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Original Article

Melatonin receptor 1B −1193T>C polymorphism is associated with diurnal preference and sleep habits

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ABSTRACT

Background: Melatonin modulates the master circadian clock through the activation of G-protein-coupled receptors MT1 and MT2. It is presumed, therefore, that genetic variations in melatonin receptors can affect both sleep and circadian phase. We investigated whether the −1193T > C (rs4753426) polymorphism in the promoter of MT2 receptor gene (*MTNR1B*) is associated with diurnal preference and sleep habits. This polymorphism was previously associated with sunshine duration, suggesting a role in circadian entrainment.

Methods: A total of 814 subjects who completed the Morningness–Eveningness and the Munich Chronotype questionnaires were genotyped for the selected polymorphism. Linear and multinomial regression were performed to test the interaction between gene variants and diurnal preference/sleep habits.

Results: The −1193C variant was associated with the extreme morningness phenotype in a codominant model (C/C vs. T/T), recessive model (C/C + C/T vs. T/T) and alleles (C vs. T). A negative correlation was found between −1193C alleles and social jetlag scores. The frequency of −1193T allele was higher in the group that stay in bed more than 8 h/night compared to the group that stay in bed less than 8 h/night on weekends.

Conclusion: To the best of our knowledge, these data provide the first insights into the role of *MTNR1B* gene in the regulation of sleep, biological rhythms, and entrainment in humans.

1. Introduction

The regulation of melatonin synthesis and release depends on rhythmic outputs from the central circadian pacemaker, the hypothalamic suprachiasmatic nucleus (SCN), via a multisynaptic pathway [1]. Melatonin, in turn, modulates the mammalian circadian system synchronizing the peripheral oscillators and providing feedback to the SCN [2]. For example, the application of exogenous melatonin can induce a phase advance of SCN neural firing rate in vitro [3] and entrain locomotor activity in nocturnal and diurnal animals [4].

In humans, melatonin is considered a chronobiotic – a substance capable of adjusting the timing of internal biological rhythms [5], with phase-advance and phase-delay effects according to the phase response curve [6]. Moreover, exogenous melatonin has an important role in the treatment of a number of circadian rhythm disorders such as shift work, jet lag, and advanced and delayed sleep phase syndromes [7,8]. Melatonin improves the sleep quality for these patients by decreasing sleep onset latency, increasing total sleep time and improving overall sleep quality [9].

The melatonin regulatory effects in the SCN are exerted through the high-affinity G-protein-coupled receptors MT1 and MT2, whose activation may lead to different cellular responses [10]. MT1 receptor activation inhibits the SCN neuronal firing rate, while MT2 activation mediates the time-dependent phase shift of the SCN neuronal firing rate, driving the entrainment of circadian rhythms

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[11]. Additionally, the MT2-selective antagonist 4P-PDOT blocks the melatonin-mediated phase advances of circadian activity rhythms in C3H/HeN mouse [12].

Assuming the importance of melatonin receptors in the modulation of circadian rhythms, genetic alterations in the corresponding genes could be involved in sleep regulation and circadian rhythms entrainment in humans. Accordingly, variants in melatonin receptor genes were associated with non-24-h sleep–wake syndrome [13], insomnia symptoms in schizophrenia patients [14] and rapid eye movement (REM) sleep latency [15]. The present study is focused on a single nucleotide polymorphism (SNP), −1193T > C (rs4753426), located in the promoter region of the *MTNR1B* gene.

The −1193T > C SNP was associated with recurrent depressive disorder risk [16] and higher fasting plasma glucose concentrations [17]. A significant correlation between the frequency of the −1193C allele and sunshine duration was observed among worldwide human populations, suggesting a potential adaptive role for this SNP and its involvement in circadian entrainment [18]. Moreover, the −1193T > C polymorphism shows a linkage disequilibrium with the rs10765576 ($r^2 = 0.97$) [19] and the rs10830963 ($r^2 = 0.41$) [17] variants. The rs10765576 variant had a significant interaction with shift work/NPAS2 and shift work/ARNTL [20], while rs10830963 was related to a substantially longer duration of elevated melatonin levels and a delayed circadian phase of dim-light melatonin offset [21]. Although there are no functional studies involving −1193T > C polymorphism, data from the GTEx database (http://www.gtexportal.org/home/) [22] suggests a transcriptional modulation of *MTNR1B* expression. The −1193C/C genotype had significantly lower expression levels of *MTNR1B* gene compared with the T homozygous genotype group in oesophagus–mucosa tissue.

The aim of the present study was to investigate the association of the −1193T > C polymorphism with diurnal preference and sleep habits in a sample of the Brazilian population.

2. Methods

2.1. Subjects

A total of 814 participants (556 females and 264 males), with a mean age of 21.36 years (standard deviation (SD) = ± 2.74) recruited from Brazilian universities (Alagoas state, latitude 9°S) were included in this study. Inclusion criteria were: (1) complete questionnaires; (2) aged between 18 and 30 years; (3) non-self-reporting of past or current history of psychiatric disorder; (4) no use of alarm clock on weekends; (5) sleep duration between 3 h and 14 h; and (6) no use of sleep or psychoactive medication [23]. Ethical approval for this study was provided by Institutional Ethics Committee at the Federal University of Alagoas (protocol number 38930914.0.0000.5013) and written informed consent was obtained from all participants.

2.2. Measurement instruments

All the subjects completed self-administered questionnaires, which included sociodemographic variables such as age, sex, personal and familial history of psychiatric disorders; the Morningness–Eveningness Questionnaire (MEQ) for assessing diurnal preference and the Munich Chronotype Questionnaire (MCTQ) for assessing sleep habits.

2.2.1. MEQ

The MEQ is a 19-item self-reported questionnaire used to assess the individual's preference with regard to the timing of daily activities, producing an overall score ranging from 16 to 86 points. Lower scores on the MEQ indicate a diurnal preference for eveningness, whereas higher scores correspond to a morningness diurnal preference [24]. The Brazilian Portuguese version of MEQ has been previously validated [25]. According to the sample distribution in quartiles (25%–50%–25%) [24], individuals were separated into three chronotypes: evening types ($N = 195$), intermediate types ($N = 411$) and morning types ($N = 208$). For genetic association analysis, we selected students with the highest and lowest MEQ scores (7% of both extreme) to create groups of the most pronounced morning and evening preferences ($N = 56$ for each), together with a control group of intermediates, who were the closest to the middle of MEQ score distribution ($N = 56$) [26]. A subset analysis with the extreme phenotypes for quantitative traits, such as MEQ scores, from large population may increase the power per genotyped individual [27].

