Is There a Link between Circadian Clock Protein PERIOD 3 (PER3) (*rs57875989*) Variant and the Severity of COVID-19 Infection?

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[Abstract] Objective: Corona Virus Disease-2019 (COVID-19) has been among the major infectious events of the century. In today's literature where COVID-19 and host factor effects are frequently examined, we aimed to examine another factor: Circadian Clock Protein PERIOD 3 (PER3). There is a significant correlation between PER3 gene polymorphism and circadian rhythm disturbances and immune system dysregulation. **Methods:** In our study, we recruited 200 patients diagnosed with COVID-19 in our hospital between April–June 2020, and 100 volunteers without known comorbidities to create a healthy control group. After comparing the initial gene polymorphisms of the patients with healthy controls, three separate clinical subgroups were formed. Gene polymorphism distribution and statistical significance were examined in the formed patient groups. **Results:** No significant difference was found between the patient group and the healthy controls (P>0.05, for all). When patients were divided into two separate clinical subgroups as exitus/alive according to their last condition during their 28-day follow-up, the 4R/5R genotype was significantly more common in patients with a mortal course (P=0.007). The PER3 4R/5R genotype was found at a significantly higher rate in the group of patients with the need for intensive care (P=0.034). **Conclusion:** The 4R/5R genotype may be associated with the need for intensive care and mortality in COVID-19 patients. These important results will be a guide for future studies.
Key words: corona virus disease 2019; circadian rhythm; PER3; prognosis

Corona virus disease 2019 (COVID-19), a pandemic that has infected many people since the first case emerged, has been among the major infectious events of the century[1]. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2) virus emerges as a resulting agent, and specifically affects patients aged 65 and over with comorbid burdens[2–4]. In particular, the patient population with hypertension, chronic respiratory and heart diseases, diabetes mellitus, renal failure and malignancy is defined as the most severely affected group[5].

In today's literature where COVID-19 and host factor effects are frequently examined. We aimed to examine another factor in our study, namely Circadian Clock Protein PERIOD 3 (rs57875989) (PER3). The circadian clock regulates both physiological and biological behavior according to the daily light and dark cycle. It is managed by the main center in mammalian physiology located in the upper chiasmatic nucleus (SCN)[6, 7]. This center interacts with the help of a complex neuro-humaral network through photic signals from the retina, daily rhythms in temperature, diet, and social stimuli. The circadian rhythm is controlled by the circadian rhythm pathway genes. At a molecular level, the mammalian circadian clock mechanism involves interlocked transcription-translation feedback loops driven by a set of genes, including CLOCK, BMAL1, NPAS2, period genes (PER1, PER2, and PER3), cryptochrome genes (CRY1 and CRY2), NR1D1 and NR1D2. Changes in the expression of these genes have been found to affect immune functions, immune cells, and cytokine plasma levels[6, 7]. The immune system is a physiological structure with a circadian rhythm of 24 h. Most of the immune system cells express circadian rhythm genes and work in a 24-h

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rhythm. Many mechanisms such as cellular function, cytokine synthesis, cytolytic functions are affected by circadian gene expressions. Therefore, the mutation or dysregulation of PER3 or other clock genes has been associated with many cancers[8].

PER3, located on chromosome 1p36.23, contains a polymorphic domain expressing 4 or 5 copies of a 54-bp tandem repeat sequence (variable number tandem repeat, VNTR). This variation results in an insertion/deletion of 18 amino acids, and it has been linked with sleep and mood disorders as well as circadian preference in humans[8]. There is a significant correlation between PER3 gene polymorphism and circadian rhythm disturbances, immune system dysregulation, and cytokine release differences[6–9]. It has been a guide in terms of both cancer biogenesis and chronic inflammatory diseases in different studies. The resulting immune dysregulation is thought to contribute to both chronic inflammatory processes and cancer development[10]. In the study of Guess *et al* the interaction of chronic inflammation-associated tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin-1 (IL-1), interleukin-6 (IL-6) and PER3 polymorphism was demonstrated. Overall, patients with 4/5 or 5/5 PER3 gene polymorphisms were shown to have higher IL-6 levels than patients with 4/4 polymorphisms[8]. In another study, 4/5 or 5/5 PER3 genotypes were shown to be more common in premenopausal breast cancer[11]. Therefore, PER3 gene polymorphisms, precancerous processes and the relationship between autoinflammatory and autoimmune disease constitute a current research area.

