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Original Article

Association of Circadian Locomotor Output Cycles Kaput rs1801260 and Hypocretin Receptor 1 rs2271933 Polymorphisms in Patients with Chronic Migraine and Sleep Disorder

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ABSTRACT

Background: Insomnia and excessive daytime sleepiness (EDS) are frequently reported as sleep disorders, especially in patients with chronic migraine (CM). The main drive of conducting a study on the relationship of genes that regulate circadian rhythm is that migraine contains a robust genetic background, and it is known that migraine attacks have circadian characteristics. This study aims to evaluate the relationship of circadian locomotor output cycles kaput (CLOCK) rs1801260 and hypocretin receptor 1 (HCRTR1) rs2271933 gene-related circadian rhythm of patients with CM and sleep disorders. **Methods:** The present study was designed prospectively in the Mersin University Neurology Clinic. Volunteer individuals aged 18–75 were included in the study in three groups. Each group was made up of 100 individuals. The first group was created among the patients diagnosed with CM. The sleep disorders of patients were evaluated by Epworth Sleep Scale and Pittsburgh Sleep Quality Scale. The second group healthy first-degree relatives of patients. Finally, the third group was formed by the other healthy volunteers who did not have blood relations with the patients. Genotyping was performed for the CLOCK rs1801260 and HCRTR1 rs2271933 genes. **Results:** Eighty-seven (87%) of the patients, 56 (56%) of the control group 1, and 50 (50%) of the control group 2 consisted of female patients. Their mean ages were 41.1 ± 11.5, 45.7 ± 15.2, and 35.9 ± 10. EDS was detected in 27% of the patients, and poor sleep quality was detected in 67%. About 21% of the patients were found to be suffering from both EDS and poor sleep quality. The CLOCK rs1801260 AG genotype was 6.71 times higher than the AA genotype in the migraine patient group with EDS compared to the second control group (odds ratio [OR]: 6.71, 95% confidence interval [CI]: 0.819–54.992, $P = 0.076$). The GG genotype, according to the AA genotype, also was found 2.87 times higher in this group (OR = 2.87, 95% CI: 0.336–24.566, $P = 0.335$). In the group of patients with CM and insomnia, the CLOCK rs1801260 AG genotype was 17.763 times higher than the AA genotype compared to the second control (OR = 17.763, 95% CI: 2.242–140.740, $P = 0.006$). **Conclusion:** When CM patients were compared with control groups, CLOCK rs1801260 gene AG genotype was associated with both insomnia and EDS. However, there was no significant relationship between patients and control groups regarding the HCRTR1 rs2271933 gene.

KEYWORDS: *Chronic migraine, circadian locomotor output cycles kaput rs1801260, excessive daytime sleepiness, hypocretin receptor 1 rs2271933, insomnia*

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Introduction

Migraine is a multifactorial complex disorder that displays a sheer genetic component (up to 50%).[1] The population-based family and twin studies have demonstrated that "the tendency to suffer from migraine" increases when a first-degree relative has migraine.[2-4] According to a study, a family history of migraine was found significantly more often in the chronic migraine (CM) than in the episodic migraine (EM) group.[5]

Migraine attacks may be provoked by environmental factors such as stress, bright light, sleep disturbances, physical effort, food and drinks, and menses.[6,7] The interconnection between migraine (particularly CM) and sleep disorders has been the core issue of various clinical and population-based researches. In a recent study, CM patients frequently report different insomnia symptoms, for example, prolonged sleep latency period, lessening total sleep time, lack of refreshment after sleep, poor sleep quality, and excessive daytime sleepiness (EDS).[8]

Considering the relationship between migraine and sleep disorders, it is eminent to associate migraine periodicity with the circadian rhythm.[9] In a study of the chronobiology of 3582 migraine attacks in 1698 adults registered in the Glaxo-Wellcome database, 48% of migraine attacks occurred between 4 and 9 am. While this result gives a clear idea of the circadian model, it is a sign that there are underlying anatomical and neurochemical potentials.[10]

The circadian rhythm comprises a 24-h physiological cycle, inclusive of glucose homeostasis, fat metabolism, thermoregulation, hormone secretion, and sleep/wake patterns in mammals, which is exclusively vital for sleep behavior.[11,12] An endogenous circadian locomotor output cycles kaput (CLOCK) that is anatomically located in the suprachiasmatic nucleus (SCN) of the hypothalamus controls the circadian rhythms. While this control is primarily synchronized by retinal solar light input, the SCN transmits signals via direct and indirect projections in the brain to synchronize oscillators in different tissues.[13]

The first gene identified with the circadian rhythm was called CLOCK, which encodes for the CLOCK protein.[14,15] Brain and muscle-Arnt-like protein gene (BMAL1) (or Aryl Hydrocarbon Receptor Nuclear Translocator gene), PERIOD (PER1, PER2, and PER3), and cryptochrome 1 and 2 (CRY1 and CRY2) are additional genes that were incorporated into the sleep–wake cycle.[16] CLOCK 3111C/T single-nucleotide polymorphism (SNP) rs1801260 is the first reported human CLOCK polymorphism that has an impact on diurnal preference. In several studies, an SNP in CLOCK rs1801260 has been explored and has been linked with sleep abnormalities such as evening preference, sleep phase delay, and insomnia.[17-19]

Another gene responsible for sleep–wake cycle regulation is the hypocretin (HCRT) gene.[20,21] HCRT gene that encodes HCRT was firstly identified in the hypothalamus. This gene is also known as orexin (OX) and provides a neuropeptide expression in various brain regions.[22] There are two isoforms, HCRT 1 (OX A) and HCRT 2 (OX B), and their receptors, OX1R and OX2R, are G-protein-coupled receptors.[21,23] These peptides produced from prepro-OX perform diverse functions such as energy metabolism, cardiovascular function, and hormone homeostasis in emotional and autonomic processes related to stress and sleep–wake behaviors.[20,24] These orexinergic neurons transmit both stimulating and inhibiting signals to the neural axis comprised the cortex, hypothalamus, brain stem, spinal cord, and presympathetic ganglionic neurons.[21,23,25] Premonitory symptoms preceding a migraine aura and a migraine attack such as yawning, mood change, fatigue, excessive sleepiness, and hunger demonstrate an involvement of different hypothalamic nuclei that can be linked with the HCRT functions. Some of the aforementioned functions regulated by HCRTs are notably impaired in patients with migraine.[26-28] Polymorphisms in the HCRT receptor 1 gene (HCRTR1) may be the risk factors in migraine pathophysiology due to their ability to affect concentrations of HCRT-1.[29] The HCRTR1 G1222A (rs2271933) polymorphism is a missense variant in exon 7. It leads to the substitution of isoleucine by valine at position 408 (Ile408Val). This polymorphism is located in the cytoplasmic tail of the receptor, and it is likely to change the signal transduction.[30] In several studies, the relationship between migraine and HCRTR1 G1222 polymorphism was analyzed.[28,29] Some sleep disorders such as secondary hypersomnia and periodic hypersomnia were found to be associated with alterations in HCRT neurotransmission.[31,32] In another study, OX-A levels were considerably on the rise in the patients with insomnia disorder, indicating an association between OX-A and insomnia.[33]