2.2.2. Munich Chronotype Questionnaire (MCTQ)

The sleep habits were assessed by the Brazilian Portuguese version of the MCTQ (http://www.bioinfo.mpg.de/wepcronotipo). Respondents were asked to self-report their bedtime (an indirect measure for sleep onset), wake up time, sleep duration and the midpoint of sleep (the halfway point between bedtime and wake up time) separately for weekdays and weekends [28]. Besides sleep habits, social jetlag was also evaluated by MCTQ. Social jetlag indicates the discrepancy between biological and social timing, and it is obtained by subtracting the midpoint of sleep on weekdays (MSW) from the midpoint of sleep on weekends (MSF), expressed in hours [29]. The MSF corrected for sleep debt accumulated during the weekdays (MSFsc) provides a quantitative measure of individual phase of entrainment based on sleep/wake behaviour [30]. Lower values indicate earlier types (individuals who sleep and wake up earlier) while higher values indicate later types (individuals who sleep and wake up later). The dimension of MSFsc is not a score but a representation of local time in hours [29]. MSFsc values were highly correlated with scores obtained by MEQ ($r = -0.73$, $p = 0.001$) [31].

2.3. Genotyping

Buccal cell samples were collected from all participants using sterile cytobrushes (Adlin, Jaraguá do Sul, SC, Brazil). The genomic DNA was extracted using the salting out adapted protocol [32]. The DNA samples were resuspended in 60 μl of TE (10 mM Tris-Cl, pH 7.6, 0.1 mM EDTA) and stored at −20 °C. The amount of genomic DNA was quantified using a spectrophotometer (Eppendorf® AG, Hamburg, Germany) and then diluted to a final concentration of 4 ng/μl. The rs4753426 (−1193T > C) SNP of *MTNR1B* was analysed using the StepOnePlus™ Real-Time polymerase chain reaction (PCR) System (Applied Biosystems, Foster City, CA, USA) using a specific TaqMan SNP Genotyping Assays (Assay ID: C_289583_10, Applied Biosystems, Foster City, CA, USA). Amplification was performed with 5.0 μL 2 × TaqMan Genotyping Master Mix, 0.125 μL TaqMan 40 × assay mix, and 4.87 μL of DNA (4 ng/μl), for 10 μl of total volume. The PCR program consisted of a 10-min denaturation step at 95 °C (one cycle), followed by 40 cycles at 92 °C for 15 s and 60 °C for 1 min. Allelic discrimination was performed for endpoint reading on StepOnePlus™ software version 2.3. A total of 814 subjects were genotyped for this study. Additionally, all genotypes were checked by two different researchers in order to confirm and validate the results.

2.4. Statistical analysis

Statistical analyses were performed using SPSS version 22.0 (Statistical Package for the Social Sciences, IBM, Armonk, New York,

USA). Correlations between MEQ scores, age and sleep habits parameters were examined by Pearson's correlation coefficient test. To verify the Hardy–Weinberg equilibrium, a Chi-squared analysis was performed. A linear regression model was conducted to explore the main effects of genotype on diurnal preference and sleep habits, according to several models of inheritance: codominant, dominant, recessive and overdominant. Additionally, a multinomial logistic regression, based on previous genetics models of inheritance, was used to confirm the genotype/allele effects in diurnal preference as a categorical variable. The significance level considered was 0.05.

3. Results

The MEQ scores ranged from 18 to 77 (mean (M) = 50.96 and SD = ± 10.91; 95% confidence interval (95% CI) [50.19, 51.69], whereas the MSFsc showed a mean of 04:03 (SD = ± 01:40 h and 95% CI [3:57–4:11 h], in a sample of 814 volunteers. The scores for both questionnaires were normally distributed (Fig. 1(a), (b)). Diurnal preference distribution demonstrated a slight tendency towards morningness (Fig. 1(a)). MEQ did not differ between sexes (females: M = 51.27 and SD = ±10.87, 95% CI [50.35–52.16]; males: M = 50.31 and SD = ± 11.00, 95% CI [48.95–51.62]; $t(818) = 1.185$, $p = 0.236$, $d = 0.08$) and no correlation was found between age and MEQ scores in our sample ($r = -0.01$, $p = 0.236$). Because there was no interaction between gender/age and the phenotype analysed, both were considered as a single sample for analysis.

MEQ scores correlated negatively with bedtime ($r = -0.56$, $p < 0.001$), wake up time ($r = -0.38$, $p < 0.001$), sleep duration ($r = -0.17$, $p < 0.001$) and midpoint of sleep ($r = -0.54$, $p < 0.001$) on weekdays and, bedtime ($r = -0.61$, $p < 0.001$), wake up time ($r = -0.64$, $p < 0.001$), and midpoint of sleep ($r = -0.69$, $p < 0.001$) on weekends. This indicates that evening types have later sleep–wake times compared to morning types during the weekdays and weekends, as expected. However, no correlation was found for sleep duration on weekends ($r = -0.02$, $p = 0.217$), indicating that morning types spend more hours sleeping than evening types on weekdays, but both have a similar sleep duration on weekends. A negative correlation was found between social jetlag and MEQ values ($r = -0.32$, $p < 0.001$), indicating that evening subjects have higher social jetlag than morning ones.

3.1. MTNR1B −1193T > C polymorphism is associated with MEQ scores

Allelic frequencies were almost equally distributed in the total sample (814 genotyped subjects), with the frequency of 51% for the −1193C allele. Moreover, the genotypes are in Hardy–Weinberg equilibrium ($\chi^2(1) = 0.310$, $p = 0.577$). Through linear regression, we investigated the effect of −1193T > C genotypes in diurnal preference according different genetic models of inheritance. From all genetic models, only the recessive model predicted diurnal preference (Table 1). The model explained 0.5% and 2.6% of the total observed variance in MEQ scores for total sample (N = 814) and a subset of diurnal preference including extreme phenotypes and intermediate controls (N = 168), respectively, with an adequate adjustment of the data [$F(1, 812) = 3.92$, $p = 0.048$ and $F(1,166) = 4.40$, $p = 0.038$]. The −1193T > C genotypes provide a positive explanation for diurnal preference, indicating that carriers of at least one −1193C allele had significantly higher MEQ scores (morning types) than T/T homozygotes for both total sample (N = 814; $\beta = 0.069$, $p = 0.048$, recessive model) and in the subset including extreme diurnal preference and intermediate controls (N = 168; $\beta = 0.161$, $p = 0.038$, recessive model). No effect of −1193T > C alleles in MEQ scores was observed for total sample ($\beta = 0.035$, $p = 0.153$) or subgroups including extreme chronotypes and intermediate controls ($\beta = 0.090$, $p = 0.101$).