In our study, we aimed to show the distribution and clinical efficacy of PER3 gene polymorphisms in COVID-19 patients.

1 MATERIALS AND METHODS

1.1 Study Design

In our study, 200 patients diagnosed with COVID-19 in pandemic clinics of Istanbul University, Faculty of Medicine, between 1 April and 1 June 2020, as well as 100 volunteers without known comorbidities were recruited to participate in this study. In addition to demographic information such as age and gender of the patients, comorbidities, clinical findings and initial symptoms, physical examination findings and initial laboratory findings were recorded. After comparing the initial gene polymorphisms of the patients with the healthy control group, three separate clinical subgroups were formed. Gene polymorphism distribution and statistical significance were examined in the following patient groups: (1) severe/mild infection, (2) exitus/alive during the 28-day follow-up, (3) presence of the need for intensive care/being only inpatient.

In the distinction between severe and mild clinical subgroups, the patient was placed in the severe category in the presence of any of the following clinical and laboratory parameters: respiratory rate more than 30 breaths/min, presence of dyspnea or peripheral oxygen saturation <90% or nasal oxygen requirement more than 5 L per min or PaO_2/FiO_2 ≤300 or lactate >2 mmol/L, presence of hypotension (if systolic blood pressure 40 mmHg lower than normal systolic blood pressure) or heart rate >100 beats/min, presence of renal, hepatic, hematological (thrombocytopenia) or cerebral (confusion) dysfunction, presence of sepsis or septic shock or skin findings such as cutis marmorata and peripheral coldness, presence of mild/severe pneumonia (bilateral infiltration and/or the presence of multiple ground glass opacities), or presence of the need for anti-cytokine therapy, and/or the presence of broad-spectrum antibacterial therapy. The exitus/alive during the 28-day follow-up subgroups were designated as patients who were exitus or alive during the 28-day follow-up period. The need for intensive care/being only inpatient groups was created during their 28-day follow-up. Two separate groups were formed as those who needed intensive care follow-up at any period during hospitalization and those who were treated as inpatient.

1.2 Case Selection

Ethical committee approval was received (Approval date and number: 21/05/2020-84539; Istanbul University, Faculty of Medicine) and the patients gave informed consent before the beginning of the study. The experimental procedures were based on the Declaration of Helsinki and relevant institutional regulations.

Patients whose nasopharyngeal swab polymerase chain reaction (PCR) result were positive or whose computed tomography (CT) were compatible with typical COVID-19 involvement were included in the study.

1.3 Isolation Method

DNA isolation from peripheral blood leukocytes of COVID-19 patients and healthy controls was performed using the saline precipitation method[12]. PER3 gene polymorphism genotypes were analyzed by the PCR method[13]. The institutional clinical research ethics committee approved the protocol (21/05/2020-84539).

1.4 Statistical Analysis

We used IBM SPSS version 21.0 (IBM Corp. USA) for all statistical analyses. Descriptive statistics included mean, standard deviation, median, minimum, and maximum for the continuous variables after assessing the normality, frequency, and percentage for the nominal variables. A Pearson Chi-square test or Fisher's exact test was used to compare discrete variables, and Bonferroni correction was used in pairwise comparisons to determine which group or

groups exhibited statistically significant results. We performed multivariate binary logistic regression analyses to determine the association between different variants of genes with study parameters. The results were adjusted for age and sex. Consequently, we report the odds ratio (OR) and 95% confidence interval (CI) for the association of gene variants with the study parameters. The Hardy Weinberg Equilibrium (HWE) was calculated using the De-Finetti program (online HWE and Association Testing-Institut für Hu-mangenetik, Germany). $P<0.05$ was considered statistically significant.

