Eur Arch Psychiatry Clin Neurosci (2014) 264:71–81
DOI 10.1007/s00406-013-0443-y

ORIGINAL PAPER

Human CLOCK gene-associated attention deficit hyperactivity disorder-related features in healthy adults: quantitative association study using Wender Utah Rating Scale

Seong Hoon Jeong · Je-Chun Yu · Chang Hwa Lee · Kyeong-Sook Choi · Jung-Eun Choi · Se Hyun Kim · Eun-Jeong Joo

Received: 10 December 2012 / Accepted: 2 September 2013 / Published online: 26 September 2013

Abstract Circadian rhythm disturbance is highly prevalent in attention deficit hyperactivity disorder (ADHD). Recently, the association between the CLOCK gene and ADHD has been demonstrated in clinical samples, and the CLOCK gene's role was thought to be mediated by rhythm dysregulation. Meanwhile, ADHD has been suggested as the extreme end of a continuously distributed trait that can be found in the general population. Therefore, we examined two possibilities: (1) an ADHD-related continuous trait may be associated with the CLOCK gene, and (2) this association may be mediated by the degree of individuals' evening preference. To explore these possibilities, we performed a quantitative trait locus association study with a sample of 1,289 healthy adults. The Wender Utah Rating Scale (WURS) and the Composite Scale of Morningness (CSM) were utilized to measure the quantitative traits. Quantitative association analysis was performed using PLINK software. We found that rs1801260 (=T3111C) was associated with WURS scores in both allele-wise ($p = 0.018$) and haplotype-wise analyses (range of p values: 0.0155–0.0171) in male participants only. After controlling for the CSM total score as a covariate, the strength of the association did not change at all, suggesting that the association was not mediated by evening preference. Despite the very weak association signal, our results provide evidence that the CLOCK gene's association with ADHD in clinical samples may be generalizable to traits measured in the normal population. However, as our results failed to show a mediating role of evening preference, ongoing efforts are needed to identify the mechanisms by which the CLOCK gene determines ADHD-related traits.

Keywords Association study · Attention deficit hyperactivity disorder · CLOCK gene · Circadian rhythm · Composite Scale of Morningness · Wender Utah Rating Scale

S. H. Jeong · J.-C. Yu · C. H. Lee · K.-S. Choi
Department of Psychiatry, Eulji University School of Medicine, Eulji University Hospital, Daejeon, Korea

J.-E. Choi
Department of Psychiatry, Seoul Metropolitan EunPyeong Hospital, Seoul, Korea

S. H. Kim
Institute of Human Behavioral Medicine, Medical Research Institute, Seoul National University, Seoul, Korea

E.-J. Joo (✉)
Department of Psychiatry, Eulji University School of Medicine, Eulji General Hospital, 28 Hangeulbiseokro, Nowon-Gu, Seoul 139-711, South Korea
e-mail: jej1303@gmail.com

Introduction

Attention deficit hyperactivity disorder (ADHD) is a common neuropsychiatric condition, with a worldwide prevalence of 5 % in school-age children [1]. A substantial number of children continue to suffer even after they have reached adulthood. The detrimental effects of ADHD are all the more apparent when the extensive comorbidity with other psychiatric conditions is considered. The rate of comorbidity in ADHD is much higher than it is in other diagnoses. Antisocial behavior and substance use disorders are frequent comorbid conditions, and rates of mood and anxiety disorders reach almost 50 % [2]. Additionally, symptomatic overlap and extensive comorbidity with bipolar disorder have been intensively discussed [3]. Moreover, a large proportion of patients exhibiting

substance abuse, mood or anxiety disorders, obesity, smoking, and illegal behavior also meet the diagnostic criteria for ADHD [4–6].

The extensive overlap between ADHD and such conditions suggests that certain features of ADHD may not be specific to ADHD but rather coextensive with a wider range of psychological and behavioral conditions. It has been proposed that ADHD may be a foundational disorder that substantially increases a person's risk of additional psychiatric impairments during his or her lifetime [7]. Alternatively, some authors have argued that ADHD may be considered an extreme end of a continuum of traits common to the general population [8]. If this is true, then a broader conceptualization of the ADHD-related features that are common and continuous traits may offer a better target for genetic studies than would a specific diagnosis.

To study such traits, the target population needs to be extended to include the general population. Additionally, the candidate genes must be selected based on their implications for similarly common phenotypes. Disrupted circadian rhythm may be one such candidate phenotype. Indeed, this is very common in the general population, and its involvement in the core pathophysiology of ADHD has been suggested [9]. Patients with ADHD have various types of sleep disturbances [10] as well as altered circadian profiles of melatonin and cortisol secretions [11]. Furthermore, the degree of the disruption in circadian rhythm has a significant impact on ADHD symptom severity, daytime functioning, and health outcomes [12].

Diurnal preference, also called morning or evening preference, reflects individual differences in circadian topology [13]. Evening preference is related to various emotional and behavioral problems, stress response patterns, and specific personality traits. Interestingly, many of these are also associated with ADHD [14]. Subjective and objective measures of attention deficits [15, 16], impulsivity, and novelty seeking [17] are among the typical examples. It should be noted that comorbid conditions other than ADHD show strong relationships with evening preference. Evening preference has been shown to be more frequent than morning preference in depression [18, 19], substance use [20], and bipolar disorder [21]. These findings support the notion that the coextensive relationship between ADHD and other comorbid conditions may be mediated by disrupted circadian rhythm or a specific diurnal preference.

Diurnal preference is largely determined by stable genetic components [22]. Thus far, the so-called clock-related genes have been believed to be responsible. The clock-related genes and their products tightly regulate the human circadian rhythm [23]. The involvement of the CLOCK gene in determining individuals' diurnal preference has been extensively studied. A study conducted by Katzenberg et al. [24] demonstrated that the rs1802160 polymorphism (T3111C) in the CLOCK gene is associated with evening preference; this finding was replicated in a Japanese population [25]. The relationship among the CLOCK gene, diurnal preference, and ADHD led to a hypothesis that changes in circadian rhythm induced by polymorphisms in the CLOCK gene may contribute to ADHD pathophysiology. An association between the CLOCK gene and ADHD has been demonstrated in German and Chinese populations [26, 27]. If this hypothesis were true, the CLOCK gene may link disrupted circadian rhythms and ADHD-related symptoms in a wide range of psychiatric conditions.

