Clinical and genetic associations of the CLOCK circadian rhythm gene and depressive disorders in patients with alcohol dependence syndrome during the period of alcohol abstinence

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About 20% of patients with depression are diagnosed with alcohol dependence, and alcohol dependent individuals are at a higher risk of developing depression. A number of authors point to the relationship of CLOCK gene activity with both affective disorders and alcohol use/dependence disorders; in particular, variations in the CLOCK gene at the evidence level link to depression and stress.

Objective: *to establish clinical and genetic associations of the CLOCK circadian rhythm gene and depressive disorders during the period of abstinence in patients with alcohol dependence syndrome.*

Patients and methods. *From June 2019 to December 2022, 402 patients (mean age 42.47±7.5 years) were examined, who underwent outpatient follow-up at a narcological dispensary not earlier than 1 month after and not later than 2 months after discharge from the hospital. All patients were diagnosed with middle-stage alcohol dependence syndrome, early remission phase. Depending on the presence of an episode of depression at the time of the examination, the main group (patients with a depressive disorder; n=128) and a comparison group (patients without a depressive disorder; n=274) were formed. Clinical interviews and psychometric study using the Montgomery–Asberg Depression Scale, were conducted for all the subjects. All subjects provided 10 ml venous blood samples for molecular, genetic and biochemical studies.*

Results and discussion. *Correlations were found between the carriage of the TT genotype of the CLOCK gene in patients with alcohol dependence and concomitant clinical depression. Carrying the TT genotype of the CLOCK gene is presumably associated with the predominance of the following symptoms of depression, which demonstrated statistically significant differences: loss of appetite, impaired concentration, apathy, as well as with a higher overall score on the MADRS depression scale. The established association between the CLOCK gene and depression in people with alcohol dependence can be considered as a vulnerability factor in relation to the development of depression in patients with the underlying disease – alcohol dependence syndrome.*

Conclusion. *The CLOCK gene is associated with the development of depression in patients suffering from alcohol dependence. Carriers of the TT genotype of the CLOCK gene (rs1801260) to a greater extent than carriers of other genotypes are characterized by the development of comorbid depression, as well as the predominance of such clinical symptoms as loss of appetite, impaired concentration, apathy, and a higher overall score on the MADRS depression scale.*

Keywords: *alcohol dependence syndrome; alcohol withdrawal syndrome; depression; CLOCK.*
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Alcohol dependence and depression make a significant contribution to the global burden of non-infectious diseases and are common mental disorders that often occur simultaneously [1]. About 20% of patients with depression are diagnosed with alcohol dependence, and people who are addicted to alcohol are at a higher risk of developing depression and, as a result, physical and social problems caused by alcohol dependence [2]. Furthermore, these co-occurring disorders are associated with a number of mutually aggravating complications, a more severe course of each of the diseases, an increased risk of suicide, and an increase in the financial burden [3]. Accordingly, the optimization of efforts for the prevention and treatment of these comorbid diseases plays a crucial role, and understanding the biological basis and genetic predisposition to these disorders is of fundamental importance in this issue.

I.S. Efremov et al. [4] found that alcohol dependence is associated with sleep disorders, and alcohol intake itself may change the circadian rhythm. Research by C.A. McClung et al.

[5] also showed that such factors as preference for and sensitivity to alcohol correlate with circadian fluctuations. The sleep-wake cycle is regulated with the participation of the circadian genes which were first identified in 1997 by Joseph Takahashi who screened for mutagenesis mice treated with N-ethyl-N-nitrosourea to identify specific mutations in key genes affecting daily activity [6]. The protein encoded by the *CLOCK* gene plays a central role in the biological regulation of circadian rhythms, and its various associations have been well-described [7]. A number of authors point to the association of the activity of the *CLOCK* gene with both affective disorders and disorders of alcohol use and/or dependence [8. 9].

The period of early remission, when the risk of resuming alcohol consumption is the highest, is crucial in the formation of remission in patients with alcohol dependence. Data of the previous studies show that the presence of depressive symptoms and insomniac disorders during this period may increase the risk of relapse. Clinical and genetic associations between depressive disorders in patients with alcohol dependence during early remission and the *CLOCK* circadian rhythm gene have not been sufficiently studied and are not represented in modern literature.

The aim of the study was to establish clinical and genetic associations between the *CLOCK* circadian rhythm gene and depressive disorders during abstinence in patients with alcohol dependence syndrome.

