 ng/mL) DNA template.

Statistical Analysis

Statistical analysis was performed using IBM SPSS version 21.0 (IBM Corp. released 2012; Armonk, NY, USA). Descriptive statistics included mean, standard deviation, frequency, percentage. Pearson chi-square test was used for the comparison of discrete variables. The Shapiro Wilk test evaluated the suitability of continuous variables to normal distribution. Intergroup comparisons of continuous variables were performed by Kruskal Wallis and Mann Whitney U testing. Statistical significance was accepted as $p<0.05$ for the results of all analyses.

RESULTS

Ninety-eight patients with BD (58 female/ 40 male) were evaluated according to their clinical characteristics, and the scale scores are presented in Table 1.

Table 1. Sociodemographic Characteristics and The Scale Scores of BD Patients

Bipolar Disorder	Mean ± SD
Age	41.44±11.79
Age of Onset	25.57±8.75
Manic episode	4.26±4.80
Dep. episode	1.44±2.45
Total episode	5.71±5.33
HAM-D	11.30±7.50
YMRS	7.73±8.66
CGI-S	4.97±0.90
CGI-I	2.03±0.84

Abbreviations: SD - standard deviation; Dep - depressive; HAM-D - Hamilton depression rating scale; YMRS - young mania rating scale; CGI-S - clinical global impression scale-severity; CGI-I - clinical global impression scale-improvement

According to the PER3 genotype distribution, 28.6% (n=28) of the patients diagnosed with BD had 4/4, 58.2% (n=57) had 4/5 and 13.3% (n=13) had 5/5 genotypes. When the PER3 genotype (4/4, 4/5, 5/5) and allele frequency (4, 5) distributions of patients with BD were compared with the control group, the PER3 genotype distribution of BD was found to be significantly different from the control group (p=0.003) (Table 2). Comparing the PER3 genotype and allele frequency distributions between the two groups according to the presence of clinical specifiers (psychotic features, atypical features, SP, mixed features, rapid cycling, peripartum onset), in the BD patient group, there was a statistically significant difference in terms of SP between PER3 genotype distributions of two groups (p=0.028) (Table 3). When the PER3 genotype and the allele frequencies of BD patients with SP were compared with the control group, the PER3 allele frequency distributions of BD were significantly different from those of the control group (p=0.047) (Table 4).

Table 2. Comparison of genotype distributions of PER3 VNTR variants in BD patients with control group

VNTR	Control Group	BD	p*
PER3			*0.003*
4/4	41 (42.3%)	28 (28.6%)	
4/5	42 (43.3%)	57 (58.2%)	
5/5	14 (14.4%)	13 (13.2%)	
PER3 (allele)			0.205
4	124 (63.9%)	113 (57.6%)	
5	70 (36.1%)	83 (42.4%)	

* Pearson chi-square

Table 3. Comparison of genotype distributions of PER3 VNTR variants in BD patients due to the seasonal pattern

	Seasonal Pattern		
VNTR	no	yes	p*
PER3			*0.028*
4/4	17 (30.4%)	11 (26.2%)	
4/5	36 (64.3%)	21 (50%)	
5/5	3 (5.4%)	10 (23.8%)	
PER3 (allele)			0.113
4	70 (62.5%)	43 (51.2%)	
5	42 (37.5%)	41 (48.8%)	

* Pearson chi-square

Table 4. Comparison of genotype distributions of PER3 VNTR variants of BD Patients with Seasonal Pattern to the Control Group

VNTR	Control Group	Seasonal Pattern	p*
PER3			0.149
4/4	41 (42.3%)	11 (26.2%)	
4/5	42 (43.3%)	21 (50%)	
5/5	14 (14.4%)	10 (23.8%)	
PER3 (allele)			*0.047*
4	124 (63.9%)	43 (51.2%)	
5	70 (36.1%)	41 (48.8%)	

* Pearson chi-square

When the PER3 genotype and allele frequency distributions of BD patients with a family history of BD were compared with the BD patients without a family history, there was a statistically significant difference between PER3 genotype distributions of two groups (p=0.030) (Table 5). When the PER3 genotype and the allele frequencies of BD patients with a family history of BD were compared with the control group, the PER3 genotype distributions of BD patients with a family history were significantly different from the healthy control group (p=0.004) (Table 6).

Comparing mean of scale scores (HAM-D, YMRS, CGI-S, CGI-I) and clinical parameters (number of manic episodes, depressive episodes, total episodes, age of onset, duration of disease and number of hospitalizetions) regarding the PER3 genotype, and the allele frequencies of BD patients, there was no statistically significant difference between PER3 genotype or the allele groups due to the mean of scale scores (p>0.05)

Table 5. Comparison of genotype distributions of PER3 VNTR variants in BD patients due to the presence of a family history of BD

	Family History of BD		
VNTR	no	yes	p*
PER3			*0.030*
4/4	21 (35.6%)	7 (17.9%)	
4/5	28 (47.5%)	29 (74.4%)	
5/5	10 (16.9%)	3 (7.7%)	
PER3 (allele)			0.561
4	70 (59.3%)	43 (55.1%)	
5	48 (40.7%)	35 (44.9%)	

* Pearson chi-square

Table 6. Comparison of genotype distributions of PER3 VNTR variants of BD Patients with Family History of BD to the Control Group

VNTR	Control Group	Family History of BD	p*
PER3			*0.004*
4/4	41 (42.3%)	7 (17.9%)	
4/5	42 (43.3%)	29 (74.4%)	
5/5	14 (14.4%)	3 (7.7%)	
PER3 (allele)			0.178
4	124 (63.9%)	43 (55.1%)	
5	70 (36.1%)	35 (44.9%)	

* Pearson chi-square

(data not shown). Again, when PER3 genotype and the allele frequencies of BD patients in the remission under lithium were compared with BD patients in remission with a non-lithium mood stabilizer, there was no statistically significant difference between PER3 genotype or the allele frequency distributions of two groups (p>0.05) (data not shown).