No genetic studies – to the best of our knowledge – have been conducted to focus on the relationship between CM and sleep disorders. It is essential to apprehend the genetic basis of CM and sleep disorders as it allows us to perceive the pathogenesis of such disorders and to develop new treatment models. For this purpose, we hypothesized that there is a relationship of CLOCK rs1801260 and HCRTR1 rs2271933 gene polymorphisms between patients with CM, sleep problems and the other factors that cause sleep disorders.

Methods

Design of the study and questionnaires

The case–control study was conducted through a database prospectively. The volunteer patients as well as the healthy people who were admitted to the Neurology Clinic of Mersin University between January 01, 2000, and February 28, 2018, took part in this study. All subjects provided written informed consent forms in accordance with the Declaration of Helsinki. Overall, 300 people were included and divided into three equal groups as patients (100), first control group (100), and second control group (100) in the study. The sample size was based on our previous experience with the design. The patients who were diagnosed with CM and complaining about sleep disorders were selected for the study. The patients aged 18–75 years were diagnosed with CM according to the International Classification of Headache Disorders (ICHD)-III criteria[34] and recorded in the www.epikriz.com database. The first control group comprised first-degree healthy relatives of patients. The reason for this selection was the genetic association of migraine, which was observed at a higher rate, especially in first-degree relatives. The second control group was composed of other healthy volunteers who did not have blood bound with the patients. This methodology was used to evaluate the differentiation of environmental factors and genetic relationship in CM patients. In addition, to the best of our knowledge, these two control groups did not take part in such a study before. The control groups were compatible to the patient group in terms of age, sex, and number. First, the patients were asked whether they used sleeping pills or not. Patients using sleeping pills were not included in the study and no questionnaires were applied. Patients' sleep disturbances were evaluated with the Epworth Sleep Scale (ESS)[35] and the Pittsburgh Sleep Quality Index (PSQI)[36] whose reliability and validity checks were done in the Turkish version. According to the tests, 26 CM patients without sleep disorders were excluded from the study. The questionnaires were designed to inquire about their tendency of dozing off or falling asleep while engaging in different activities, habitual bedtimes, falling asleep times, wake-up times, and frequency of specific sleep-related problems. Aforementioned questionnaires included a set of multiple-choice questions about the frequency and severity of diverse sleep symptoms. Thus, the patients' tendencies to insomnia and/or EDS were evaluated. In addition, some factors that may affect sleep characteristics in the participants such as shift work, smoking, and alcohol consumption were questioned. There were no missing data in the questionnaires that were applied to the patient and control groups of the study. At this study, the ethics committee approval was received from Mersin University Rectorate, Clinical Research Ethics Committee, and the aforementioned study was supported by Mersin University Faculty of Medicine, Individual Research Project (Project No: 2018-2-AP4-2932).

Genotyping

Circadian locomotor output cycles kaput and hypocretin receptor 1 polymorphisms

Genomic DNA was extracted from 10 ml of EDTA anticoagulated whole blood using The PureLink™ Genomic DNA Mini Kit (Thermo Cat No: K182002, USA). The polymorphisms of CLOCK and HCRTR1 genes were genotyped using TaqMan SNP Genotyping assays (Applied Biosystems, Thermo cat No: 4351379) and TaqMan Genotyping Master Mix II (Thermo cat No: 4440038). Genotyping was performed using LightCyler 480 II real-time polymerase chain reaction (PCR) (Roche). The PCR was performed in a 20 µl reaction mixture and a 96-well Real-Time PCR System (Bio-Rad Laboratories, Hercules, CA, USA). The reaction mixture included 5 µl genomic DNA, 1 ml of primer-probe assay (for CLOCK [rs1801260; https://www.ncbi.nlm.nih.gov/snp/rs1801260],[37] TaqMan SNP Genotyping Assay, C__8746719_20, Thermo, USA and for HCRTR1 [rs2271933; https://www.ncbi.nlm.nih.gov/snp/rs2271933],[38] TaqMan SNP Genotyping Assay, C__15961465_10 Thermo, USA). The amplification protocol was as follows: Initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s (denaturation), 60°C for 60 s (annealing), and 40°C for 30 s (cooling).

Statistical analysis

The sample size was calculated in the G*Power program. A total of 143 individuals were calculated with 80% power and 5% type 1 error, with an effect size of 0.3 (moderate) (degrees of freedom = 5) between genotype distributions by groups. It was considered appropriate to include at least 72 people in each group, with a 1:1 allocation number. This study is the primary analysis of these data. IBM SPSS Statistics 21 program was used to analyze the data. Student's *t*-test (two-tailed) was used for comparisons of means between two independent groups. The Chi-square test (χ^2) was used in the analysis of categorical data and verifying Hardy–Weinberg equilibrium. Two ratio comparisons were applied for the comparisons, and they were found significant in line with the results of the χ^2 test. The variables found to be significant in univariate analysis were analyzed using advanced statistical methods. Univariate logistic regression analysis was applied for SNPs and found to have a significant relationship between the patients and second control groups. Multiple logistic regression (MLP)

was used to evaluate the effects of defining variables and SNPs together. With the MLP backward elimination method, the final model was created with descriptive variables for the SNPs and for the patients with CM who were also suffering from EDS or insomnia. MLP analyses were also conducted with descriptive variables and SNPs for the patients with CM suffering from insomnia and the control group 2. Descriptive statistics variables were expressed in terms of frequency (percentage), mean, standard deviation, odds ratio (OR), and 95% confidence interval (CI) for OR depending on the structure of the data and analysis. The statistical significance level was taken as 0.05 in all analyzes.