In this context, we aimed to investigate the genetic association between the CLOCK gene and ADHD-related features and to demonstrate the possible mediating role of evening preference in the relationship between these two factors. To this end, we typed the CLOCK gene polymorphisms in a sample of healthy adults and simultaneously measured diurnal preference and ADHD-related symptoms using the Composite Scale of Morningness (CSM) and the Wender Utah Rating Scale-25 (WURS-25), respectively. We expect that this study will help to clarify the possible mediating role of diurnal preference in the association between the CLOCK gene and ADHD-related symptoms.

Materials and methods

Study participants

Study participants were recruited from two different sources, referred to as subset #1 and subset #2 in the following discussion. Subset #1 included workers and students in university-affiliated general hospitals, and subset #2 consisted of workers in government offices. A description of the study's purpose and procedure was advertised through posters and leaflets. All participants were provided with a detailed description of the study before written consent was obtained. Brief psychiatric interviews for screening purposes were performed by trained psychiatric nurses. Participants with a lifetime history of major psychiatric illness or brain trauma were excluded. Participants with a lifetime history of bipolar or unipolar mood disorder severe enough to require hospitalization were also excluded. Other psychiatric difficulties such as anxiety, minor depression, and substance problems were not considered as exclusion criteria.

Although no structured assessment tools were used, participants who reported severe attention or behavioral problems in childhood were excluded. A total of 1,289 participants were included. All participants were unrelated

to one another, and all were ethnically Korean. Basic demographic data and information on current mood, sleep quality, and daily life stress were collected using self-administered questionnaires. This study was approved by the ethics committees of Eulji General Hospital and Eulji University Hospital.

Measurement of childhood ADHD features

ADHD-related features were measured with the WURS-25, which was developed for retrospective diagnosis of ADHD symptoms experienced before the age of 12 years [28]. Although the original WURS contained 61 items, the short version consisted of 25 items selected from the original version. The Korean version of the WURS-25 was developed and standardized using female Korean adults [29]. In this standardization study, the mean total score was 19.39 ± 14.21. A gender difference could not be ascertained because only female subjects participated in that study.

Measurement of circadian rhythm

The participants' degree of evening preference was measured using the CSM, a 13-item questionnaire that assesses individual differences related to the time of day at which a person prefers to carry out various activities [30]. Higher scores represent greater morning preference. Previous studies have demonstrated good test–retest reliability and adequate external validity for the CSM [31]. The Korean version had also been standardized [32]. In this standardization study, the mean total scores were 34.00 ± 6.32 in male and 34.37 ± 5.47 in female. There was no significant difference between the two sexes.

Genotyping

DNA was extracted from blood samples using a DNA isolation kit (Roche, Mannheim, Germany). We selected five SNPs in the CLOCK gene based on the minor allele frequencies reported in Asians and the linkage disequilibrium pattern (Table 1). Relevant information was consulted in Entrez SNP of NCBI. Even though the number of SNPs was chosen to cover the whole CLOCK gene, we tried to include SNPs that were representative of the whole gene and selected the SNPs closest to the markers previously studied regarding psychiatric phenotypes. Genotyping was performed using the TaqMan method (Applied Biosystems, Foster City, CA, USA) [33]. The chromosomal location of SNPs was referenced from the UCSC Genome Browser on Human Assembly and Entrez SNP of NCBI in February, 2009 (GRCh37.p3/hg19).

Statistical analysis

R statistical software for Windows version 2.15.1 was used for descriptive statistics, linear regression, and exploratory factor analysis [34]. Demographic variables such as age and the total scores of the CSM and WURS-25 were compared by sex using Student's *t*-test. The influence of age and sex on CSM and WURS-25 total scores was analyzed with linear regression. Because the summary statistics for male and female participants were so different, the data set for each sex was analyzed separately from this point forward.

The exploratory factor analysis was performed to extract major factors representing the latent dimensions of the WURS-25. The principal component method was used for factor extraction. The number of factors was decided by eigenvalue criteria (≥1). The extracted factors were rotated by promax rotation with Kaiser normalization. After the rotation, the factor scores of each participant were calculated by regression. Partial correlation coefficients were obtained to examine the relationship among CSM and WURS-25 total scores and extracted factor scores on the WURS-25, controlling for age.

The genetic analysis was performed using PLINK software [35]. The linkage disequilibrium structure was investigated using the Haploview software [36], and

Table 1 Five SNP markers in the CLOCK gene that were subjected to association analysis

rs No.	Gene location	Chromosomal position	Alleles	MAF	HWE *p* value
rs1801260	UTR-3	56301369	C/T	0.1047	0.530
rs3805148	Intron	56306810	A/C	0.3479	0.162
rs12504300	Intron	56348527	C/G	0.3503	0.097
rs4864542	Intron	56354087	C/G	0.3503	0.097
rs12649507	Intron	56380484	A/G	0.3503	0.084

MAF and Hardy–Weinberg equilibrium analyses are also included

rs No. indicates SNP identification in SNP site of NCBI

Chromosomal position was referenced from UCSC genome browser (http://genome.ucsc.edu/)

A adenine, *T* thymine, *C* cytosine, *G* guanine, *MAF* minor allele frequency, *HWE* Hardy–Weinberg equilibrium

separate haplotype blocks were determined based on confidence bounds on D′ [37]. The quantitative association analyses were performed as implemented in the PLINK software. The statistical framework was basically a linear regression analysis. The scores for each individual were entered as response variables, and all SNP markers or reconstructed haplotypes were entered as explanatory variables. Given that age had been shown to be a very strong confounding variable for both CSM and WURS-25 scores, age was included as a covariate in all subsequent analyses using the "—covar" option in the "—linear" command.

The sliding window approach was used for haplotype construction in the haplotype-wise association analysis. Of the five SNP markers in the CLOCK gene, four two-adjacent-marker haplotypes, three three-adjacent-marker haplotypes, two four-adjacent-marker haplotypes, and one five-marker haplotype were constructed (15 in total). Nonadjacent marker haplotypes were not considered. When performing haplotype-based association analyses, the "-hap-omnibus" option was used to simultaneously estimate all haplotype effects at a single locus.