2 RESULTS

Of the 200 patients included in the study, 87 were female (43.5%), and 113 were male (56.5%). The median age of the patients was 49 years (range: 19–92). The most common comorbidity was hypertension (54 patients, 26%). Initial physical examination findings, laboratory data and treatment preferences are shown in table 1. During the 28-day follow-up, mortality was 4.5% with 9 patients, and the rate of need for intensive care was 8% with 16 patients.

2.1 Distribution of Genotypes of PER3 Between Patients and Healthy Controls

Statistical analysis was performed in terms of the distribution of PER3 genotypes and alleles between the patient group and healthy controls. When the PER3 gene 4R/4R, 4R/5R, 5R/5R genotypes and 4R, 5R alleles were examined, no significant differences were found between the patient group and the healthy controls ($P>0.05$, for all) (table 2).

2.2 Distribution of Genotypes of PER3 among Clinical Subgroups

Statistical analysis was expanded by dividing

Table 1 Clinical features, laboratory results and treatment preferences of patients

	COVID-19 [*n*=200 (%)]	Median		COVID-19 [*n*=200 (%)]	Median
Age (years)		49 (19–92)	Laboratory		
Gender			Hemoglobin (g/dL)		13.1 (6.3–16.8)
Female/Male	87/113 (43.5/56.5)		Leukocyte (/mm^3)		7115 (220–28 300)
Comorbidities			Thrombocyte (×10^3/mm^3)		237 (66–576)
Hypertension	54 (26)		Lymphocyte (/mm^3)		1340 (290–4500)
Diabetes mellitus	31 (15.5)		Lymphocyte (<800/mm^3)	42 (21)	
COPD	22 (116)		Eosinophil (/mm^3)		30 (10–2780)
CAD	14 (7.0)		Urea (mg/dL)		14 (5–107)
CHF	4 (28)		Creatinine (mg/dL)		0.8 (0.4–6)
Solid malignancy	22 (112)		Na		139 (113–172)
Hematological malignancy	5 (2.5)		K		4.4 (3–6)
Clinical findings			Glucose		108 (68–496)
Severe/mild	94/106 (47/53)		AST		22 (10–409)
Cough	125 (62.5)	2 (0–20)	ALT		22 (2–493)
Fever	107 (53.5)	1 (0–14)	GGT		22 (5–744)
Myalgia	104 (52)	1 (0–15)	ALP		73 (33–400)
Dyspnoea	62 (31)	1 (0–15)	LDH (IU/L)		205 (78–731)
Nausea, vomiting	23 (11.5)	1 (0–15)	T. Protein (g/L)		7.4 (5–9)
Diarrhea	16 (8.1)		Albumin (g/L)		4.0 (2–5)
Anosmia	7 (3.5)		C-reactive protein (mg/dL)		20 (1–363)
Sputum	1 (0.4)		Procalcitonin		0.06 (0.20–50.0)
Initial examination			Ferritin		179 (6–6656)
Fever	94/106 (47/53)	36.7 (36–40)	D-Dimer		620 (190–20 000)
SpO_2		97 (80–100)	proBNP		58 (5–35 000)
Systolic blood pressure		128 (90–200)	Troponin		4 (3–848)
Diastolic blood pressure		75 (50–100)	Fibrinogen		437 (204–1053)
Heart rate (/min)		93 (60–160)	INR		0.9 (0.8–3.8)
Respiratory rate (/min)		16 (12–40)	APTT		28 (21–53)
pH		7.41 (7–8)	Treatment		
pO_2		63 (35–86)	Favipravir	61 (30.5)	
pCO_2		38 (23–58)	Tocilizumab	11 (5.5)	
HCO_3		24 (15–30)	28-days mortality	9 (4.5)	
Lactate		1.40 (1–5)	Need for intensive care	16 (8)	