Regarding diurnal preference as a categorical variable, a multinomial logistic regression analysis revealed that −1193T > C genotypes or alleles do not confer significant association with chronotypes in the total sample (N = 814; $p > 0.05$) (Table 2). However, an association was found between diurnal preference and −1193T > C genotypes for both codominant and recessive models of inheritance in the subset of diurnal preference including extreme morning/evening types and intermediate controls (N = 168) (Tables 2 and 3). In the codominant model, extreme morning types showed a higher frequency of C/C (38%) and T/C (48%) genotypes than T/T (14%) when compared to extreme evening types (T/T = 34%; C/C vs. T/T: odds ratio (OR) = 3.11, 95% CI [1.08–8.92], $p = 0.034$ and C/T vs. T/T: OR = 3.05, 95% CI [1.11–8.33], $p = 0.029$) (Fig. 2(a)). Additionally, there was a low frequency of heterozygotes (T/C = 34%) in intermediate types compared to morning ones (T/C = 48%) for the codominant model (OR = 0.31, 95% CI [0.11–0.86], $p = 0.025$).

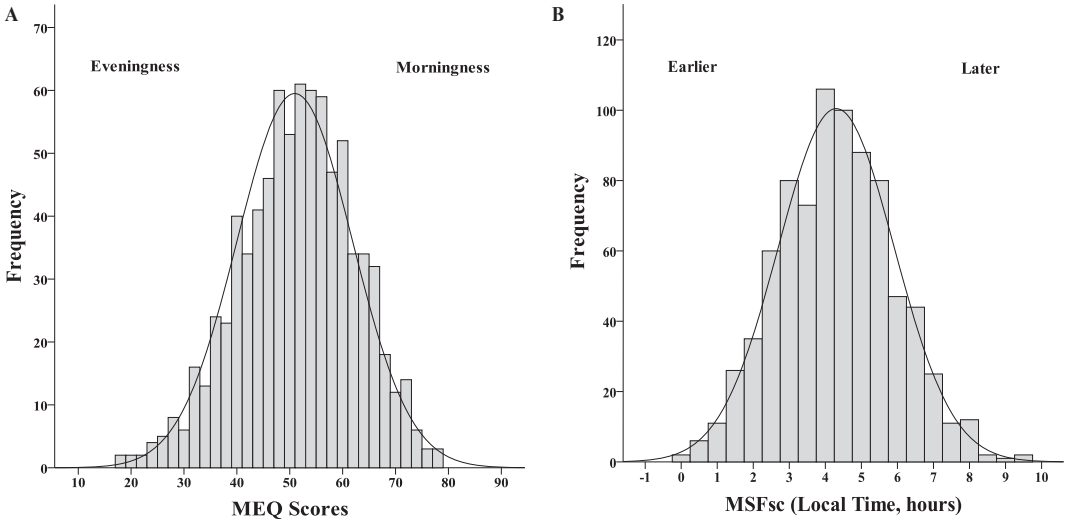

Fig. 1. Histograms of the Morningness–Eveningness Questionnaire (MEQ) (a) and the Munich Chronotype Questionnaire (MCTQ) (b) scores distribution and the corresponding normal curves.

Table 1
Regression model predicting diurnal preference and sleep habits according to different models of heritance/alleles (−1193T > C SNP) for the total sample ($N = 814$), and a subset including extreme morning/evening types and intermediate controls ($N = 168$).