Results

Scale results

While 87 (87%) of the patients consisted of female patients, the mean age was 41.1 ± 11.5. Fixty-six (56%) of the patients were female in the control group 1, whereas 50 (50%) of them were female in the control group 2. Their mean ages were 45.7 ± 15.2 and 35.9 ± 10, respectively. EDS was detected in 27% of the patients, and poor sleep quality was detected in 67% of the patients. Only 21% of the patients were found to be suffering from both EDS and poor sleep quality. About 81.4% of patients with EDS and 89.6% of patients with impaired sleep quality entailed female patients, but it was not statistically significant. There was no significant relationship between sleep problems and the age of onset of a migraine, aura, and patients with autonomic symptoms accompanying migraine attacks. The factors of being Turkish ($P = 0.015$) and being a smoker ($P = 0.026$) have been associated with insomnia. However, these findings were not significant in the MLP analysis. The other parameters used in the study also are specified in Table 1.

Genetic analyses

Frequencies of hypocretin receptor 1 rs2271933 and circadian locomotor output cycles kaput rs1801260 polymorphisms

A statistically significant association was not found between the migraine and control 1 group in terms of CLOCK rs1801260 genotypes and alleles. However, a significant association was found between the migraine and control 2 groups ($P = 0.001$). Accordingly, while CLOCK rs1801260 homozygous (AA and GG) genotypes were higher in the control 2 group, heterozygous (AG) genotype was found higher in the migraine group. In addition, no statistically significant association was found between alleles of these two groups ($P = 0.675$). The AG genotype in the migraine group was statistically significant 6.32 times more than the AA genotype compared to the control group 2 (OR = 6.32; 95% CI = 1.95–20.43; $P = 0.002$). The GG genotype was found 2.55 times higher than the AA genotype but was not statistically significant (OR = 2.55; 95% CI = 0.78–8.40; $P = 0.123$). According to the groups, no statistically significant association was found between the OX1R rs2271933 genotypes ($P = 0.128$) and alleles ($P = 0.125$) [Table 2].

Sleepiness relationship of hypocretin receptor 1 rs2271933 and circadian locomotor output cycles kaput rs1801260 polymorphisms

Among the groups ESS scores ≥11 and ESS scores <11, no statistically significant association was found in terms of CLOCK rs1801260 and HCRTR1rs2271933 genotypes and alleles [Table 3]. Age, gender, race, shift work, smoke addiction, alcohol consumption, CLOCK rs1801260, and HCRTR1rs2271933 genotypes and alleles were included in the logistic regression model created for ESS in migraine patients. Male gender was 8.58 times higher than female (OR = 8.58; 95% CI: 1.33–55.2, $P = 0.024$), being Kurdish was 12.5 times higher than Turkish (OR = 12.5; 95% CI: 1.66–93.2, $P = 0.014$), shift work was 9.30 times higher (OR = 9.30; 95% CI: 1.18–93.3, $P = 0.034$), and smoking was 4.21 times higher (OR = 4.21; 95% CI: 1.05–16.9, $P = 0.042$); they were found to be associated with EDS.

Between the migraine patient group with EDS and the first control group,; no statistically significant association was found in terms of CLOCK rs1801260 and HCRTR1 rs2271933 genotypes or alleles. Furthermore, no statistically significant association was found between the migraine patient group with EDS and second control group in terms of HCRTR1 rs2271933 genotypes or alleles but was found in terms of CLOCK rs1801260 genotypes ($P = 0.047$). Accordingly, CLOCK rs1801260 gene AG genotype was lower in the second control group. The CLOCK, rs1801260 AG genotype was 6.71 times higher than the AA genotype in the migraine patient group with EDS compared to the second control group (OR = 6.71, 95% CI: 0.819–55.0, $P = 0.076$). The GG genotype was 2.87 times higher than the AA genotype in this group (OR = 2.87, 95% CI: 0.336–24.6, $P = 0.335$), but it is not statistically significant [Table 4]. Age, gender, race, shift work, smoke addiction, alcohol consumption, CLOCK rs1801260, and HCRTR1 rs2271933 genotypes and alleles were included in the logistic regression model created between CM patients with EDS and control groups. No significant relationship was found.

Insomnia relationship of hypocretin receptor 1 rs2271933 and circadian locomotor output cycles kaput rs1801260 polymorphisms

There was no statistically significant association between CLOCK rs1801260 genotypes ($P = 0.171$) and

Table 1: Classification of the Epworth Sleep Scale and Pittsburgh Sleep Quality Index scores of the migraine patient group according to the sociodemographic and clinical characteristics of the patients

	ESS scores≥11 (%)	ESS scores<11 (%)	*P*	PSQI scores≥6 (%)	PSQI scores<6 (%)	*P*
n	27	73		67	33	
Age	42.2±9.9	40.7±12.1	0.556	40.9±11.8	41.6±11.2	0.761
Gender						
Female	22 (81.4)	65 (89)	0.329*	60 (89.6)	27 (81.8)	0.280*
Male	5 (18.6)	8 (11)		7 (10.4)	6 (18.2)	
The year of education	12.6±7.3	12.7±6.6	0.951	13.1±6.4	12.2±7.6	0.536
Age of onset of migraine	27.7±10.5	26.6±10.6	0.65	27.2±10.4	26.3±10.7	0.701
Ethnicity						
Turkish	7 (53.8)	46 (82.1)	0.071*	35 (89.7)	18 (60)	0.015*
Kurdish	5 (38.5)	7 (12.5)		3 (7.7)	9 (30)	
Arab	1 (7.7)	3 (5.4)		1 (2.6)	3 (10)	
Shift work						
No	22 (84.6)	62 (87.3)	0.742*	57 (87.7)	27 (84.4)	0.652*
Yes	4 (15.4)	9 (12.7)		8 (12.3)	5 (15.6)	
Smoke						
No	13 (54.2)	45 (66.2)	0.486*	32 (53.3)	26 (81.3)	0.026*
Yes	10 (41.7)	22 (32.4)		26 (43.3)	6 (18.8)	
Left	1 (4.2)	1 (1.5)		2 (3.3)	0	
Alcohol						
No	18 (81.8)	53 (80.3)	1*	42 (75)	29 (90.6)	0.095*
Yes	4 (18.2)	13 (19.7)		14 (25)	3 (9.4)	
Aura						
No	17 (63.0)	48 (66.7)	0.813*	44 (65.7)	21 (65.6)	0.996*
Yes	10 (37.0)	24 (33.3)		23 (34.3)	11 (34.4)	
Autonomic findings						
No	5 (18.5)	29 (40.3)	0.057*	20 (29.9)	14 (43.8)	0.173*
Yes	22 (81.5)	43 (59.7)		47 (70.1)	18 (56.2)	