COPD: chronic obstructive pulmonary disease, CAD: coronary artery disease, CHF: congestive heart failure, SpO_2: capillary oxygen saturation, pO_2: partial pressure of oxygen, pCO_2: partial pressure of carbon dioxide, HCO_3: bicarbonate, Na: natrium, K: potassium, AST: aspartate aminotransferase, ALT: alanine transaminase, GGT: gamma-glutamyl transferase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase, T.Protein: total protein, proBNP: pro brain natriuretic peptide, INR: international normalized ratio, aPTT: activated partial thromboplastin time

the patients into the three subgroups of severe/mild infection (1), exitus/alive during the 28-day follow-up (2), and presence of the need for intensive care/being inpatient (3). When patients were divided into two separate clinical subgroups as severe and mild, no significant differences were found in or group in terms of the distribution of PER3 genotypes (P>0.05, for all) (table 3). When patients were divided into two separate clinical subgroups as exitus/alive according to their last condition during their 28-day follow-up, the 4R/5R genotype was significantly more common in patients with a mortal course (P=0.007) (table 4). When patients were divided into two separate clinical subgroups as those with intensive care unit follow-up or not during their hospitalization, the rate of PER3 4R/5R genotype was found to be significantly higher in the group of patients with the need for intensive care (P=0.034) (table 5).

3 DISCUSSION

COVID-19 and host factor relationship has become a very important research topic recently. Specifically, studies on genetic characteristics, disease course, and mortality are quite extensive. In a recently published study, Medetalibeyoglu *et al*[14] studied the course of mannose-binding lectin 2 (MBL2) and COVID-19 and found that the B variant of this gene was associated with lower MBL 2 levels and more severe disease. Our study is the first to study the relationship between clock genes and the course of COVID-19.

Many studies have shown that clock gene-related sleep disorders increase inflammatory mediators[15, 16]. In particular, the increase in TNF-α, IFN-γ, IL-6 and IL-1 is associated with both chronic inflammatory diseases and carcinogenesis. In the study of Guess *et al*. based on IL-6 and PER3 polymorphism, IL-6 levels of patients with 4/5 and 5/5 genotypes were higher than patients with the 4/4 genotype. In another study from 2018, there was a significant correlation between high PER3 expression and increased inflammatory monocytes and CD16+ natural killer (NK) cells[17]. In our study, patients with the COVID-19 4R/5R genotype had a significantly higher need for intensive care and

Table 2 Comparison of genotype and allele frequencies between patients and healthy controls

	Genotype	COVID-19 patients [n=200 (%)]	Healthy control [n=100 (%)]	OR Exp (B)	95% CI	P^*
Genotypes						
PER3	4R/4R	79 (39.5)	39 (39)	1.223*	0.589–2.538*	0.590*
	4R/5R	94 (47)	45 (45)	1.262*	0.617–2.583*	0.524*
	5R/5R	27 (13.5)	16 (16)	0.776&	0.401–1.502&	0.491&
Allele						
	4R	252 (63)	123 (61.5)			
	5R	148 (37)	77 (38.5)	0.938&	0.661–1.331&	0.721&
HWEp		0.908	0.618			

*: OR (95%CI) was adjusted by age and sex; &Fisher's Exact Test

Table 3 Distribution of PER3 genotypes between clinical subgroups: severe or mild infection

	Genotype	Severe [n=94 (%)]	Mild [n=106 (%)]	OR Exp (B)	95% CI	P^*
Genotypes						
PER3	4R/4R	34 (36.2)	45 (2.5)	1.019*	0.408–3.012*	0.839*
	4R/5R	48 (51.1)	46 (43.4)	0.649*	0.243–17.37*	0.390*
	5R/5R	12 (12.7)	15 (15.1)	0.888&	0.393–2.007&	0.838&