Variables	Models	Total sample ($N = 814$)					Subset of diurnal preference ($N = 168$)				
		r	B[SE]	β	t	p	r	B[SE]	β	t	p
MEQ score											
	Codominant	0.062	1.53[1.07]	0.062	1.43	0.151	0.039	5.51[3.42]	0.151	1.60	0.110
	Dominant	−0.012	−0.30[0.87]	−0.012	0.353	0.724	−0.039	−1.42[2.82]	−0.039	0.502	0.616
	Recessive	**0.069**[a]	**1.74[0.88]**[1]	**0.069**	**1.98**	**0.048**	**0.161**[a]	**6.23[2.97]**[2]	**0.161**	**2.09**	**0.038**
	Overdominat	0.049	1.07[0.76]	0.049	1.40	0.161	0.108	3.78[2.70]	0.108	1.39	0.164
	Allele	0.035	0.77[0.54]	0.035	1.40	0.153	0.090	3.08[1.87]	0.090	1.64	0.101
Bedtime weekdays											
	Codominant	−0.014	−9.84[8.45]	−0.050	1.16	0.245	−0.058	−0.57[21.25]	−0.003	0.027	0.978
	Dominant	0.014	2.64[6.88]	0.014	0.385	0.700	−0.058	−13.05[17.38]	−0.058	0.751	0.454
	Recessive	−0.053	−10.50[6.97]	−0.053	1.50	0.132	−0.053	−12.67[18.51]	−0.053	0.685	0.495
	Overdominat	−0.034	−5.82[6.04]	−0.034	0.96	0.335	−0.104	−22.46[16.67]	−0.104	1.34	0.180
	Allele	−0.029	−4.95[4.26]	−0.029	1.16	0.246	0.004	0.83[11.60]	0.004	0.072	0.943
Wake time weekdays											
	Codominant	−0.018	−2.28[8.76]	−0.011	0.26	0.754	−0.025	−3.50[19.12]	−0.017	0.184	0.854
	Dominant	0.018	3.60[7.12]	0.018	0.506	0.613	−0.025	−5.10[15.61]	−0.025	0.327	0.744
	Recessive	0.002	0.50[7.22]	0.002	0.070	0.944	−0.044	−9.46[16.61]	−0.044	0.569	0.570
	Overdominat	0.018	3.15[6.25]	0.018	0.504	0.614	−0.064	−12.47[15.01]	−0.064	0.831	0.407
	Allele	−0.007	−1.19[4.42]	−0.007	0.271	0.786	−0.008	−1.44[10.40]	−0.008	0.139	0.890
Sleep duration weekdays											
	Codominant	0.008	7.30[8.36]	0.038	0.873	0.383	−0.046	−4.01[18.21]	−0.021	0.221	0.826
	Dominant	0.008	1.49[6.81]	0.008	0.22	0.826	0.046	8.88[14.85]	0.046	0.598	0.551
	Recessive	0.057	11.20[6.89]	0.057	1.62	0.105	0.013	2.60[15.85]	0.013	0.164	0.870
	Overdominat	0.056	9.54[5.96]	0.056	1.59	0.110	0.056	10.36[14.28]	0.056	0.725	0.469
	Allele	0.021	3.61[4.22]	0.021	0.857	0.392	−0.016	−2.94[9.90]	−0.016	0.297	0.767
Midpoint of sleep weekdays											
	Codominant	−0.016	−5.93[7.52]	−0.034	0.789	0.430	−0.050	−1.54[18.07]	−0.008	0.085	0.932
	Dominant	0.016	2.85[6.11]	0.016	0.467	0.641	−0.050	−9.53[14.78]	−0.050	0.645	0.524
	Recessive	−0.029	−5.09[6.20]	−0.029	0.821	0.412	−0.053	−10.79[15.73]	−0.053	0.686	0.424
	Overdominat	−0.011	−1.61[5.37]	−0.011	0.300	0.764	−0.096	−17.66[14.18]	−0.096	1.24	0.215
	Allele	−0.020	−3.00[5.28]	−0.020	0.793	0.428	−0.000	0.00[9.85]	−0.000	0.001	0.999
Bedtime weekends											
	Codominant	−0.006	−6.18[0.55]	−0.025	0.590	0.555	−0.034	−28.83[2717]	−0.101	1.06	0.289
	Dominant	0.006	1.35[8.52]	0.006	0.159	0.874	0.034	−9.69[22.19]	0.034	0.237	0.663
	Recessive	−0.028	−6.09[8.64]	−0.028	0.799	0.424	−0.100	−30.55[23.51]	−0.100	1.12	0.196
	Overdominat	−0.019	−4.13[7.48]	−0.019	0.552	0.589	−0.058	−16.00[21.34]	−0.058	0.750	0.454
	Allele	−0.015	3.10[5.26]	−0.015	0.558	557	−0.061	−16.36[14.76]	−0.061	1.10	0.268
Wake time weekends											
	Codominant	−0.023	−8.76[10.36]	−0.037	0.847	0.397	−0.045	−32.30[28.34]	−0.108	1.14	0.256
	Dominant	0.023	5.55[8.42]	0.023	0.658	0.510	0.045	13.41[23.19]	0.045	0.578	0.564
	Recessive	−0.026	−6.23[8.42]	−0.026	0.729	0.466	−0.100	−31.90[24.59]	−0.100	1.29	0.196
	Overdominat	−0.002	−0.38[7.40]	−0.002	0.053	0.477	−0.047	−13.67[22.33]	−0.047	0.612	0.541
	Allele	−0.021	4.47[5.22]	−0.021	0.855	0.393	−0.066	−18.55[15.43]	−0.066	1.20	0.230
Sleep duration weekends											
	Codominant	−0.025	−3.17[8.57]	−0.016	0.370	0.711	−0.028	−4.54[16.29]	−0.027	0.279	0.781
	Dominant	0.025	4.98[6.57]	0.025	0.715	0.475	0.028	4.78[13.28]	0.028	0.360	0.719
	Recessive	0.003	0.68[7.07]	0.003	0.097	0.923	−0.010	−1.84[14.14]	−0.010	0.131	0.896
	Overdominat	−0.025	4.35[6.12]	−0.025	0.712	0.477	0.018	2.92[12.78]	0.018	0.229	0.819
	Allele	−0.010	−1.16[4.32]	−0.010	0.385	0.700	−0.018	−2.86[8.84]	−0.018	0.324	0.746
Midpoint of sleep weekends											
	Codominant	−0.014	−7.21[4.21]	−0.033	0.764	0.923	−0.039	−30.03[26.52]	−0.107	1.13	0.259
	Dominant	0.014	3.06[7.69]	0.014	0.398	0.691	0.039	11.01[21.71]	0.039	0.507	0.613
	Recessive	−0.030	−6.59[7.60]	−0.030	0.845	0.399	0.104	−30.98[23.00]	0.104	1.34	0.180
	Overdominat	−0.013	−2.57[6.75]	−0.013	0.381	0.703	0.056	−15.13[20.89]	0.056	0.724	0.613
	Allele	−0.019	−3.64[4.77]	−0.019	0.764	0.445	0.065	−17.12[14.44]	0.065	1.18	0.237
Social jetlag											
	Codominant	−0.004	−0.77[7.57]	−0.004	0.097	0.923	−0.125	−34.93[17.45]	−0.188	2.00	0.047
	Dominant	0.004	0.66[6.15]	0.004	0.108	0.914	−0.125	23.15[14.28]	−0.125	1.62	0.107
	Recessive	−0.002	−0.31[6.24]	−0.002	0.051	0.959	−0.135	−26.63[15.18]	−0.135	1.75	0.081
	Overdominat	−0.002	0.27[5.40]	−0.002	0.051	0.959	−0.002	−0.32[13.86]	−0.002	0.023	0.981
	Allele	−0.002	0.37[3.82]	−0.002	0.099	0.922	**−0.119**[a]	**−20.82[9.52]**[3]	**−0.119**	**2.18**	**0.237**

Values in bold presented significance in statistical analysis. r = Pearson's correlation; B = unstandardized coefficients; SE = standard error; β = standardized coefficients. MEQ, Morningness–Eveningness Questionnaire.
Notes:**1**: $R^2 = 0.005$; adjusted $R^2 = 0.004$; $F(1,812) = 3.92$, $p = 0.048$. **2**: $R^2 = 0.026$; adjusted $R^2 = 0.020$; $F(1,166) = 4.40$, $p = 0.038$. **3**: $R^2 = 0.014$; adjusted $R^2 = 0.020$; $F(1,334) = 4.78$, $p = 0.029$.
[a] $p < 0.05$.

In the recessive model, the extreme morning group also demonstrated more −1193C carriers (C/C + C/T = 86%) than T/T individuals (14%) compared to the extreme evening group (C/C + C/T = 66% and T/T = 34%; OR = 3.08, 95% CI [1.21–7.81], $p = 0.018$) and intermediate controls (C/C + C/T = 68% and T/T = 32%; OR = 0.35, 95% CI [0.13–0.89], $p = 0.029$) (Fig. 2(b)). Furthermore, the −1193C allele frequency was significantly higher in the morning group (C = 62% and T = 38%) compared to the evening group

Table 2
Multinomial logistic regression analysis performed for association between the −1193T > C genotypes and diurnal preference for total sample ($N = 814$), and a subset including extreme chronotypes and intermediate controls ($N = 168$).