P value: Student's *t*-test. *Chi-square test. ESS: Epworth Sleepiness Scale, PSQI: Pittsburgh Sleep Quality Index

Table 2: Genotype and allele frequencies of hypocretin receptor 1 rs2271933 and circadian locomotor output cycles kaput rs1801260 polymorphisms

	Migraine patients	Control Group 1	*P*	Control Group 2	*P*
CLOCK rs1801260					
Genotypes					
AA	4 (4.0)	10 (10.0)	0.128	15 (15.0)*	0.001
AG	64 (64.0)	67 (67.0)		38 (38.0)*	
GG	32 (32.0)	23 (23.0)		47 (47.0)*	
Alleles					
A	72 (36.0)	87 (43.5)	0.125	68 (34.0)	0.675
G	128 (64.0)	113 (56.5)		132 (66.0)	
Hardy-Weinberg equilibrium	<0.001	<0.001		<0.130	
HCRTR1 rs2271933					
Genotypes					
AA	25 (25.0)	26 (26.0)	0.982	23 (23.0)	0.880
AG	30 (30.0)	29 (29.0)		33 (33.0)	
GG	45 (45.0)	45 (45.0)		44 (44.0)	
Alleles					
A	80 (40.0)	81 (40.5)	0.919	79 (39.5)	0.919
G	120 (60.0)	119 (59.5)		121 (60.5)	
Hardy-Weinberg equilibrium	<0.001	<0.001		<0.002	

*It shows the rate that differs from the migraine group. *P* value: Chi-square test. CLOCK: Circadian locomotor output cycles kaput, HCRTR1: Hypocretin receptor 1

Table 3: According to Epworth Sleepiness Scale and Pittsburgh Sleep Quality Index scores in patients with chronic migraine genotype and allele frequencies of hypocretin receptor 1 rs2271933 and circadian locomotor output cycles kaput rs1801260 polymorphisms

	ESS scores≥11	ESS scores<11	*P*	PSQI scores≥6	PSQI scores<6	*P*
CLOCK rs1801260						
Genotypes						
AA	1 (3.7)	3 (4.1)	0.983	1 (1.5)	3 (9.1)	0.171
AG	17 (63.0)	47 (64.4)		45 (67.2)	19 (57.6)	
GG	9 (33.3)	23 (31.5)		21 (31.3)	11 (33.3)	
Alleles						
A	19 (35.2)	53 (36.3)	0.884	47 (35.1)	25 (37.9)	0.698
G	35 (64.8)	93 (63.7)		87 (64.9)	41 (62.1)	
Hardy-Weinberg equilibrium	0.050	<0.001		<0.001	0.200	
HCRTR1 rs2271933						
Genotypes						
AA	6 (22.2)	19 (26.0)	0.881	12 (17.9)	13 (39.4)	0.058
AG	9 (33.3)	21 (28.8)		23 (34.3)	7 (21.2)	
GG	12 (44.4)	33 (45.2)		32 (47.8)	13 (39.4)	
Alleles						
A	21 (38.9)	59 (40.4)	0.845	47 (35.1)	33 (50)	0.043
G	33 (61.1)	87 (59.6)		87 (64.9)	33 (50)	
Hardy-Weinberg equilibrium	0.120	<0.001		0.040	<0.001	

P value: Chi-square test. CLOCK: Circadian locomotor output cycles kaput, HCRTR1: Hypocretin receptor 1, ESS: Epworth Sleepiness Scale

Table 4: Genotype and allele frequencies of hypocretin receptor 1 rs2271933 and circadian locomotor output cycles kaput rs1801260 polymorphisms between patients with chronic migraine whose Epworth Sleepiness Scale or insomnia and control groups

	ESS scores≥11 (patients Group)	Control Group 1	*P*	Control Group 2	*P*	PSQI scores≥6 (patients Group)	Control Group 1	*P*	Control Group 2	*P*
CLOCK rs1801260										
Genotypes										
AA	1 (3.7)	10 (10.0)	0.382	15 (15.0)	0.047	1 (1.5)	10 (10.0)	0.065	15 (15.0)†	<0.001
AG	17 (63.0)	67 (67.0)		38 (38.0)*		45 (67.2)	67 (67.0)		38 (38.0)†	
GG	9 (33.3)	23 (23.0)		47 (47.0)		21 (31.3)	23 (23.0)		47 (47.0)†	
Alleles										
A	19 (35.2)	87 (43.5)	0.272	68 (34.0)	0.871	47 (35.1)	87 (43.5)	0.124	68 (34.0)	0.839
G	35 (64.8)	113 (56.5)		132 (66.0)		87 (64.9)	113 (56.5)		132 (66.0)	
Hardy-Weinberg equilibrium	0.050	<0.001		0.130		<0.001	<0.001		0.130	
HCRTR1 rs2271933										
Genotypes										
AA	6 (22.2)	26 (26.0)	0.880	23 (23.0)	0.996	12 (17.9)	26 (26.0)	0.452	23 (23.0)	0.726
AG	9 (33.3)	29 (29.0)		33 (33.0)		23 (34.3)	29 (29.0)		33 (33.0)	
GG	12 (44.4)	45 (45.0)		44 (44.0)		32 (47.8)	45 (45.0)		44 (44.0)	
Alleles										
A	21 (38.9)	81 (40.5)	0.830	79 (39.5)	0.935	47 (35.1)	81 (40.5)	0.317	79 (39.5)	0.413
G	33 (61.1)	119 (59.5)		121 (60.5)		87 (64.9)	119 (59.5)		121 (60.5)	
Hardy-Weinberg equilibrium	0.120	<0.001		0.002		0.040	<0.001		0.002	