*: OR (95%CI) was adjusted by age and sex; &Fisher's Exact Test

Table 4 Distribution of PER3 genotypes between clinical subgroups: exitus or alive during the 28 day follow-up

	Genotype	28-days mortality [n=200 (%)]	Alive [n=191 (%)]	OR Exp (B)	95% CI	P^*
Genotypes						
PER3	4R/4R	1 (11.1)	78 (40.8)	6.133&	0.761–49.394&	0.092&
	4R/5R	8 (88.9)	86 (45.0)	0.090&	0.011–0.724&	0.007&
	5R/5R	0 (0)	27 (14.2)	1.165&	1.100–1.234&	0.612&

*: OR (95%CI) was adjusted by age and sex; &Fisher's Exact Test

Table 5 Distribution of PER3 genotypes between clinical subgroups: need for intensive care or being inpatient

	Genotype	Need for intensive care [n=16 (%)]	Inpatient [n=184 (%)]	OR Exp (B)	95% CI	P^*
Genotypes						
PER3	4R/4R	4 (15)	75 (40.7)	2.064&	0.641–6.645&	0.290&
	4R/5R	12 (75)	82 (44.6)	0.268&	0.083–0.862&	0.034&
	5R/5R	0 (0)	27 (14.7)	1.172&	1.004–1.244&	0.136&

*: OR (95%CI) was adjusted by age and sex; &Fisher's Exact Test

a higher 28-day mortality. This is a unique result that should be emphasized.

There are many literature reports in which polymorphisms of biological circadian rhythm are associated with carcigonenesis[11, 18–21]. The relationship between clock gene polymorphisms and vascular endothelial growth factor (VEGF) has been examined in these studies, and it has been revealed that the increased VEGF level contributes to carcinogenesis when circadian rhythm is affected[8, 15]. The relationship between COVID-19 and VEGF is also a very current area of discussion. Aside from studies showing an increase in VEGF in the course of COVID-19[22, 23], there are also studies in which VEGF is used as a biomarker in terms of severe patient group and disease progression[24]. In this context, it is possible to say that another point which might be related to our study results is the relationship between the 4R/5R genotype and VEGF, which is associated with high PER3 expression.

Besides clock genes and cancer biology, the autoimmune/auto-inflammatory disease relationship is another remarkable area of research. In the study of Helvacı *et al*[10], the role of PER3 *rs2797685* polymorphism in autoimmune thyroiditis was investigated and found to be associated with Graves' disease. In another study conducted in inflammatory bowel diseases, the same polymorphism was shown to be associated with aggressive Crohn's disease[25]. In terms of the current literature where the cytokine storm and inflammatory response devel-oping during the COVID-19 process constitutes a current and new field of study, the relationship between PER3 polymorphism and inflammatory response has an important place. Although significant statistical data could not be obtained in terms of severe-mild disease, the 4R/5R genotype emerges as a very important finding for the group that has a deadly course and needs intensive care.

Our study has important limitations that should be noted. When divided into clinical subgroups, the statistical analysis may have been biased due to the decreased number of patients, and no significant results were obtained in terms of the 5R/5R genotype. Measuring the inflammatory cytokine levels simultaneously with gene polymorphisms could also contribute to the emergence of more meaningful results.

In conclusion, in our study the PER3 gene polymorphism, one of the COVID-19 host factors, was examined, and it was shown that the 4R/5R genotype may be associated with the need for intensive care and mortality in COVID-19 patients. These important results will be a guide for future studies.

Acknowledgements

We respectfully remember all the colleagues we lost in the COVID-19 fight.

Conflict of Interest Statement

The authors declare that they have no competing interests.

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(Received May 14, 2021; accepted Aug. 3, 2021)