Models	Total sample ($N = 814$)						Subset of diurnal preference ($N = 168$)					
	E* vs. I OR (95% CI)	*P*	E* vs. M OR (95% CI)	*p*	M* vs. I OR (95% CI)	*p*	E* vs. I OR (95% CI)	*p*	E* vs. M OR (95% CI)	*p*	M* vs. I OR (95% CI)	*P*
Codominant												
CC	1.05 (0.65−1.67)	0.838	1.50 (0.86−2.61)	0.152	0.70 (0.43−1.13)	0.417	1.25 (0.49−3.16)	0.558	**3.11 (1.08−8.92)**[a]	**0.034**	0.40 (0.14−1.13)	0.086
CT	0.96 (0.63−1.46)	0.868	0.94 (0.58−1.51)	0.068	0.68 (0.44−1.04)	0.082	0.95 (0.39−2.33)	0.633	**3.05 (1.11−8.33)**[b]	**0.029**	**0.31 (0.11−0.86)**[e]	**0.025**
TT	1.00		1.00		1.00		1.00		1.00		1.00	
Dominant												
CC	0.97 (0.67−1.47)	0.980	1.09 (0.70−1.70)	0.680	0.90 (0.62−1.32)	0.620	1.28 (0.57−2.86)	0.541	1.50 (0.67−3.31)	0.316	1.16 (0.53−2.53)	0.693
CT + TT	1.00		1.00		1.00		1.00		1.00		1.00	
Recessive												
CC + CT	1.07 (0.73−1.57)	0.710	1.56 (0.98−2.48)	0.060	1.45 (0.96−2.17)	0.072	1.08 (0.49−2.38)	0.841	**3.08 (1.21−7.81)**[c]	**0.018**	**0.35 (0.13−0.89)**[f]	**0.029**
TT	1.00		1.00		1.00		1.00		1.00		1.00	
Overdominant												
C/C + T/T	0.94 (0.66−1.32)	0.728	0.78 (0.52−1.15)	0.212	1.20 (0.86−1.68)	0.269	1.16 (0.53−2.53)	0.693	0.64 (0.30−1.36)	0.253	1.81 (0.84−3.88)	0.126
C/T	1.00		1.00		1.00		1.00		1.00		1.00	
Alleles												
C	1.02 (0.80−1.30)	0.830	1.21 (0.52−1.60)		0.84 (0.66−1.07)	0.162	1.15 (0.68−1.94)	0.593	**1.78 (1.05−3.04)**[d]	**0.032**	0.64 (0.38−1.09)	0.107
T	1.00		1.00		1.00		1.00		1.00		1.00	

E, evening types; I, intermediate types; M, morning types; OR, odds ratio; CI, confidential interval; 1.00, Reference category. * Reference group. Values in bold presented significance in statistical analysis.
[a] B = 1.13 and SE = 0.53.
[b] −B = 1.11 and SE = 0.51.
[c] B = 1.12 and SE = 0.47.
[d] B = 0.58 and SE = 0.27.
[e] B = −1.16 and SE = 0.52.
[f] B = −1.04 and SE = 0.47.

Table 3
Genotypic and allelic frequencies of −1193T > C polymorphism distribution among the three diurnal preference groups for total sample ($N = 814$) and a subset including extreme morning/evening types and intermediate controls ($N = 168$).

Chronotypes	Total sample						Subset of diurnal preference					
	N	C/C	C/T	T/T	C	T	*N*	C/C	C/T	T/T	C	T
Evening	195	0.25	0.47	0.28	0.49	0.51	56	0.29	0.37	0.34	0.47	0.53
Intermediate	411	0.26	0.48	0.26	0.50	0.50	56	0.34	0.34	0.32	0.51	0.49
Morning	208	0.27	0.53	0.20	0.54	0.46	56	0.38	0.48	0.14	0.62	0.38
Combined	814	0.26	0.49	0.25	0.51	0.49	112	0.33	0.43	0.24	0.55	0.45