Patients and methods. We conducted a comparative cross-sectional study of patients with alcohol dependence syndrome (F10.2) and concomitant depressive disorders during abstinence. All patients signed voluntary informed consent. The study was approved by the local Ethics Committee of the BSMU of the Ministry of Health of the Russian Federation (Report No. 2 of 27.02.2019).

Inclusion, non-inclusion and exclusion criteria were developed to form the sample. *Inclusion criteria:* the presence of a verified diagnosis of F10.2 "Alcohol dependence syndrome"; signed voluntary informed consent; age not younger than 18 and not older than 55 years; at least one month of confirmed abstinence; clinical survey and laboratory examination of the level of gamma-glutamyl transferase (GGT; reference values 10-71 U/L) and total carbohydrate deficient transferrin (CDT; reference values less than 1.3%); not taking psychotropic drugs at the time of examination. *Criteria for non-inclusion:* alcohol consumption in the month before the inclusion in the study; dependence on psychoactive substances other than alcohol and nicotine; presence of objective reasons making the verbal contact difficult; presence of comorbid mental pathology – schizophrenia, schizotypal states, delusional disorders (F20–F29), dementia (F00–F03), mental retardation (F70–F79), somatic pathology in the decompensation stage; absence of clinically and laboratory confirmed abstinence for at least one month; levels of GGT and CDT above the reference values. *Exclusion criteria:* refusal to participate in the study after its start, identification of non-inclusion criteria during clinical interviewing.

Characteristics of the sample. The examination of the patients was carried out from June 2019 to December 2021. Patients were selected in accordance with the criteria of alcohol dependence syndrome (F10.2) presented in the International Classification of Diseases of the 10th revision (ICD-10). All patients underwent outpatient observation in a drug abuse dispensary no earlier than a month and no later than two months after discharge from the hospital, all were diagnosed with alcohol dependence syndrome of the middle stage, the phase of early remission. The state of sobriety in all patients was confirmed by laboratory tests. At the time of the study the patients did not take any psychotropic drugs. All patients underwent neurological examination; no severe neurological pathology was detected. 446 patients were screened, 44 were not included in the study in accordance with the criteria of non-inclusion. The final sample consisted of 402 patients, (mean age 42.47±7.5 years), of whom 94 (23.4%) were women, 308 (76.6%) were men, which generally corresponds to the distribution by gender in the general population of alcohol addicts. The sample can be considered representative of the surveyed population group.

Depending on the presence of an episode of depression at the time of examination, the patients were divided into two groups: the main group (patients with depressive disorders) and the control group (patients without depressive disorders). The main group consisted of 128 subjects, 88 (69%) of whom were males. The control group included 274 patients, 220 (80%) of whom were men. The groups did not differ in age, but there were differences in gender: in the group of patients with depression, women were more common than men (χ^2=6.487; p=0.011). There were no sex differences in the genotype distribution of the CLOCK gene (rs1801260).

Research methods. When assessing the presence and severity of depressive disorders, we relied on clinical signs in accordance with the ICD-10 criteria (F32.0–F32.2. F33.0–F33.2), which, in addition to the method of clinical interviewing, were evaluated using the Montgomery–Asberg Depression Rating Scale (MADRS) [10].

Venous blood samples in the amount of 10 ml were taken from all the subjects using Vacutainer vacuum systems for molecular-genetic and biochemical studies. Venous blood samples for biochemical examination (GGT, CDT) were obtained in the morning on an empty stomach after 10-12 hours of fasting. Venous blood samples for molecular genetic analysis were frozen (-20 °C) and transferred to V.M. Bekhterev National Research Medical Center for Psychiatry and Neurology, where the study was continued.

Molecular genetic testing. Preparation of blood samples for the isolation of dexoxyribonucleic acid (DNA) was carried out using a reagent for the pretreatment of whole peripheral and umbilical cord blood "Hemolytic" (AmpliSens ®, Russia). DNA extraction was carried out using the Ribot-PREP kit (AmpliSens®, Russia). Genotyping for determining single nucleotide variants of the *CLOCK* gene (rs1801260) was performed using a real-time polymerase chain reaction on a RotorGene 6000 amplifier (Quigen, Germany) using a set of reagents manufactured by Syntol (Russia). Genetic examination was carried out in both groups: the main group - patients with alcohol dependence syndrome and comorbid depression, and the control group – patients with alcohol dependence syndrome.

Statistical processing was carried out using software packages Statistica 6.1 (StatSoft Inc., USA, Serial number AXXR902E261711FAN4), Microsoft Excel, IBM SPSS Statistics 22. The Shapiro–Wilk test was used as a method for determining the normality of the distribution of quantitative variables. For the frequency analysis, we used χ^2 test (Pearson chi-squared test), for the comparison of quantitative variables in several independent groups – the Kruskal–Wallis test. Correspondence of the genotype frequency distribution of the studied loci to Hardy–Weinberg equilibrium was evaluated using Fisher's exact test.