All the reported p values were nominal p values without any adjustment. However, since multiple markers and phenotypes were analyzed simultaneously, the inflation of family-wise error rate was expected to be considerable. Meanwhile, strictly applying Bonferroni's correction would be too conservative, because the tests involved were not mutually independent. In addition, since this study purported to be rather an exploratory study than a confirmatory study, controlling for type-II error was also essential. In order to compromise these dilemmas, we employed the false discovery rates (FDRs) method proposed by Storey [38]. FDR controls the expected proportion of null results that are falsely identified as significant in a set of predictions. In order to decide on the acceptable significance threshold, we calculated FDR at each level of significance level and set the significance level with the lowest FDR value as the significance threshold. FDR values were calculated with R-package "QVALUE."

Results

Demographic variables and the influence of sex and age on CSM and WURS-25 scores

The number of male and female participants and their mean ages are shown in Table 2. About two-thirds of the participants were female; female participants were significantly younger than male participants (males: 27.37 ± 8.33 years, females: 23.70 ± 3.53 years, $t = 10.988$, $p < 0.001$). Average CSM total scores were significantly higher in male participants, suggesting a relative morning preference in this group (males: 32.25 ± 6.18, females: 29.74 ± 5.69, $t = 7.415$, $p < 0.001$). Additionally, average WURS-25 total scores were 19.49 ± 15.65 and 19.31 ± 14.23 in male and female participants, respectively, revealing no significant sex differences.

As it has been shown that diurnal preference is highly dependent on age and sex [39], univariate linear regression was performed with sex as a fixed factor and age as a covariate to determine the influence of sex, age, and their interaction on CSM and WURS-25 scores. In the case of the CSM, not only were the two main effects of sex ($F = 58.29$, $df = 1$, $p = 4.39 \times 10^{-14}$) and age ($F = 69.68$, $df = 1$, $p = 2.2 \times 10^{-16}$) significant, but the interaction effect between them was also highly significant ($F = 9.718$, $df = 1$, $p = 0.00187$). This significant interaction was due to the fact that CSM scores were strongly correlated with age in male participants only (Pearson's correlation coefficient $R = 0.37$, $p = 2.2 \times 10^{-16}$). In the case of WURS-25 scores, only the main effect of age ($F = 12.44$, $df = 1$, $p = 0.000435$) was significant; neither the main effect of sex ($F = 0.0409$, $df = 1$, $p = 0.840$) nor the interaction ($F = 0.721$, $df = 1$, $p = 0.396$) was significant.

Relationship between WURS-25 and CSM total scores

WURS-25 total scores were negatively correlated with CSM scores even after age was controlled as a confounding

Table 2 Constitution of the study participants and the total CSM and WURS-25 scores in male and female participants

	Whole	Male	Female	Student's t-test betw. male and female	
Number of participants	1,289	476	813		
Age	25.06 ± 6.05[a]	27.37 ± 8.33	23.70 ± 3.53	$t = 10.99$	$p < 0.001$
CSM	30.67 ± 6.00	32.25 ± 6.18	29.74 ± 5.69	$t = 7.415$	$p < 0.001$
WURS-25	19.38 ± 14.76	19.49 ± 15.65	19.31 ± 14.23	$t = 0.201$	$p = 0.840$

Age, CSM, and WURS-25 total scores were compared between the sexes using Student's t-test

[a] Mean ± standard deviation

variable, suggesting the existence of more ADHD-related features in both male and female evening-preference individuals (partial correlation coefficient, male: $R = -0.182$, $p = 5.93 \times 10^{-5}$; female: $R = -0.084$, $p = 0.0154$).

Allele frequency, Hardy–Weinberg equilibrium, and linkage disequilibrium structure

All SNP markers from the CLOCK gene had minimum allele frequencies (MAFs) greater than 10 % and did not deviate from Hardy–Weinberg equilibrium (Table 1). All pair-wise D′ values among the markers were greater than 0.90. According to the criteria developed by Gabriel et al. [37], the data suggested that a single haplotype block covered 73 kb, which included rs3805148, rs12504300, rs4864542, and rs12649507. The remaining marker, rs1801260, was not included in this block.

Calculation of false discovery rates

In the overall analysis, the total number of markers considered was 15 (5 SNPs and their haplotypes), and the total number of phenotypes was 5 (2 for CSM, WURS total scores and 3 for factors of WURS). These tests were done separately in male and female, such that the grand total number of performed statistical tests was 150 (=15 × 5 × 2). Submitting the obtained p values to FDR calculation algorithm, we could obtain FDRs associated with each p values. The lowest FDR was 0.0695 and the associated nominal p value was 0.0180. Even though the lowest FDR (7 %) was well above the traditional acceptable risk of 5 %, we decided to use 0.0180 as a significance threshold for the following analyses.

Quantitative trait-association analysis: CSM and WURS-25 total scores

None of the markers of the CLOCK gene showed a significant association with CSM total scores in either male or female participants, neither allelewise nor haplotypewise (the lowest p value: $p = 0.0194$) (Table 3). On the other hand, marker rs1801260 showed a weakly significant association with WURS-25 total scores in male participants only ($p = 0.0180$). Additionally, all the haplotypes including rs1801260 showed similar significant associations with WURS-25 total scores (rs1801260–rs3805148: $p = 0.0150$; rs1801260–rs12504300: $p = 0.0170$; rs1801260–rs4864542: $p = 0.0170$; rs1801260–rs12649507: $p = 0.0171$).

Extraction of factors from the WURS-25

To determine which components of the WURS-25 were responsible for the demonstrated association signal, exploratory factor analysis was performed to extract the relevant factors of the WURS-25. Three factors were extracted by the eigenvalue criteria. The combined extracted factors explained 55.22 % of the total variance (1st factor: 40.17 %, 2nd factor: 8.34 %, 3rd factor: 6.71 %). Comparable to similar factor analyses performed by other investigators and according to the loading pattern, three factors were tentatively named "impulsivity and defiant behavior" (1st factor: IMP), "inattention" (2nd factor: INATT), and "mood instability and anxiety" (3rd factor: MOOD) [40, 41].

The factor scores obtained for the WURS-25 were subjected to quantitative trait-association analysis using the same methodology as above. The allele-wise analysis revealed that none of the factors showed a significant association with rs1801260. In contrast, all markers except rs1801260 showed weak associations with factor INATT (rs3805148: $p = 0.0147$, rs12504300: $p = 0.0168$, rs4864542: $p = 0.0168$, rs12649507: $p = 0.0152$). In the haplotype-wise analysis, all of the constructed haplotypes showed weak associations with factor INATT at the significance threshold of $p = 0.0180$ (Table 4).