DISCUSSION

Although the PER3 gene contributes to the pathogenesis of psychiatric disorders is still unclear, a probable explanation for mood disorders is that in affected people, the circadian clock may perform weak adjustability to different seasons. Another possible way a polymorphism of PER3 could affect mood is by altering sleep (Karthikeyan et al. 2014). Genetic association researches have reported some positive results of the PER3 gene and BD plus specific clinical sub-phenotypes, although there have been preceding studies that detected no evidence of the relation between the PER3 gene and BD (Benedetti et al. 2008, Dallaspezia et al. 2011, Karthikeyan et al. 2014, Mansour et al. 2009, Mansour et al. 2006, Nievergelt et al. 2006). In the present study, whereas the distribution of the PER3 genotype of the BD patients with SP was significantly different from BD patients without SP, the PER3 allele frequency distributions of BD with SP was found to be significantly different from the control group. Although seasonality and BD's genetic background has not yet been fully known, numerous studies suggest that

both situations have an inherited component. A polymorphism in the CLOCK gene that is known to affect diurnal preference in ordinary cases, also affects BD patients, leading to worsening of insomnia, higher evening activity, and prolonged sleep onset (Garbazza & Benedetti 2018). A VNTR of the PER3 gene was published to influence the age of onset (Benedetti et al. 2008) and a postpartum depressive onset of the BD (S Dallaspezia et al. 2011). PER3 was associated with an increased choice for the evening hours in daily activity among patients diagnosed with BD, too (Kripke et al. 2009). Again, Johansson et al. published circadian clock-related variants in susceptibility to SAD and diurnal preference. PER3 647 Val/Gly was associated with self-reported morningness-eveningness scores, with higher scores found in individuals with at least one glycine allele (Johansson et al. 2003).

The PER3 genotype distributions of BD patients with a family history of BD were found to be significantly different from BD patients without a family history or the control group in our study. Different earlier studies suggest that the dysfunction of circadian rhythms might increase susceptibility to BD. Although recently, two researcher groups have reported that ARNTL and PER3 show evocative evidence for association with BD families (Mansour et al. 2006; Nievergelt et al. 2006), Shi et al. have not detected similar association signals (Shi et al. 2008). Johansson et al. reported that a significant difference between patients and controls for neuronal PAS domain protein 2 (NPAS2) 471 Leu/Ser (a functional analog of CLOCK), indicating a recessive effect of the leucine allele on BD susceptibility (Johansson et al. 2003). The recent findings also show the association of the PER3 gene variants with improvement in depressive symptoms, with a family history of suicidal behavior, and with sleep disruptions in various examples of BD patients (Sara Dallaspezia et al. 2016; Dmitrzak-Weglarz et al. 2015; Pawlak et al. 2015). In the present study, when PER3 genotype distributions of BD patients in the remission under lithium were compared with BD patients in remission with a non-lithium mood stabilizer, there was no statistically significant difference between genotype distributions of two groups. In contrast to our study, Osland et al. have recently shown that lithium alters the expression of the PER3 gene in laboratory cultures of fibroblasts; therefore, lithium significantly reduced the expression of PER3 (Osland et al. 2011).

While the strengths of this study lie in the fact that it is the first report examining potential associations between the SP in Turkish BD patients and the VNTR polymorphic region of PER3 (rs57875989) if the study's limitations should be considered, results must be read with attention as a comparatively small sample size can restrict the statistical power. Again this research must be studied in different ethnic groups with a larger individual size to verify outcomes. Only the VNTR polymerphic region of PER3 (rs57875989) was examined in our study, but it was not possible to know the other coding region variants.

The variants of the core clock genes and the light-dark cycle's seasonal changes heavily impact patients' mood disorders. The association between biological clock and behavior suggests these patients' specific sensibility to psychobiological factors that can modify the circadian timing system, such as environmental synchronizers (light phase and seasonal photoperiod changes), and conditions directly disturbing the clock (sleep deprivation, or phase advance/delay). These approaches can trigger or worsen the severity level of affective disorders or be successfully used to treat manic and depressive episodes.

CONCLUSION

In conclusion, we found that a VNTR variant in the PER3 gene has been related to SP and family history of BD in patients diagnosed with BD. Future studies in this area may show promise towards a greater understanding of SP's etiopathology in BD, and confirmation of the present findings with other coding region variants in different ethnic populations covering more extensive regions will contribute to a better understanding of the relationship between PER3 and SP of BD.

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Statement of interest

All authors declare not to have any conflicts of interest that might be interpreted as influencing the manuscript's content.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the relevant national and institutional committees' ethical standards on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008 (Williams 2008).

Conflict of interest: None to declare.

Contribution of individual authors:

Hasan Mervan Aytac, Mustafa Pehlivan & Sacide Pehlivan developed the hypothesis designed for the study.

Hasan Mervan Aytac is responsible for the formulation of overarching research goals and aims, integrity of the data, and data analysis accuracy.

Hasan Mervan Aytac, Yasemin Oyaci & Sacide Pehlivan are responsible provisions of study materials and laboratory samples.

All authors discussed the results and contributed to the final manuscript.

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