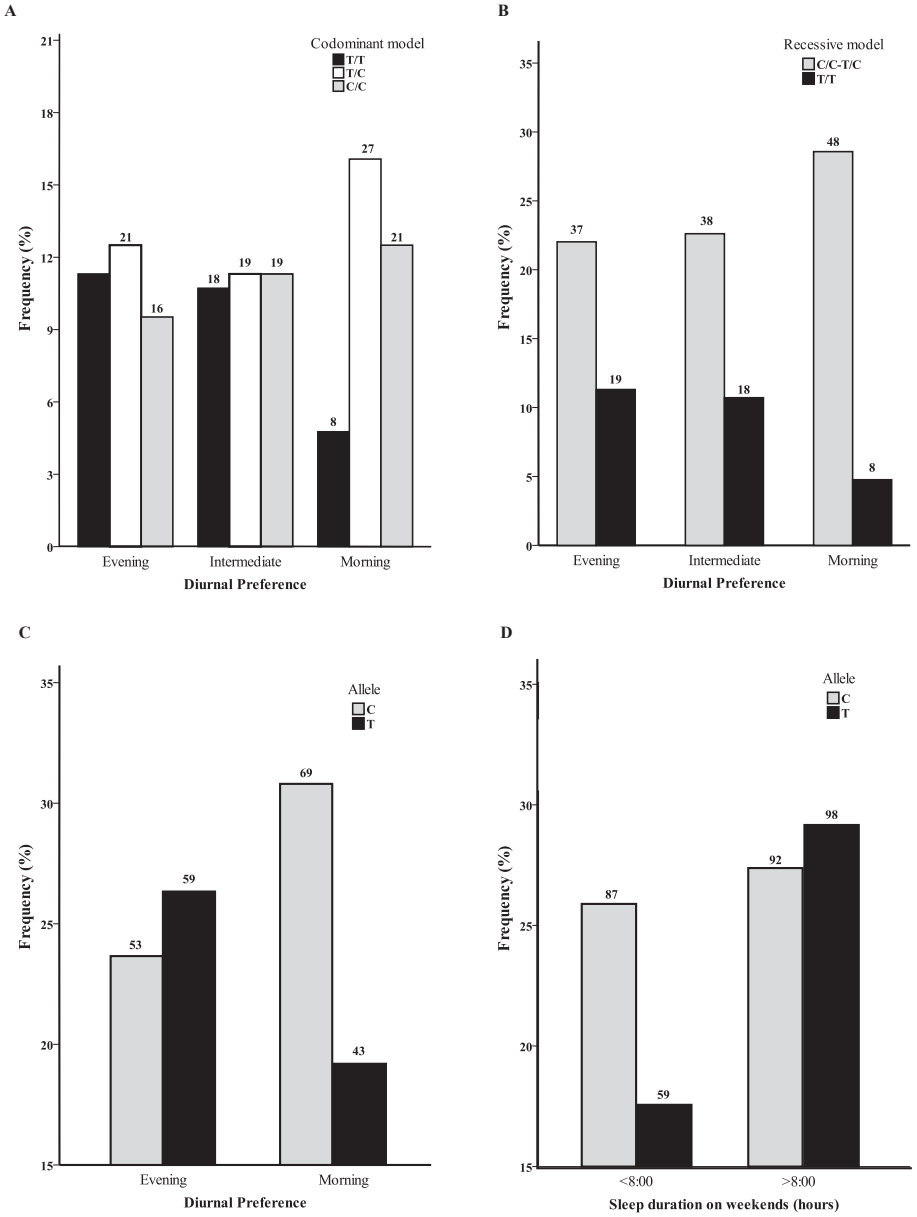

Fig. 2. Association of the −1193T > C polymorphism with diurnal preference and sleep duration. (a) The number of T/T, T/C, and C/C genotypes in the extreme morning/evening types and intermediate control group according to the codominant model. There are more C/C and T/C genotypes within the extreme morning than evening group (odds ratio (OR) = 3.11, 95% confidence interval (CI) (1.08–8.92), $p = 0.034$ and OR = 3.05, 95% CI (1.11–8.33), $p = 0.029$). (b) The number of C/C + C/T and T/T genotypes in extreme morning/evening subjects and intermediate controls according to the recessive model. There is a higher number of C/C + C/T genotypes in the extreme morning compared to the extreme evening group (OR = 3.08, 95% CI (1.21–7.81), $p = 0.018$). (c) The number of −1193T > C alleles in extreme morning/evening chronotypes. The C allele is more frequent in the morning group (OR = 1.78, 95% CI (1.05, 3.04), $p = 0.032$). (d) The number of −1193T > C alleles in the groups with more than 8 h and less than 8 h of sleep. The T allele frequency is higher in the group that stay in bed more than 8 h/night (OR = 1.60, 95% CI (1.04–2.47), $p = 0.032$).

(C = 47% and T = 53%) (OR = 1.78, 95% CI [1.05–3.04], $p = 0.032$) (Fig. 2(c)). However, no difference was found in allele distribution comparing morning vs. intermediate controls.

3.2. MTNR1B −1193T > C polymorphism and sleep habits

A linear regression analysis conducted to evaluate the −1193T > C SNP effect in sleep habits revealed that there was no −1193T > C genotype influence on sleep habits in the total sample ($N = 814$, $p > 0.05$) (Table 1). However, in the subset including extreme chronotypes and intermediate controls ($N = 168$), the −1193C allele was negatively correlated with social jetlag scores. The model accounts for 1.4% of the explained variability and adequately adjusted to the data [$F(1,334) = 4.78$, $p = 0.029$]. The −1193C allele negatively predicted the social jetlag ($\beta = -0.12$, $p = 0.029$).

Additionally, a logistic regression was used to investigate whether the −1193T > C frequencies differed regarding the sleep duration on weekdays/weekends in the total sample ($N = 814$) and in the subgroup including extreme chronotypes and intermediate controls ($N = 168$). The results showed that allelic frequency is similar for individuals who sleep more or less than 8 h/night on weekdays (OR = 1.07, 95% CI [0.59–1.91], $p = 0.819$). However, on weekends, a significant difference in allelic frequencies was observed between individuals who sleep more than 8 h/night (T = 52% and C = 48%) and individuals who sleep less than 8 h/night (T = 40% and C = 60%), with a higher −1193T allele frequency in the group that stay in bed more than 8 h/night (OR = 1.60, 95% CI [1.04–2.47], $p = 0.032$) (Fig. 2(d)).

4. Discussion

SCN regulates the synthesis and release of melatonin by the pineal gland, which feeds back to modulate sleep and circadian phase through the activation of MT1 and MT2 receptors [33]. Studies in vitro [12,34] and in vivo [35,36] have established that MT2 receptor mediates phase advanced effect of melatonin on circadian rhythms in the SCN, depending on the dose and timing of sensibility [3]. In addition, endogenous melatonin facilitates the re-entrainment of the circadian locomotor activity rhythm after phase advance by acting upon MT2 receptors [36]. In humans, a diurnal rhythm in MT2 expression was described in the SCN, with a peak around midnight [37]. The MT2-dependent phase advance seems to be mediated through the increase in protein kinase C (PKC) activity [38], which is necessary for melatonin-induced changes in *Per1* and *Per2* expression at dusk (CT 10) [39].

In the present study, we hypothesized that the −1193T > C variation in the MT2 gene could be associated with diurnal preference and sleep habits. Our data show that the −1193C/C and −1193T/C genotypes, in a codominant model of inheritance, and −1193C allele frequency are significantly higher in the group of extreme morning types compared to the extreme evening group. Curiously, a previous research found a negative correlation between −1193C allele frequency and sunshine duration among worldwide populations [18]. The authors suggested that the selection of this derived allele would provide an advantage to populations adapting to shorter sunshine durations and, consequently, a more efficient adaptation to seasonal variations. If we consider that shorter sunshine length leads to an increased duration of melatonin secretion [40], a possible physiological adaptation could be the selection of genetic variants in the receptors that balance this increased melatonin, potentially impacting the circadian rhythm plasticity and sleep homeostasis.

Additionally, we found an association of the −1193T allele with the group that stayed in bed more than 8 h/night on weekends, suggesting that −1193T could modulate the melatonin role in circadian regulation of sleep [41]. The sleep-promoting effects of melatonin result from phase advancing the circadian clock [42] or inhibiting the drive for wakefulness [43]. Thus, the −1193T allele could be responsible for higher MT2 receptors levels and an earlier onset of melatonin signal, resulting in a longer sleep duration compared to the −1193C allele. On weekends, students are free from timetable restrictions, so the sleep habits reflect their endogenous circadian tendencies [44]. Furthermore, diurnal preference did not differ in sleep duration on weekends, in accordance with previous studies [41,45].

Morning–evening people differ in their intrinsic circadian period, with shorter periods associated with morning-types and longer periods associated with evening-types [46]. Since sleep propensity is under circadian regulation [47], it is expected that morning types and evening types go to bed and wake up at different times [48]. In fact, our results showed a robust and consistent difference in sleep/wake times between morning and evening types in accordance with large studies [49,50]. Evening types presented a higher social jetlag compared to morning types, as expected [23], and regarding the analysed polymorphism, the −1193T allele was associated with longer social jetlag (20 min) in the subset including extreme chronotypes and intermediate controls.

A possible explanation for these results is that, because the −1193T > C polymorphism is located in the promoter region, the −1193C/C genotype could be responsible for lower MT2 levels, causing a delay in melatonin signalling onset. On the other hand, if the −1193T/T genotype contributes to higher MT2 receptor level and a slower desensitization of endogenous MT2 receptors, the −1193T allele may contribute to a prolonged transduction of the melatonin signal, leading to an extended sleep duration [51]. In fact, gene expression data from −1193T > C genotypes in GTEx database (http://www.gtexportal.org/home/) show that C homozygous has a significantly lower mRNA level compared to C/T and T/T genotypes in the oesophagus–mucosa tissue ($p < 0.001$) (Supplementary Fig. S1).