Quantitative trait-association analysis of the WURS-25 when the CSM was controlled as a covariate

Of particular interest was the possibility that the demonstrated association between the CLOCK gene and the WURS-25 total score might be mediated by evening preference. To examine this possibility, the CSM total score was controlled as a covariate with age in the updated model, and the analysis was repeated (original analysis vs. CSM controlled analysis). Even though the allele-wise p value from the CSM controlled analysis ($p = 0.0241$) was somewhat higher than the original analysis ($p = 0.0180$), all the p values obtained from updated haplotype-wise analyses were lower than the original analyses (Table 5).

Discussion

We had initially proposed two linked hypotheses: (1) that genetic polymorphisms in the CLOCK gene may be associated with ADHD-related features in the general population, and (2) that this association may be mediated by individuals' evening preference. To examine these

Table 3 Results (nominal *p*-values) of the allele- and haplotype-based (sliding window approach) quantitative trait-association analysis results between (1) CSM and WURS-25 total scores and (2) five SNP markers in the CLOCK gene

Clock	Male				Female			
	CSM		WURS		CSM		WURS	
	Allele wise	Haplotype wise	Allele wise	Haplotype wise	Allele wise	Haplotype wise	Allele wise	Haplotype wise
rs1801260	0.426		0.018		0.825		0.19	
①-②-③-④-⑤		0.602		0.0155		0.942		0.214
①-②-③-④-⑤		0.594		0.0170		0.974		0.292
①-②-③-④-⑤		0.594		0.0170		0.974		0.292
①-②-③-④-⑤		0.609		0.0171		0.980		0.315
rs3805148	0.224		0.152		0.899		0.651	
①-②-③-④-⑤		0.204		0.176		0.972		0.513
①-②-③-④-⑤		0.204		0.176		0.972		0.513
①-②-③-④-⑤		0.204		0.176		0.943		0.488
rs12504300	0.194		0.166		0.972		0.513	
①-②-③-④-⑤		0.194		0.166		0.972		0.513
①-②-③-④-⑤		0.194		0.166		0.943		0.488
rs4864542	0.194		0.166		0.972		0.513	
①-②-③-④-⑤		0.194		0.166		0.943		0.488
rs12649507	0.258		0.155		0.932		0.500	

* ①-②-③-④-⑤ : Series of SNP markers in the CLOCK gene. The circled numbers denote each SNP marker of the CLOCK gene and the surrounding rectangle denoted the markers used in constructing the haplotypes to be tested. Only the adjacent markers were used and the actual PLINK command was issued using sliding window specification.

①: rs1801260, ②: rs3805148, ③: rs12504300, ④: rs4864542, ⑤: rs12649507

Table 4 Results (nominal *p*-values) of the allele- and haplotype-based (sliding window approach) quantitative trait-association analysis between WURS-25 factor scores and five SNP markers in the CLOCK gene

Clock	Male						Female					
	Impulsivity		Inattention		Instability		Impulsivity		Inattention		Instability	
	Allele wise	Haplotype wise	Allele wise	Haplotype wise	Allele wise	Haplotype wise	Allele wise	Haplotype wise	Allele wise	Haplotype wise	Allele wise	Haplotype wise
rs1801260	0.0324		0.170		0.0340		0.747		0.0836		0.161	
①-②-③-④-⑤		0.0990		0.0150		0.0279		0.877		0.0921		0.170
①-②-③-④-⑤		0.0907		0.0164		0.0357		0.907		0.146		0.207
①-②-③-④-⑤		0.0907		0.0164		0.0357		0.907		0.146		0.207
①-②-③-④-⑤		0.0906		0.0162		0.0383		0.903		0.176		0.222
rs3805148	0.526		0.0147		0.550		0.757		0.626		0.761	
①-②-③-④-⑤		0.564		0.0178		0.588		0.641		0.485		0.680
①-②-③-④-⑤		0.564		0.0178		0.588		0.641		0.485		0.680
①-②-③-④-⑤		0.564		0.0178		0.588		0.628		0.498		0.610
rs12504300	0.541		0.0168		0.580		0.641		0.485		0.680	
①-②-③-④-⑤		0.541		0.0164		0.580		0.641		0.485		0.680
①-②-③-④-⑤		0.541		0.0164		0.580		0.628		0.498		0.610
rs4864542	0.541		0.0168		0.580		0.641		0.485		0.680	
①-②-③-④-⑤		0.541		0.0164		0.580		0.628		0.498		0.610
rs12649507	0.537		0.0152		0.546		0.629		0.544		0.596	

hypotheses, we performed a quantitative trait-association study between polymorphic markers in the CLOCK gene and ADHD-related features in healthy adults, with diurnal preference controlled as a mediating variable.

We obtained the following results. First, we found a suggestive association between WURS-25 total scores and a single SNP marker in the 3′-UTR region of the CLOCK gene (rs1802160). Second, WURS-25 total scores were

Table 5 Results (nominal *p*-values) of the allele- and haplotype-based (sliding window approach) quantitative trait-association analysis between WURS-25 total scores and rs1801260 in the CLOCK gene 1) before CSM had been controlled and 2) after CSM had been controlled as a covariate

Clock	Male			
	WURS		WURS (CSM controlled)	
	Allele wise	Haplotype wise	Allele wise	Haplotype wise
rs1801260	0.018		0.0241	
①-②-③-④-⑤		0.0155		0.0124
①-②-③-④-⑤		0.0170		0.0131
①-②-③-④-⑤		0.0170		0.0131
①-②-③-④-⑤		0.0171		0.0131

strongly correlated with CSM scores, with far more ADHD-related features found in evening-preference individuals. Third, the inclusion of the CSM score as a mediating variable did not much reduce the strength of the association between WURS-25 total scores and rs1801260. Consistent with this observation, no association was observed between the CSM score and the CLOCK gene. The latter two negative findings suggest that degree of evening preference does not mediate the association between the CLOCK gene and ADHD-related features. To summarize, our results support the first hypothesis, but not the second.