*It shows the difference from the group whose ESS scores≥11, †It shows the difference from the group whose PSQI scores≥6. *P* value: Chi-square test. CLOCK: Circadian locomotor output cycles kaput, HCRTR1: Hypocretin receptor 1, ESS: Epworth Sleepiness Scale, PSQI: Pittsburgh Sleep Quality Index

alleles ($P = 0.698$) according to patients with CM whose PSQI scores ≥6 and <6. While there was no statistically convincing association between the HCRTR1rs2271933 genotypes according to the patients with CM whose PSQI scores ≥6 and <6, a statistically significant association was found between alleles ($P = 0.043$) [Table 3]. Age, gender, race, shift work, smoke addiction, alcohol consumption, CLOCK rs1801260, and HCRTR1rs2271933 genotypes were included in the logistic regression model created for PSQI in migraine

patients. Being Kurdish was 0.029 times lower compared to being Turkish (OR = 0.029; 95% CI: 0.002–0.456, $P = 0.012$), being Arab was 0.064 times lower compared to being Turkish (OR = 0.064; 95% CI: 0.004–0.928, $P = 0.044$), smoking was 4.19 times higher (OR = 4.19; 95% CI: 1.02–17.2, $P = 0.047$), having HCRT AG genotype compared to have AA genotype was 6.06 times higher (OR = 6.06; 95% CI: 1.04–35.5, $P = 0.045$), and having a GG genotype compared to have AA genotype was 5.47 times higher (OR = 5.47; 95% CI: 1.16–25.8, $P = 0.032$); which were associated with poor sleep quality.

No statistically significant association was found between the group of patients with CM with PSQI ≥6 and the first control groups in terms of CLOCK rs1801260 and HCRTR1rs2271933 genotypes and alleles. A statistically significant association was found between CLOCKrs1801260 genotypes between the group of patients with CM with PSQI ≥6 and the second control groups ($P < 0.001$). Accordingly, the AA and GG genotypes were higher in the second control group, and the AG genotype had a lower rate. In the group of CM patients with poor sleep quality, the AG genotype was 17.8 times higher than the AA genotype compared to the second control group which was statistically significant (OR = 17.8, 95% CI: 2.24–140.7, $P = 0.006$). There was no statistically significant association between the CLOCK rs1801260 alleles between the patient group with CM suffering from poor sleep quality and the second control group ($P = 0.839$). According to the groups, no statistically significant association was found between HCRTR1rs2271933 genotypes ($P = 0.726$) and alleles ($P = 0.413$) [Table 4]. Age, gender, race, shift work, smoking, alcohol use, CLOCK rs1801260, and HCRTR1 rs2271933 genotypes and alleles were included in the logistic regression model created between CM patients with insomnia and control groups. In CM patients, the risk of developing insomnia increased 1.07 times as age increased compared to the second control group (OR = 1.07, 95% CI: 1.01–1.14, $P = 0.017$). This risk was observed 17.2 times higher in female gender (OR = 17.2, 95% CI: 1.99–142.9, $P = 0.01$).

Discussion

There is a veiled vicious cycle that links sleep disorders with migraine.[8] The poor quality or insufficient duration of sleep could be a provoking cause of migraine attacks,[7,39] on the other hand, coping behaviors of migraineurs with headache attacks such as going to bed early with an intention of sleeping just to relieve migraine attacks can be the factor that results in sleep disturbances themselves.[40] Especially in patients with CM (in over half of the patients) sleep disturbances such as EDS and insomnia have been reported with a higher rate compared to EM patients.[9,40] In our study, poor sleep quality was detected in 67% of the patients with CM, and EDS was detected in 27% of the patients with CM. In 21% of the patients with CM, both poor sleep quality and EDS were found. The women with CM (81%–92%) were reported to have significantly higher frequencies of nonrestorative sleep and unstable sleep patterns.[41] In our study, also 81.4% of patients with EDS and 89.6% of patients with impaired sleep quality were composed of female patients; however, the finding was not statistically significant.

In the study of Barbanti *et al*., logistic regression analysis indicated that ESS scores adjusted for gender, age, and alcohol were not significantly higher in patients when compared to the control groups.[42] In the study of Kristoffersen *et al*., gender and age were not associated with EDS.[43] In our study, logistic regression analysis showed that male gender, Kurdish race, shift work, and smoking were found to be associated with EDS. It was highlighted that EDS was independently associated with an elevated risk of metabolic syndrome in men with obstructive sleep apnea (OSA).[44] Obesity, OSA, and metabolic syndrome are likely to justify the higher incidence of EDS in men in our study, but these factors were not evaluated.

Shift work has been associated with several sleep disorders, such as EDS, due to the rapidly changing circadian clock and erratic exposure to light, and this indirectly leads to poor health outcomes.[45] The results from the "In the Middle of the Night" project displayed that the three hormone melatonin, cortisol, and testosterone all responded distinctively to an increasing number of consecutive night shifts and develop adaptation to the new sleep/wake cycle with different speeds in a real-life setting.[46] The relationship between migraine (especially CM) and EDS, which was found in some studies, may be due to the presence of other triggering factors in addition to the fact that sleep disorders in shift workers can trigger migraine attacks.[47,48] To exemplify, many researches had demonstrated that shift workers are more often obese than daytime workers[49] and smokers.[50,51] In several studies, it has been found that the prevalence of migraine increases in obese people, smokers, and women.[52-54] In our study, in the logistic regression analysis of migraine patients, smoking was found to be associated with both EDS and insomnia. Smoking was associated with sleep problems such as falling asleep, maintaining sleep, and daytime sleepiness.[55] Moreover, the working environment of shift workers involves exposure to more psychological stress, and therefore, migraines are more difficult to tolerate.[56]

Our logistic regression analysis showed that the Kurdish race was found to be associated with EDS. There are not enough researches on racial/ethnic variation in sleep disturbances. In one study, it was emphasized that race may be effective on sleep disorders.[57] Impaired sleep hygiene, male gender, excess weight, urban residing, depressive symptoms, physical activity lacks, and functional impairment may be the causes of EDS.[58] In a study carried out with black patients, mean ESSs in patients with VitDd were higher and 25OHD levels were lower ($P < 0.05$).[59] In another study, the findings reveal a measurement of race/ethnic differences in EDS which may prompt conflicting estimates of race/ethnic variation.[60] Indeed in our study, being Kurdish was associated with EDS, while being Turkish was associated with insomnia. It is difficult to evaluate these data due to the lack of academic research.