These hypotheses could also explain two other findings previously discussed: (1) a higher frequency of −1193C variant in the morning group, which would correspond to an earlier signalling and a faster inactivation of the melatonin pathway; (2) a lower frequency of the same allele in populations exposed to longer sunshine durations [18], potentially leading to increased MT2 levels, which compensates for less melatonin synthesis in this environmental condition. However, further studies incorporating functional evaluations are warranted to confirm the association and to clarify the potential biological mechanisms of this polymorphism in diurnal preference and sleep habits.

Although MEQ and MCTQ scores have been significantly correlated with the internal period (τ) [52], a potential limitation of our study is related to the use of self-report questionnaires, which are an indirect measure of the circadian phenotypes. Moreover, we selected in some analyses subjects who are extreme morning or evening types in order to reduce the sample variance and increase the power per genotyped individual. This method, however, has been used for association studies regarding diurnal preference [26]. Another limitation of this study is not taking into account the exposure to artificial light at night (late night reading, TV, computer or smartphones) as exclusion criteria. Nocturnal light decreases pineal melatonin production and secretion [53]. This suppression could alter melatonin receptor expression, because the hormone regulates its own receptor levels [54], and therefore modify sleep response. Urban areas are bathed in light which can also contribute to lower melatonin levels in relation to rural areas [55].

One further possible limitation is that our sample was composed mainly of females. Sex differences in melatonin rhythms have been described [56,57], with women tending to have a higher melatonin

amplitude and an earlier onset of peak melatonin levels compared to men. Although MEQ scores did not differ between males and females in our sample, an earlier timing of melatonin onset (DLMO) could be observed in females [56,57], which can explain sex differences in some sleep parameters such as bedtime on weekdays/weekends and midpoint of sleep on weekdays ($p \leq 0.001$ data not shown). Thus, other studies with mixed populations living in the same or different latitudes, and considering the influence of artificial light and sex differences, would be necessary for determining the replication of this association.

5. Conclusion

In summary, this study demonstrated that the −1193C allele was associated with morningness and −1193T influences sleep habits. To our knowledge, this is the first report associating a polymorphism in a melatonin receptor gene with diurnal preference, adding to previous information regarding melatonin's function in circadian rhythm regulation. In addition, this genetic variability may affect the action of melatonin and its agonists used in the treatment of sleep and mood disorders.

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Conflict of interest

All authors declare no conflicts of interest for this article.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: https://doi.org/10.1016/j.sleep.2018.09.023.