If our results are to be considered valid, our strategy of using WURS-25 as a measure of ADHD-related features should be justified beforehand. WURS was originally developed for diagnostic purposes. However, the limitations of WURS as a diagnostic tool have been repeatedly discussed. A correlation between WURS scores and an objective measure of attention has been difficult to demonstrate [42], and the sensitivity and specificity of the WURS with the standard cutoff point are not adequate to permit its use as a reliable diagnostic tool [43]. Rather, it has been more often used to measure general character traits common to a broad range of psychiatric diagnoses [44]. In the psychiatric literature, the WURS has been widely used to investigate the effect of ADH-related features on the severity and prognosis of other psychiatric and behavioral problems such as PTSD [45], eating disorder [46], and borderline personality [47]. Thus, it can be argued that our use of WURS-25 as a proxy measure of ADHD-related features conformed to previous literature.

Unlike the two previous reports demonstrating the association between rs1801260 in the CLOCK gene and ADHD in clinical samples [26, 27], we tried to demonstrate this in the general population. For this purpose, a broader concept of ADHD-related features was postulated, and such features were assumed to be continuously distributed among the general population. The boundary between attention deficits as a clinical symptom and as a normal variation cannot be clearly defined. This difficulty is reflected in the remarkable variation in ADHD prevalence depending on the phenotypic definition used (between 2.5 and 42.3 %) [48]. Additionally, if a biological substratum of the broader concept of ADHD-related features can be found, it will help us to understand the relationship between ADHD and other comorbid conditions. Although the association signal observed in this study was very weak, ours is the first attempt to ascertain the role of CLOCK gene polymorphisms in the variability of ADHD-related features in the general population.

Despite the fact that the WURS-25 includes several component factors, only the total score has been used in most previous research, and it has usually been equated with ADHD severity or nonspecific impulsivity [26]. Although nonspecific impulsivity actually explained the greatest proportion of the variance in our study, other factors also substantially contributed to the remaining variance. A three-factor solution has generally been accepted [40, 41]. The specific names of these factors may differ, but the underlying concepts are very similar. Recently, Joo et al. [41] named them IMP (impulsivity), INATT (inattention), and MOOD (mood instability).

In our results, although the strengths of the association signals were at most suggestive, some points warrant attention. First, all markers used except rs1801260 showed evidence of association with the INATT factor (*p* values: 0.0147–0.0168). Second, this finding was further supported by the fact that all the constructed haplotypes showed similar association with the INATT factor, but not with other factors (*p* values: 0.0150–0.0178) (Table 4). Although these findings cannot be regarded as rigorous evidence, they may indicate a possible direction for further research. It is generally accepted that although impulsivity is the most prominent symptom in ADHD, cognitive impairments are the most fundamental problems. Thus, the CLOCK gene's contribution to ADHD-related features may operate via more fundamental cognitive impairments rather than via general impulsivity. To clarify this matter, further research focused on the relationship between cognitive impairments in ADHD patients and the CLOCK gene is needed.

According to the original guidelines developed by Baron and Kenny [49], if a variable functions as a mediator, then

(1) it is simultaneously related to both independent and dependent variables, and (2) the strength of the relationship between the independent and dependent variables is greatly reduced when the effect of the variable is controlled. Therefore, to demonstrate that evening preference serves as a mediator, we would have to demonstrate both the relationship between the CLOCK gene and evening preference ("A" in Fig. 1) and that between evening preference and ADHD-related features ("B" in Fig. 1). Our result corroborates previous reports showing that evening preference is related to ADHD-related symptoms in both clinical and nonclinical samples [14, 16]. However, we could not confirm the previously reported association between rs1801260 and evening preference [24]. Although a few reports have successfully replicated this association [25], a greater number of similar studies have failed to do so [50–53]. Even a study done with Korean college students failed to find such an association [54]. Therefore, only relationship "B," but not relationship "A," could be demonstrated (Fig. 1).

Furthermore, our results also failed to meet Baron and Kenny's second requirement in that controlling for the CSM score had only a minimal effect on association strength; that is, the p value changed from 0.018 to 0.024. Undoubtedly, evening preference is just one tiny aspect of the full manifestation of circadian rhythm. Therefore, the finding that evening preference did not mediate the association does not lead to the conclusion that circadian rhythm disruption cannot be a mediating link. However, previous studies have reported that diurnal preference is correlated with a wide range of the manifestations of circadian rhythm, including the sleep–wake cycle, as well as with the circadian phase and the amplitude of the phase [55]. Diurnal preference has also been associated with various human behaviors. Not only sleep habits or disturbances but also emotional problems [56], psychopathology, and personality [57] are affected by diurnal preference. Thus, although diurnal preference does not represent the whole system of circadian rhythm manifestations, it may be the most versatile indicator of the circadian system. Therefore, the search for a mediating link between the CLOCK gene and ADHD-related features need not be limited to the circadian system. The so-called noncircadian effects of the CLOCK gene may also need to be considered [58].

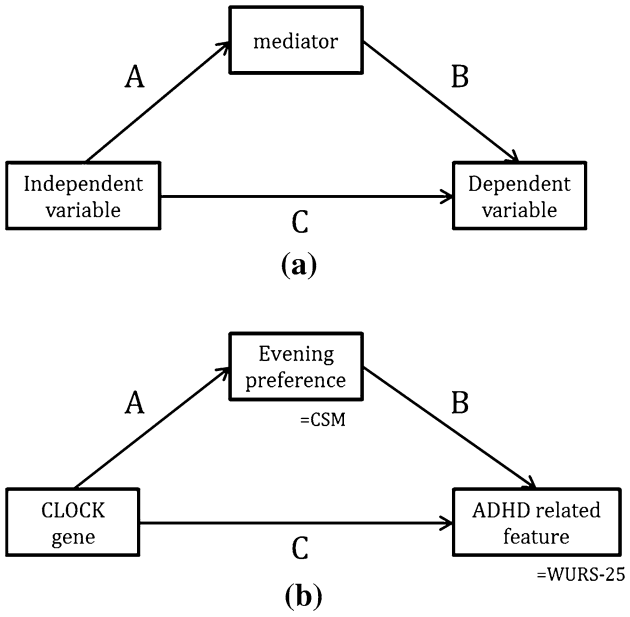

Fig. 1 Conceptual representation of the mediation model. A mediating relationship occurs when a mediator variable (evening preference in this study) can account for either all or some of the observed relationship between the independent and dependent variables (the CLOCK gene and ADHD-related features in this study)

Our study found several sex-specific results. The linear relationship between age and CSM scores was observed only in male subjects. Likewise, the association between the CLOCK gene and WURS-25 scores was found only in male subjects. Clearly, marked sex differences exist in the prevalence and symptom manifestation of ADHD. Studies of ADHD symptoms using the WURS-25 in healthy college students have also noted that male students scored higher than did females [59]. The two studies that demonstrated an association between the CLOCK gene and ADHD both used only male participants [26, 27]. It could be that the influence of the CLOCK gene on ADHD-related features has some sex-specific quality; however, it is also possible that the higher prevalence of attention problems in male participants renders detection of a weak association easier.