Furthermore, in the logistic regression analysis performed in CM patients, in the carriers of the HCRTR1 rs2271933 G allele, sleep quality was impaired. There was no significant relationship between migraine patients with EDS or insomnia and control groups in terms of HCRTR1 rs2271933 alleles and genotypes. OXs have a vital part in the regulation of the sleep–wake cycle.[33] Some premonitory symptoms such as excessive sleepiness and autonomic processes before migraine attacks are associated with HCRT dysfunction, suggesting that they may be related to the pathophysiology of the disease.[20,61] A number of researches have scrutinized the relationship between polymorphisms of the HCRT1 G12222A gene and migraine. Rainero *et al*. also found AA genotype of the HCRTR1 gene as a risk factor in migraine patients without aura (MO).[26] Similarly, Kowalska *et al*. found the A allele of the HCRT1 G12222A gene more frequently in MO patients. They also suggested that hypocretinergic systems may play a role in the development of migraine by altering HCRT-1 concentrations.[29] Several studies have focused on the role of OXs in narcolepsy. While OX deficiency causes narcolepsy and cataplexy, CSF OX-A levels in affected patients are lower than normal controls or undetectable.[62] Another study found that OX-A levels increased significantly in insomnia patients. This suggests an association between OX-A levels and insomnia. However, in this study, no significant difference was found in the allelic or genotypic frequencies of the OX1R gene (rs2271933) polymorphism in patients with insomnia compared to normal sleepers.[33] Kowalska *et al*. found the A allele of HCRT1 G1222A tended to decrease both 5-HT and hypocretin-1 levels in healthy individuals.[29] Peres *et al*. researched HCRT-1 levels in the CSF of patients with CM and could not find a significant difference compared to controls.[63] Sarchielli *et al*. found high levels of HCRT-1 in the CSF in patients with CM and drug overuse headache.[64] Considering all these, the fact that the G allele of HCRT-1 G12222A detected in our study is associated with insomnia in patients with CM brings to mind the question of whether this polymorphism increases OX-A levels in patients with CM.

When CM patients are compared with control groups, CLOCK rs1801260 gene AG genotype is associated with EDS among the second control group and CM patients with EDS, while the presence of an AA genotype is protective. On the other hand, when the CM patients are compared with the control groups, the presence of AG genotype for CLOCK rs1801260 gene is associated with insomnia among the second control group and CM patients with insomnia, while homozygous genotypes (AA and GG) are protective. Mishima *et al*. were found that the CLOCK rs1801260 CC genotype was associated with EDS in Japanese. In the same study, this polymorphism also was found associated with delayed sleep onset and shorter total sleep duration.[18] Carrying the CLOCK rs1801260 C allele was found associated with delayed sleep onset and insomnia in patients with bipolar disorder.[19,65] In contrast, Ziv-Gal *et al*. found that the selected SNP of CLOCK (rs1801260) was not associated with insomnia or early awakening.[66] The CLOCK gene plays an essential role in circadian rhythm regulation. The observations found between the rs1801260 variant and sleep behavior can be justified by the fact that this polymorphism produces changes in the stability and half-life of mRNA, which influences mRNA translocation.[67] Besides, this is likely to be the reason for the fact that CLOCK interacts with other factors in the AHR-signaling pathway.[66] Therefore, CLOCK protein levels can affect circadian clock organization, sleep features, and, consequently, migraine attacks. In addition, some neurotransmitter systems such as dopamine, serotonin, glutamate, and GABA show circadian fluctuations.[68,69] Some authors have suggested that serotonin plays a role in the pathogenesis of migraine. This effect can be related to its direct action upon the cranial vasculature, its role in central pain control pathways, or cerebral cortical projections of brainstem serotonergic nuclei.[70,71] When all these are evaluated together, there may be an underlying indirect relationship between migraine and sleep with the CLOCK gene.

In the regression analysis, age and female gender were found to be associated with insomnia between CM patients with insomnia and the second control group. Gender-related evaluations have been mentioned above. In our study, the risk of insomnia was found to increase

with the age. In the study, a longer migraine history and shorter sleep time were reported with a higher rate in elderly individuals, and the onset of migraine at night was observed with a higher rate in these patients. The reason for this was disrupted sleep rhythm in the elderly with a high frequency.[72]

To the best of our knowledge, our study is the initial academic work exploring the genetic relationship in patients with sleep disorders and CM. Additional studies in different populations are needed to confirm the findings. The limitations of the study are the relatively small sample size, the inability to objectively evaluate sleep characteristics with PSG and actigraphy because of practical difficulty, and the difficulties in determining temporary relationships due to the lack of functional studies of HCRT and CLOCK. The violation of the HardyWeinberg Equilibrium may be due to the diverse ethnic origins of the participants in the study. As there are no data available on rs1801260 and rs2271933 alleles frequencies in the 1000 Genomes Project database for our country, there would not be any comparisons with respect to the rs1801260 and HCRTR1 rs2271933 frequencies in the control group 2. In addition, there is no guarantee that young patients in the control group shall not suffer from migraine in the future.

Conclusion

HCRTR1 rs2271933 gene G allele carriers have been associated with insomnia in patients with CM. When CM patients were compared with control groups, CLOCK rs1801260 gene AG genotype was found to be associated with both insomnia and EDS. Besides, CLOCK rs1801260 gene AA genotype stands out as a protective factor against both sleep disorders. This relationship was significant only between the second control group and migraine patients, and it suggests that the impact of environmental factors may be more than genetic factors. Furthermore, as far as the highly polygenic genetic architecture of migraine is concerned, it may be important to evaluate gene–gene and gene–environment interactions in explaining this relationship.

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Conflicts of interest

There are no conflicts of interest.