Appendix A. Supplementary data

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References

[1] Tsang AH, Barclay JL, Oster H. Interactions between endocrine and circadian systems. J Mol Endocrinol 2014;52:R1–16.
[2] Pfeffer M, Korf HW, Wicht H. Synchronizing effects of melatonin on diurnal and circadian rhythms. Gen Comp Endocrinol 2018;258:215–21.
[3] McArthur AJ, Hunt AE, Gillette MU. Melatonin action and signal transduction in the rat suprachiasmatic circadian clock: activation of protein kinase C at dusk and dawn. Endocrinology 1997;138:627–34.
[4] Schuhler S, Pitrosky B, Kirsch R, et al. Entrainment of locomotor activity rhythm in pinealectomized adult Syrian hamsters by daily melatonin infusion. Behav Brain Res 2002;133:343–50.
[5] Arendt J, Skene DJ. Melatonin as a chronobiotic. Sleep Med Rev 2005;9(1):25–39.
[6] Keijzer H, Smits MG, Duffy JF, et al. Why the dim light melatonin onset (DLMO) should be measured before treatment of patients with circadian rhythm sleep disorders Sleep. Med Rev 2014;18:333–9.
[7] Sack RL, Auckley D, Auger RR, et al. Circadian rhythm sleep disorders: part I, basic principles, shift work and jet lag disorders. Sleep 2007;30:1460–83.
[8] Sack RL, Auckley D, Auger RR, et al. Circadian rhythm sleep disorders: part II, advanced sleep phase disorder, delayed sleep phase disorder, free-running disorder, and irregular sleep–wake rhythm. Sleep 2007;30:1484–501.
[9] Ferracioli-Oda E, Qawasmi A, Bloch MH. Meta-analysis: melatonin for the treatment of primary sleep disorders. PloS One 2013;8, e63773.
[10] Pevet P. Melatonin receptors as therapeutic targets in the suprachiasmatic nucleus. Expert Opin Ther Targets 2016;20:1209–18.
[11] Dubocovich ML. Melatonin receptors: role on sleep and circadian rhythm regulation. Sleep Med 2007;8:34–42.
[12] Hunt AE, Al-Ghoul WM, Gillette MU, et al. Activation of MT(2) melatonin receptors in rat suprachiasmatic nucleus phase advances the circadian clock. Am J Physiol Cell Physiol 2001;280:C110–8.
[13] Ebisawa T, Kajimura N, Uchiyama M, et al. Allelic variants of human melatonin 1a receptor: function and prevalence in subjects with circadian rhythm sleep disorders. Biochem Biophys Res Commun 1999;262:832–7.
[14] Park HJ, Park JK, Kim SK, et al. Association of polymorphism in the promoter of the melatonin receptor 1A gene with schizophrenia and with insomnia symptoms in schizophrenia patients. J Mol Neurosci 2011;45:304–8.
[15] Chang AM, Bjonnes AC, Aeschbach D, et al. Circadian gene variants influence sleep and the sleep electroencephalogram in humans. Chronobiol Int 2016;33:561–73.
[16] Galecka E, Szemraj J, Florkowski A, et al. Single nucleotide polymorphisms and mRNA expression for melatonin MT(2) receptor in depression. Psychiatr Res 2011;189:472–4.
[17] Staiger H, Machicao F, Schafer SA, et al. Polymorphisms within the novel type 2 diabetes risk locus MTNR1B determine beta-cell function. PloS One 2008;3:e3962.
[18] Ji LD, Xu J, Wu DD, et al. Association of disease-predisposition polymorphisms of the melatonin receptors and sunshine duration in the global human populations. J Pineal Res 2010;48:133–41.
[19] Deming SL, Lu W, Beeghly-Fadiel A, et al. Melatonin pathway genes and breast cancer risk among Chinese women. Breast Cancer Res Treat 2012;132:693–9.
[20] Rabstein S, Harth V, Justenhoven C, et al. Polymorphisms in circadian genes, night work and breast cancer: results from the GENICA study. Chronobiol Int 2014;31:1115–22.
[21] Lane JM, Chang AM, Bjonnes AC, et al. Impact of common diabetes risk variant in MTNR1B on sleep, circadian, and melatonin physiology. Diabetes 2016;65:1741–51.
[22] Consortium GT. The genotype-tissue expression (GTEx) project. Nat Genet 2013;45:580–5.
[23] Levandovski R, Dantas G, Fernandes LC, et al. Depression scores associate with chronotype and social jetlag in a rural population. Chronobiol Int 2011;28:771–8.
[24] Horne JA, Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol 1976;4:97–110.
[25] Benedito-Silva AA, Menna-Barreto L, Marques N, et al. A self-assessment questionnaire for the determination of morningness-eveningness types in Brazil. Prog Clin Biol Res 1990;341B:89–98.
[26] Robilliard DL, Archer SN, Arendt J, et al. The 3111 Clock gene polymorphism is not associated with sleep and circadian rhythmicity in phenotypically characterized human subjects. J Sleep Res 2002;11:305–12.
[27] Ronin Y, Korol A, Weller J. Selective genotyping to detect quantitative trait loci affecting multiple traits: interval mapping analysis. Theor Appl Genet 1998;97:1169–78.
[28] Roenneberg T, Wirz-Justice A, Merrow M. Life between clocks: daily temporal patterns of human chronotypes. J Biol Rhythm 2003;18:80–90.
[29] Wittmann M, Dinich J, Merrow M, et al. Social jetlag: misalignment of biological and social time. Chronobiol Int 2006;23:497–509.
[30] Roenneberg T. What is chronotype? Sleep Biol Rhythm 2012;10:75–6.
[31] Zavada A, Gordijn MC, Beersma DG, et al. Comparison of the Munich chronotype questionnaire with the Horne-Ostberg's morningness-eveningness score. Chronobiol Int 2005;22:267–78.
[32] Aljanabi SM, Martinez I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res 1997;25:4692–3.
[33] Pevet P. The internal time-giver role of melatonin. A key for our health. Rev Neurol 2014;170:646–52.
[34] Dubocovich ML, Markowska M. Functional MT1 and MT2 melatonin receptors in mammals. Endocrine 2005;27:101–10.
[35] Dubocovich ML, Yun K, Al-Ghoul WM, et al. Selective MT2 melatonin receptor antagonists block melatonin-mediated phase advances of circadian rhythms. FASEB J 1998;12:1211–20.
[36] Pfeffer M, Rauch A, Korf HW, et al. The endogenous melatonin (MT) signal facilitates reentrainment of the circadian system to light-induced phase advances by acting upon MT2 receptors. Chronobiol Int 2012;29:415–29.
[37] Wu YH, Ursinus J, Zhou JN, et al. Alterations of melatonin receptors MT1 and MT2 in the hypothalamic suprachiasmatic nucleus during depression. J Affect Disord 2013;148:357–67.
[38] Rivera-Bermudez MA, Masana MI, Brown GM, et al. Immortalized cells from the rat suprachiasmatic nucleus express functional melatonin receptors. Brain Res 2004;1002:21–7.
[39] Kandalepas PC, Mitchell JW, Gillette MU. Melatonin signal transduction pathways require E-box-mediated transcription of Per1 and Per2 to reset the SCN clock at dusk. PloS One 2016;11, e0157824.
[40] Adamsson M, Laike T, Morita T. Annual variation in daily light exposure and circadian change of melatonin and cortisol concentrations at a northern latitude with large seasonal differences in photoperiod length. J Physiol Anthropol 2016;36:6.
[41] Gandhi AV, Mosser EA, Oikonomou G, et al. Melatonin is required for the circadian regulation of sleep. Neuron 2015;85:1193–9.
[42] Arendt J. Importance and relevance of melatonin to human biological rhythms. J Neuroendocrinol 2003;15:427–31.
[43] Scheer FA, Czeisler CA. Melatonin, sleep, and circadian rhythms. Sleep Med Rev 2005;9:5–9.
[44] Arrona-Palacios A, Díaz-Morales JF. Morningness–eveningness and sleep habits at school: a comparative study between Mexico and Spain. Biol Rhythm Res 2016;48:175–88.
[45] Roepke SE, Duffy JF. Differential impact of chronotype on weekday and weekend sleep timing and duration. Nat Sci Sleep 2010;2:213–20.

[46] Duffy JF, Rimmer DW, Czeisler CA. Association of intrinsic circadian period with morningness-eveningness, usual wake time, and circadian phase. Behav Neurosci 2001;115:895–9.
[47] Lavie P. Sleep-wake as a biological rhythm. Annu Rev Psychol 2001;52:277–303.
[48] Mongrain V, Lavoie S, Selmaoui B, et al. Phase relationships between sleep-wake cycle and underlying circadian rhythms in morningness–eveningness. J Biol Rhythm 2004;19:248–57.
[49] Paine SJ, Gander PH. Differences in circadian phase and weekday/weekend sleep patterns in a sample of middle-aged morning types and evening types. Chronobiol Int 2016;33:1009–17.
[50] Bakotic M, Radosevic-Vidacek B, Koscec Bjelajac A. Morningness–eveningness and daytime functioning in university students: the mediating role of sleep characteristics. J Sleep Res 2017;26:210–8.
[51] Gerdin MJ, Masana MI, Rivera-Bermudez MA, et al. Melatonin desensitizes endogenous MT2 melatonin receptors in the rat suprachiasmatic nucleus: relevance for defining the periods of sensitivity of the mammalian circadian clock to melatonin. FASEB J 2004;18:1646–56.
[52] Kantermann T, Sung H, Burgess HJ. Comparing the morningness-eveningness questionnaire and Munich ChronoType questionnaire to the dim light melatonin onset. J Biol Rhythm 2015;30:449–53.
[53] Gooley JJ, Chamberlain K, Smith KA, et al. Exposure to room light before bedtime suppresses melatonin onset and shortens melatonin duration in humans. J Clin Endocrinol Metab 2010;96:E463–72.
[54] Bedrosian TA, Herring KL, Walton JC, et al. Evidence for feedback control of pineal melatonin secretion. Neurosci Lett 2013;542:123–5.
[55] Stevens RG, Zhu Y. Electric light, particularly at night, disrupts human circadian rhythmicity: is that a problem? Phil Trans R Soc Lond B Biol Sci 2015;370:20140120.
[56] Cain SW, Dennison CF, Zeitzer JM, et al. Sex differences in phase angle of entrainment and melatonin amplitude in humans. J Biol Rhythm 2010;25:288–96.
[57] Gunn PJ, Middleton B, Davies SK, et al. Sex differences in the circadian profiles of melatonin and cortisol in plasma and urine matrices under constant routine conditions. Chronobiol Int 2016;33:39–50.