This study had several limitations. The most problematic was that we could not adequately control the inflation of family-wise error rate. The lowest false discovery rate of our study was about 7 %. It meant that if all the p values lower than 0.0180 were called significant then, there would be 7 % chance of falsely rejecting null hypotheses overall. If this risk cannot be deemed acceptable, then all the results in our study are just inconclusive findings. The only justification would be that this study was an explorative one aiming to prove the concept for future studies.

Another significant drawback was the fact that the participants were recruited from two different sources. Despite the difference in sex ratio and age distribution, we had to combine the two subsets of participants and analyze the whole set because the second subset was almost exclusively male. However, when we analyzed the first subset separately, the results were similar to those described above (data not shown). The generalizability of our results is also limited. The participants were almost exclusive in their 20s or 30s. The genetic composition of such a narrow

age group could be affected by the cohort effect, and the character phenotypes could be influenced by a generation effect. Therefore, findings in a specific age group may not be valid in other age groups.

Presuming a direct link between a gene (CLOCK) and a phenotype (ADHD-related feature) mediated by another single phenotype (eveningness) would be a too simplistic approach to real phenomena. A great number of additional variables had to be accounted for to properly understand the given data. Especially among those variables, the sleep pattern and the seasonality have to be considered. Evening preference cannot substitute the actual sleep pattern. What determines the individual's psychiatric difficulties may not be the evening preference, but the actual sleep-rhythm or sleep quality [60, 61]. Though we did not measure participants' sleep patterns or qualities, it would be an essential step for the further studies. Meanwhile, the individual's seasonality is another important variable that has to be considered. Seasonality refers to a seasonal variation in mood and behaviors and represents a longer biological rhythm than circadian. It was also found to be associated with mood disorders, ADHD, and cognitive function fluctuations [15, 62]. Since seasonality is found to be associated both with circadian preference and ADHD symptoms, it may be another candidate variable for mediating CLOCK gene and ADHD-related symptoms.

In conclusion, we found that a polymorphic marker (rs1801260) in the CLOCK gene showed male-specific associations with WURS-25 total scores in a large sample of healthy adults and that this association was not mediated by the degree of evening preference. Although the association signal identified in our study was very weak, this is the first study to ascertain the role of the CLOCK gene in the variation in ADHD-related features in a nonclinical sample. It is not yet clear what kinds of underlying mechanism are responsible for this association; however, the search for a mediating link may need to include the noncircadian effects of the CLOCK gene.

Acknowledgments This work was supported by National Research Foundation of Korea Grant funded by the Korean Government (2011-0003164). The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: (http://www.textcheck.com/certificate/2TN2pL).

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Polanczyk G, de Lima MS, Horta BL, Biederman J, Rohde LA (2007) The worldwide prevalence of ADHD: a systematic review and metaregression analysis. Am J Psychiatry 164(6):942–948. doi:10.1176/appi.ajp.164.6.942
2. Kessler RC, Adler L, Barkley R, Biederman J, Conners CK, Demler O, Faraone SV, Greenhill LL, Howes MJ, Secnik K, Spencer T, Ustun TB, Walters EE, Zaslavsky AM (2006) The prevalence and correlates of adult ADHD in the United States: results from the National Comorbidity Survey Replication. Am J Psychiatry 163(4):716–723. doi:10.1176/appi.ajp.163.4.716
3. Nierenberg AA, Miyahara S, Spencer T, Wisniewski SR, Otto MW, Simon N, Pollack MH, Ostacher MJ, Yan L, Siegel R, Sachs GS (2005) Clinical and diagnostic implications of lifetime attention-deficit/hyperactivity disorder comorbidity in adults with bipolar disorder: data from the first 1000 STEP-BD participants. Biol Psychiatry 57(11):1467–1473. doi:10.1016/j.biopsych.2005.01.036
4. Downey KK, Pomerleau CS, Pomerleau OF (1996) Personality differences related to smoking and adult attention deficit hyperactivity disorder. J Subst Abuse 8(1):129–135
5. Altfas JR (2002) Prevalence of attention deficit/hyperactivity disorder among adults in obesity treatment. BMC Psychiatry 2:9
6. Wilens TE, Kwon A, Tanguay S, Chase R, Moore H, Faraone SV, Biederman J (2005) Characteristics of adults with attention deficit hyperactivity disorder plus substance use disorder: the role of psychiatric comorbidity. Am J Addict/Am Acad Psychiatr Alcohol Addict 14(4):319–327. doi:10.1080/10550490591003639
7. Brown T (2008) ADHD comorbidities: handbook for ADHD complications in children and adults. American Psychiatric Publishing, USA
8. Levy F, Hay DA, McStephen M, Wood C, Waldman I (1997) Attention-deficit hyperactivity disorder: a category or a continuum? Genetic analysis of a large-scale twin study. J Am Acad Child Adolesc Psychiatry 36(6):737–744. doi:10.1097/00004583-199706000-00009
9. Walters AS, Silvestri R, Zucconi M, Chandrashekariah R, Konofal E (2008) Review of the possible relationship and hypothetical links between attention deficit hyperactivity disorder (ADHD) and the simple sleep related movement disorders, parasomnias, hypersomnias, and circadian rhythm disorders. J Clin Sleep Med 4(6):591–600
10. Gruber R, Sadeh A, Raviv A (2000) Instability of sleep patterns in children with attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 39(4):495–501. doi:10.1097/00004583-200004000-00019
11. Blomqvist M, Holmberg K, Lindblad F, Fernell E, Ek U, Dahllof G (2007) Salivary cortisol levels and dental anxiety in children with attention deficit hyperactivity disorder. Eur J Oral Sci 115(1):1–6. doi:10.1111/j.1600-0722.2007.00423.x
12. Imeraj L, Sonuga-Barke E, Antrop I, Roeyers H, Wiersema R, Bal S, Deboutte D (2012) Altered circadian profiles in attention-deficit/hyperactivity disorder: an integrative review and theoretical framework for future studies. Neurosci Biobehav Rev 36(8):1897–1919. doi:10.1016/j.neubiorev.2012.04.007
13. Kerkhof GA (1985) Inter-individual differences in the human circadian system: a review. Biol Psychol 20(2):83–112
14. Caci H, Bouchez J, Bayle FJ (2009) Inattentive symptoms of ADHD are related to evening orientation. J Atten Disord 13(1):36–41. doi:10.1177/1087054708320439
15. Rybak YE, McNeely HE, Mackenzie BE, Jain UR, Levitan RD (2007) Seasonality and circadian preference in adult attention-deficit/hyperactivity disorder: clinical and neuropsychological correlates. Compr Psychiatry 48(6):562–571. doi:10.1016/j.comppsych.2007.05.008
16. Bae SM, Park JE, Lee YJ, Cho IH, Kim JH, Koh SH, Kim SJ, Cho SJ (2010) Gender difference in the association between adult attention deficit hyperactivity disorder symptoms and morningness–eveningness. Psychiatry Clin Neurosci 64(6):649–651