References

1. Russell MB, Iselius L, Olesen J. Migraine without aura and migraine with aura are inherited disorders. Cephalalgia 1996;16:305-9.
2. Russell MB, Olesen J. Increased familial risk and evidence of genetic factor in migraine. BMJ 1995;311:541-4.
3. Ulrich V, Gervil M, Fenger K, Olesen J, Russell MB. The prevalence and characteristics of migraine in twins from the general population. Headache 1999;39:173-80.
4. Ziegler DK, Hur YM, Bouchard TJ Jr., Hassanein RS, Barter R. Migraine in twins raised together and apart. Headache 1998;38:417-22.
5. Ferrari A, Leone S, Vergoni AV, Bertolini A, Sances G, Coccia CP, *et al.* Similarities and differences between chronic migraine and episodic migraine. Headache 2007;47:65-72.
6. Kelman L. The triggers or precipitants of the acute migraine attack. Cephalalgia 2007;27:394-402.
7. Levy D, Strassman AM, Burstein R. A critical view on the role of migraine triggers in the genesis of migraine pain. Headache 2009;49:953-7.
8. Zhu Z, Fan X, Li X, Tan G, Chen L, Zhou J. Prevalence and predictive factors for poor sleep quality among migraineurs in a tertiary hospital headache clinic. Acta Neurol Belg 2013;113:229-35.
9. Ferini-Strambi L, Andrea G, Romina C. Sleep disorder-related headaches. Neurol Sci 2019;40:107-13.
10. Fox AW, Randy LD. Migraine chronobiology. Headache 1998;38:436-41.
11. Dibner C, Schibler U. Circadian timing of metabolism in animal models and humans. J Intern Med 2015;277:513-27.
12. Lamont EW, Legault-Coutu D, Cermakian N, Boivin DB. The role of circadian clock genes in mental disorders. Dialogues Clin Neurosci 2007;9:333-42.
13. Reppert SM, David RW. Coordination of circadian timing in mammals. Nature 2002;418:935-41.
14. Vitaterna MH, King DP, Chang AM, Kornhauser JM, Lowrey PL, McDonald JD, *et al.* Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. Science 1994;264:719-25.
15. Steeves TD, King DP, Zhao Y, Sangoram AM, Du F, Bowcock AM, *et al.* Molecular cloning and characterization of the human CLOCK gene: Expression in the suprachiasmatic nuclei. Genomics 1999;57:189-200.
16. Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, King DP, *et al.* Role of the CLOCK protein in the mammalian circadian mechanism. Science 1998;280:1564-9.
17. Katzenberg D, Young T, Finn L, Lin L, King DP, Takahashi JS, *et al.* A CLOCK polymorphism associated with human diurnal preference. Sleep 1998;21:569-76.
18. Mishima K, Tozawa T, Satoh K, Saitoh H, Mishima Y. The 3111T/C polymorphism of hClock is associated with evening preference and delayed sleep timing in a Japanese population sample. Am J Med Genet Part B Neuropsychiatr Genet 2005;133:101-4.
19. Serretti A, Benedetti F, Mandelli L, Lorenzi C, Pirovano A, Colombo C, *et al.* Genetic dissection of psychopathological symptoms: Insomnia in mood disorders and CLOCK gene polymorphism. Am J Med Genet Part B Neuropsychiatr Genet 2003;121:35-8.

20. Sutcliffe JG, Luis de L. The hypocretins: Excitatory neuromodulatory peptides for multiple homeostatic systems, including sleep and feeding. J Neurosci Res 2000;62:161-8.
21. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, *et al.* Orexins and orexin receptors: A family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998;92:573-85.
22. Gautvik KM, de Lecea L, Gautvik VT, Danielson PE, Tranque P, Dopazo A, *et al.* Overview of the most prevalent hypothalamus-specific mRNAs, as identified by directional tag PCR subtraction. Proc Natl Acad Sci U S A 1996;93:8733-8.
23. De Lecea L, Kilduff TS, Peyron C, Gao XB, Foye PE, Danielson PE, *et al.* The hypocretins: Hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci 1998;95:322-7.
24. Smart D, Jeffrey CJ. The physiology and pharmacology of the orexins. Pharmacol Ther 2002;94:51-61.
25. Nuñez A, Rodrigo-Angulo ML, Andrés ID, Garzón M. Hypocretin/Orexin neuropeptides: Participation in the control of sleep-wakefulness cycle and energy homeostasis. Curr Neuropharmacol 2009;7:50-9.
26. Rainero I, Rubino E, Gallon, S, Fenoglio P, Picci LR, Giobbe L, *et al.* Evidence for an association between migraine and the hypocretin receptor 1 gene. J Head Pain 2011;12:193-9.
27. Alstadhaug KB. Migraine and the hypothalamus. Cephalalgia 2009;29:809-17.
28. Giffin NJ, Ruggiero L, Lipton RB, Silberstein SD, Tvedskov JF, Olesen J, *et al.* Premonitory symptoms in migraine: An electronic diary study. Neurology 2003;60:935-40.
29. Kowalska M, Kapelusiak-Pielok M, Grzelak T, Wypasek E, Kozubski W, Dorszewska J. The New *G29A and G1222A of HCRTR1, 5-HTTLPR of SLC6A4 polymorphisms and hypocretin-1, serotonin concentrations in migraine patients. Front Mol Neurosci 2018;11:191.
30. Meerabux J, Iwayama Y, Sakurai T, Ohba H, Toyota T, Yamada K, *et al.* Association of an orexin 1 receptor 408Val variant with polydipsia-hyponatremia in schizophrenic subjects. Biol Psychiatry 2005;58:401-7.
31. Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, *et al.* The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999;98:365-76.
32. Bourgin P, Jamie MZ, Emmanuel M. CSF hypocretin-1 assessment in sleep and neurological disorders. Lancet Neurol 2008;7:649-62.
33. Tang S, Huang W, Lu S, Lu L, Li G, Chen X, *et al.* Increased plasma orexin – A levels in patients with insomnia disorder are not associated with prepro-orexin or orexin receptor gene polymorphisms. Peptides 2017;88:55-61.
34. International Classification of Headache Disorders, 3rd Edition/Chronic Migraine. Available from: https://ichd-3.org/1-migraine/1-3-chronic-migraine/. [Last accessed on 2021 Sep 16].
35. Izci B, Ardic S, Firat H, Sahin A, Altinors M, Karacan I. Reliability and validity studies of the Turkish version of the Epworth Sleepiness Scale. Sleep Breath 2008;12:161-8.
36. Agargun MY, Kara H, Anlar O. The reliability and validity of Turkish version of Pittsburgh Sleep Quality Index. Turk Psycol J 1996;7:107-15.
37. National Library of Medicine, dbSNP, Reference SNP (rs) Report, rs1801260. Available from: https://www.ncbi.nlm.nih.gov/snp/rs1801260. [Last accessed on 2021 Mar 30].
38. National Library of Medicine, dbSNP, Reference SNP (rs) Report, rs2271933. Available from: https://www.ncbi.nlm.nih.gov/snp/rs2271933. [Last accessed on 2021 Mar 30].
39. Lin YK, Lin GY, Lee JT, Lee MS, Tsai CK, Hsu YW, *et al.* Associations between sleep quality and migraine frequency: A cross-sectional case-control study. Medicine (Baltimore) 2016;95:e3554.
40. Ong JC, Park M. Chronic headaches and insomnia: Working toward a biobehavioral model. Cephalalgia 2012;32:1059-70.
41. Calhoun AH, Ford S, Finkel AG, Kahn KA, Mann JD. The prevalence and spectrum of sleep problems in women with transformed migraine. Headache 2006;46:604-10.
42. Barbanti P, Aurilia C, Egeo G, Fofi L, Vanacore N. A case-control study on EDS in CM. Sleep Med 2013;14:278-81.
43. Kristoffersen ES, Stavem K, Lundqvist C, Russell MB. Excessive daytime sleepiness in chronic migraine and chronic tension-type headache from the general population. Cephalalgia 2018;38:993-7.
44. Fu Y, Xu H, Xia Y, Qian Y, Li X, Zou J, *et al.* Excessive daytime sleepiness and metabolic syndrome in men with obstructive sleep apnea: A large cross-sectional study. Oncotarget 2017;8:79693-702.
45. Drake CL, Roehrs T, Richardson G, Walsh JK, Roth T. Shift work sleep disorder: Prevalence and consequences beyond that of symptomatic day workers. Sleep 2004;27:1453-62.
46. Jensen MA. Working in the "Middle of the Night." University of Copenhagen. (PhD Thesis); 2015.
47. Peres MF, Stiles MA, Siow HC, Silberstein SD. EDS in migraine patients. J Neurol Neurosurg Psychiatry 2005;76:1467-8.
48. Sancisi E, Cevoli S, Vignatelli L, Nicodemo M, Pierangeli G, Zanigni S, *et al.* Increased prevalence of sleep disorders in chronic headache: A case-control study. Headache 2010;50:1464-72.
49. Herichova I. Changes of physiological functions induced by shift work. Endocr Regul 2013;47:159-70.
50. Nabe-Nielsen K, Quist HG, Garde AH, Aust B. Shiftwork and changes in health behaviors. J Occup Environ Med 2011;53:1413-7.
51. Bøggild H, Anders K. Shift work, risk factors and cardiovascular disease. Scand J Work Environ Health 1999;25:85-99.
52. Chen TC, Leviton A, Edelstein S, Ellenberg JH. Migraine and other diseases in women of reproductive age. The influence of smoking on observed associations. Arch Neurol 1987;44:1024-8.
53. López-Mesonero L, Márquez S, Parra P, Gámez-Leyva G, Muñoz P, Pascual J. Smoking as a precipitating factor for migraine: A survey in medical students. J Headache Pain 2009;10:101-3.
54. Peterlin BL, Rosso AL, Rapoport AM, Scher AI. Obesity and migraine: The effect of age, gender and adipose tissue distribution. Headache 2010;50:52-62.
55. Phillips BA, Frederick JD. Cigarette smoking and sleep disturbance. Arch Intern Med 1995;155:734-7.
56. Jakobsen GS, Timm AM, Hansen ÅM, Garde AH, Nabe-Nielsen K. The association between shift work and treatment-seeking migraine in Denmark. Ergonomics 2017;60:1207-17.
57. Chen X, Wang R, Zee P, Lutsey PL, Javaheri S, Alcántara C, *et al.* Racial/ethnic differences in sleep disturbances: The multi-ethnic study of atherosclerosis (MESA). Sleep 2015;38:877-88.
58. Hays JC, Dan GB, Daniel JF. Risk of napping: EDS and mortality in an older community population. J Am Geriatr Soc 1996;44:693-8.
59. McCarty DE, Reddy A, Keigley Q, Kim PY, Marino AA. Vitamin D, race, and excessive daytime sleepiness. J Clin Sleep Med 2012;8:693-7.