17. Caci H, Robert P, Boyer P (2004) Novelty seekers and impulsive subjects are low in morningness. Eur Psychiatry 19(2):79–84. doi:10.1016/j.eurpsy.2003.09.007
18. Kitamura S, Hida A, Watanabe M, Enomoto M, Aritake-Okada S, Moriguchi Y, Kamei Y, Mishima K (2010) Evening preference is related to the incidence of depressive states independent of sleep-wake conditions. Chronobiol Int 27(9–10):1797–1812. doi:10.3109/07420528.2010.516705
19. Abe T, Inoue Y, Komada Y, Nakamura M, Asaoka S, Kanno M, Shibui K, Hayashida K, Usui A, Takahashi K (2011) Relation between morningness–eveningness score and depressive symptoms among patients with delayed sleep phase syndrome. Sleep Med 12(7):680–684. doi:10.1016/j.sleep.2010.12.017
20. Negriff S, Dorn LD, Pabst SR, Susman EJ (2011) Morningness/eveningness, pubertal timing, and substance use in adolescent girls. Psychiatry Res 185(3):408–413. doi:10.1016/j.psychres.2010.07.006
21. Kripke DF, Nievergelt CM, Joo E, Shekhtman T, Kelsoe JR (2009) Circadian polymorphisms associated with affective disorders. J Circadian Rhythms 7:2. doi:10.1186/1740-3391-7-2
22. Hur YM (2007) Stability of genetic influence on morningness–eveningness: a cross-sectional examination of South Korean twins from preadolescence to young adulthood. J Sleep Res 16(1):17–23. doi:10.1111/j.1365-2869.2007.00562.x
23. Mansour HA, Monk TH, Nimgaonkar VL (2005) Circadian genes and bipolar disorder. Ann Med 37(3):196–205. doi:10.1080/07853890510007377
24. Katzenberg D, Young T, Finn L, Lin L, King DP, Takahashi JS, Mignot E (1998) A CLOCK polymorphism associated with human diurnal preference. Sleep 21(6):569–576
25. Mishima K, Tozawa T, Satoh K, Saitoh H, Mishima Y (2005) The 3111T/C polymorphism of hClock is associated with evening preference and delayed sleep timing in a Japanese population sample. Am J Med Genet B Neuropsychiatr Genet 133B(1):101–104. doi:10.1002/ajmg.b.30110
26. Kissling C, Retz W, Wiemann S, Coogan AN, Clement RM, Hunnerkopf R, Conner AC, Freitag CM, Rosler M, Thome J (2008) A polymorphism at the 3′-untranslated region of the CLOCK gene is associated with adult attention-deficit hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet 147(3):333–338. doi:10.1002/ajmg.b.30602
27. Xu X, Breen G, Chen CK, Huang YS, Wu YY, Asherson P (2010) Association study between a polymorphism at the 3′-untranslated region of CLOCK gene and attention deficit hyperactivity disorder. Behav Brain Funct 6:48. doi:10.1186/1744-9081-6-48
28. Ward MF, Wender PH, Reimherr FW (1993) The Wender Utah Rating Scale: an aid in the retrospective diagnosis of childhood attention deficit hyperactivity disorder. Am J Psychiatry 150(6):885–890
29. Koo YJ, Choi KS, Bang YW, Kim OS, Song HJ, Joo EJ (2009) Factor structure of Wender Utah Rating Scale (Korean) in normal female adults. J Soc Korean Women Psychiatr 7:63–67
30. Smith CS, Reilly C, Midkiff K (1989) Evaluation of three circadian rhythm questionnaires with suggestions for an improved measure of morningness. J Appl Psychol 74(5):728–738
31. Caci H, Nadalet L, Staccini P, Myquel M, Boyer P (2000) The composite scale of morningness: further psychometric properties and temporal stability. Eur Psychiatry 15(4):278–281
32. Kook SH, Yoon JS, Lee HY (1999) Cross validation of the Korean Translation of Composite Scale (KtCS) to measure morningness–eveningness. J Korean Neuropsychiatr Assoc 38:297–305
33. McGuigan FE, Ralston SH (2002) Single nucleotide polymorphism detection: allelic discrimination using TaqMan. Psychiatr Genet 12(3):133–136
34. R-core-Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
35. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81(3):559–575. doi:10.1086/519795
36. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21(2):263–265. doi:10.1093/bioinformatics/bth457
37. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D (2002) The structure of haplotype blocks in the human genome. Science 296(5576):2225–2229. doi:10.1126/science.1069424
38. Storey JD (2002) A direct approach to false discovery rates. J Roy Stat Soc B 64:479–498
39. Adan A, Natale V (2002) Gender differences in morningness–eveningness preference. Chronobiol Int 19(4):709–720
40. Caci HM, Bouchez J, Bayle FJ (2010) An aid for diagnosing attention-deficit/hyperactivity disorder at adulthood: psychometric properties of the French versions of two Wender Utah Rating Scales (WURS-25 and WURS-K). Compr Psychiatry 51(3):325–331. doi:10.1016/j.comppsych.2009.05.006
41. Joo EJ, Greenwood TA, Schork N, McKinney RA, Sadovnick AD, Remick RA, Keck PE, McElroy SL, Kelsoe JR (2010) Suggestive evidence for linkage of ADHD features in bipolar disorder to chromosome 10p14. Am J Med Genet B Neuropsychiatr Genet 153B(1):260–268. doi:10.1002/ajmg.b.31005
42. Mackin RS, Horner MD (2005) Relationship of the Wender Utah Rating Scale to objective measures of attention. Compr Psychiatry 46(6):468–471. doi:10.1016/j.comppsych.2005.03.004
43. Suhr J, Zimak E, Buelow M, Fox L (2009) Self-reported childhood attention-deficit/hyperactivity disorder symptoms are not specific to the disorder. Compr Psychiatry 50(3):269–275. doi:10.1016/j.comppsych.2008.08.008
44. Hill BD, Pella RD, Singh AN, Jones GN, Gouvier WD (2009) The Wender Utah Rating Scale: adult ADHD diagnostic tool or personality index? J Atten Disord 13(1):87–94. doi:10.1177/1087054708320384
45. Feigelson ED, Babu GJ (2013) Statistical challenges in modern astronomy V. Lecture notes in statistics, vol 209. In: Proceedings. Springer, New York
46. Yilmaz Z, Kaplan AS, Zai CC, Levitan RD, Kennedy JL (2011) COMT Val158Met variant and functional haplotypes associated with childhood ADHD history in women with bulimia nervosa. Prog Neuropsychopharmacol Biol Psychiatry 35(4):948–952. doi:10.1016/j.pnpbp.2011.01.012
47. Ferrer M, Andion O, Matali J, Valero S, Navarro JA, Ramos-Quiroga JA, Torrubia R, Casas M (2010) Comorbid attention-deficit/hyperactivity disorder in borderline patients defines an impulsive subtype of borderline personality disorder. J Pers Disord 24(6):812–822. doi:10.1521/pedi.2010.24.6.812
48. Simon V, Czobor P, Balint S, Meszaros A, Bitter I (2009) Prevalence and correlates of adult attention-deficit hyperactivity disorder: meta-analysis. Br J Psychiatry 194(3):204–211. doi:10.1192/bjp.bp.107.048827
49. Baron RM, Kenny DA (1986) The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J Pers Soc Psychol 51(6):1173–1182
50. Johansson C, Willeit M, Smedh C, Ekholm J, Paunio T, Kieseppa T, Lichtermann D, Praschak-Rieder N, Neumeister A, Nilsson LG, Kasper S, Peltonen L, Adolfsson R, Schalling M, Partonen T