60. Baron KG, Liu K, Chan C, Shahar E, Hasnain-Wynia R, Zee P. Race and ethnic variation in excessive daytime sleepiness: The multi-ethnic study of atherosclerosis. Behav Sleep Med 2010;8:231-45.
61. Ellis J, Pediani JD, Canals M, Milasta S, Milligan G. Orexin-1 receptor-cannabinoid CB1 receptor heterodimerization results in both ligand-dependent and -independent coordinated alterations of receptor localization and function. J Biol Chem 2006;281:38812-24.
62. Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, *et al.* The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002;59:1553-62.
63. Peres MF, Vieira DS, Masruha MR, Gonçalves AL, Mercante JP, da Graça Naffah-Mazzacoratti M. Orexin-A CSF levels correlate with anxiety but not excessive daytime sleepiness in chronic migraine. Headache Med 2011:41-5.
64. Sarchielli P, Rainero I, Coppola F, Rossi C, Mancini M, Pinessi L, *et al.* Involvement of corticotrophin-releasing factor and orexin-A in chronic migraine and medication-overuse headache: Findings from cerebrospinal fluid. Cephalalgia 2008;28:714-22.
65. Benedetti F, Dallaspezia S, Fulgosi MC, Lorenzi C, Serretti A, Barbini B, *et al.* Actimetric evidence that CLOCK 3111 T/C SNP influences sleep and activity patterns in patients affected by bipolar depression. Am J Med Genet B Neuropsychiatr Genet 2007;144B: 631-5.
66. Ziv-Gal A, Flaws JA, Mahoney MM, Miller SR, Zacur HA, Gallicchio L. Genetic polymorphisms in the aryl hydrocarbon receptor-signaling pathway and sleep disturbances in middle-aged women. Sleep Med 2013;14:883-7.
67. Iwase T, Kajimura N, Uchiyama M, Ebisawa T, Yoshimura K, Kamei Y, *et al.* Mutation screening of the human Clock gene in circadian rhythm sleep disorders. Psychiatry Res 2002;109:121-8.
68. Castañeda TR, de Prado BM, Prieto D, Mora F. Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: Modulation by light. J Pineal Res 2004;36:177-85.
69. Matheson GJ, Schain M, Almeida R, Lundberg J, Cselényi Z, Borg J, *et al.* Diurnal and seasonal variation of the brain serotonin system in healthy male subjects. Neuroimage 2015;112:225-31.
70. Hamel E, Currents H. Serotonin and migraine: biology and clinical implications. Cephalalgia 2007;27:1293-300.
71. Panconesi A. Serotonin and migraine: A reconsideration of the central theory. J Headache Pain 2008;9:267-76.
72. de Tommaso M, Marianna D. Circadian rhythms of migraine attacks in episodic and chronic patients: A cross sectional study in a headache center population. BMC Neurol 2018;18:1-10.