(2003) Circadian clock-related polymorphisms in seasonal affective disorder and their relevance to diurnal preference. Neuropsychopharmacology 28(4):734–739. doi:10.1038/sj.npp.1300121

51. Pedrazzoli M, Louzada FM, Pereira DS, Benedito-Silva AA, Lopez AR, Martynhak BJ, Korczak AL, Koike Bdel V, Barbosa AA, D'Almeida V, Tufik S (2007) Clock polymorphisms and circadian rhythms phenotypes in a sample of the Brazilian population. Chronobiol Int 24(1):1–8. doi:10.1080/07420520601139789
52. Choub A, Mancuso M, Coppede F, LoGerfo A, Orsucci D, Petrozzi L, DiCoscio E, Maestri M, Rocchi A, Bonanni E, Siciliano G, Murri L (2011) Clock T3111C and Per2 C111G SNPs do not influence circadian rhythmicity in healthy Italian population. Neurol Sci 32(1):89–93. doi:10.1007/s10072-010-0415-1
53. Chang AM, Buch AM, Bradstreet DS, Klements DJ, Duffy JF (2011) Human diurnal preference and circadian rhythmicity are not associated with the CLOCK 3111C/T gene polymorphism. J Biol Rhythms 26(3):276–279. doi:10.1177/0748730411402026
54. Lee HJ, Paik JW, Kang SG, Lim SW, Kim L (2007) Allelic variants interaction of CLOCK gene and G-protein beta3 subunit gene with diurnal preference. Chronobiol Int 24(4):589–597. doi:10.1080/07420520701534632
55. Mongrain V, Lavoie S, Selmaoui B, Paquet J, Dumont M (2004) Phase relationships between sleep–wake cycle and underlying circadian rhythms in morningness–eveningness. J Biol Rhythms 19(3):248–257. doi:10.1177/0748730404264365
56. Gelbmann G, Kuhn-Natriashvili S, Pazhedath TJ, Ardeljan M, Wober C, Wober-Bingol C (2012) Morningness: protective factor for sleep-related and emotional problems in childhood and adolescence? Chronobiol Int 29(7):898–910. doi:10.3109/07420528.2012.686946
57. Hsu CY, Gau SS, Shang CY, Chiu YN, Lee MB (2012) Associations between chronotypes, psychopathology, and personality among incoming college students. Chronobiol Int 29(4):491–501. doi:10.3109/07420528.2012.668995
58. Rosenwasser AM (2010) Circadian clock genes: non-circadian roles in sleep, addiction, and psychiatric disorders? Neurosci Biobehav Rev 34(8):1249–1255. doi:10.1016/j.neubiorev.2010.03.004
59. Norvilitis JM, Ingersoll T, Zhang J, Jia S (2008) Self-reported symptoms of ADHD among college students in China and the United States. J Atten Disord 11(5):558–567. doi:10.1177/1087054707308496
60. Van Veen MM, Kooij JJ, Boonstra AM, Gordijn MC, Van Someren EJ (2010) Delayed circadian rhythm in adults with attention-deficit/hyperactivity disorder and chronic sleep-onset insomnia. Biol Psychiatry 67(11):1091–1096. doi:10.1016/j.biopsych.2009.12.032
61. Voinescu BI, Szentagotai A, David D (2012) Sleep disturbance, circadian preference and symptoms of adult attention deficit hyperactivity disorder (ADHD). J Neural Transm 119(10):1195–1204. doi:10.1007/s00702-012-0862-3
62. Amons PJ, Kooij JJ, Haffmans PM, Hoffman TO, Hoencamp E (2006) Seasonality of mood disorders in adults with lifetime attention-deficit/hyperactivity disorder (ADHD). J Affect Disord 91(2–3):251–255. doi:10.1016/j.jad.2005.11.017