Results. When analyzing the obtained molecular genetic data, we found associations between the carriage of the TT genotype and the presence of clinical depression. Carriers of the TT genotype were more likely to develop comorbid depression than carriers of other genotypes. The results are shown in Table 1.

To determine the association between the severity of depressive symptoms and the carriage of various genotypes of the *CLOCK* gene (rs1801260), we compared the severity of depressive manifestations on the MADRS scale using the Kruskal–Wallis test. The results are presented in Table 2.

Discussion. The results of our study revealed correlations between the carriage of the TT genotype of the *CLOCK* gene in patients with alcohol dependence and concomitant clinical depression. The carriage of the TT genotype of the *CLOCK* gene is presumably associated with the predominance of the following symptoms of depression, which demonstrated statistically significant differences - reduced appetite, concentration difficulties, lassitude, as well as an overall higher score on the MADRS scale. It is known that people with depression often have a disrupted circadian rhythm, and many physiological phenomena, including the sleep-wake cycle and hormonal profiles, are also disrupted [11]. It is important to note that people who are addicted to psychoactive substances have circadian changes, such as sleep disorders [12]. A number of researchers have found evidence that ethanol significantly affects circadian regulation [4]. Preference for and sensitivity to alcohol also seem to vary depending on the time of day [13].

Accordingly, the suggestive association established in our work between the *CLOCK* gene and depression in people with alcohol dependence can be considered a vulnerability factor for the development of depression in patients with the main disease – alcohol dependence syndrome. This conclusion is identical to the results of T. Partonen [14], who showed that mutations of the *CLOCK* gene are associated with alcohol use disorders only if they accompany depressive disorders. At the same time, a study by C.B. Forsyth et al. [15] demonstrates a new mechanism of intestinal hyperpermeability caused by alcohol and provides direct evidence of the central role of circadian genes, and above all, the *CLOCK* gene, in the regulation of intestinal permeability and the development of alcohol dependence. Furthermore, S. Leclercq et al. [2] revealed the importance of microbiota disorders associated with alcohol dependence in the development of depression in these patients. I.C. Webb [12], who investigated the genes of circadian rhythms, including the *CLOCK* gene, found that they are also expressed in dopaminergic cells of the ventral tegmental area and in mesolimbic target areas, where they can directly modulate the neurophysiology and behavior associated with the reward system, which is also dysfunctional in depression. A number of researchers have found that the effects of the *CLOCK* gene are associated with seasonal fluctuations and stress, which further explains the mechanism of influence of the circadian system on the clinical picture of mood disorders [8-10. 16. 17]. Other studies have also shown that variants of the *CLOCK* gene may be associated with the effectiveness of selective serotonin reuptake inhibitors in patients with depression [18. 19]. The results obtained by us – in the context of the review of the available literature – allow us to suggest the role of pathogenetic, evidence-based pharmacotherapy of depression and alcohol dependence using selective serotonin reuptake inhibitors, taking into account personalized variations of the *CLOCK* gene and normalization of circadian rhythms.

Conclusion. Based on the analysis of the results obtained, we can conclude that the CLOCK gene is associated with the development of depression in patients suffering from alcohol dependence. It has been revealed that carriers of the TT genotype of the *CLOCK* gene (rs1801260) are more likely to develop comorbid depression than carriers of other genotypes. Moreover, carriers of the TT genotype are characterized by the predominance of such clinical symptoms as reduced appetite, concentration difficulties, lassitude, and an overall higher score on the depression scale MADRS.

Table 1. *Frequency of occurrence of depressive disorder in carriers of different genotypes of the CLOCK gene (rs1801260)*

***CLOCK* (rs1801260)**	**Genotypes, n (%)**			**χ^2**	**d.f.**	**p-value**
	CC	**CT**	**TT**			
Comparison of genotypes	4/21 (19)	44/174 (25)	80/207 (39)	9,446	2	0,009*
TT vs CC, CT	48/195 (25)		80/207 (39)	9,11	1	0,003*

Note. * – p<0,05.

Table 2. *Severity of depressive symptoms in carriers of different genotypes of the CLOCK gene (rs1801260)*

Symptom (MADRS scale)	**CLOCK genotypes (rs1801260), mean rank value**			**H**	**p-value**
	CC	**CT**	**TT**		
Apparent sadness	171,98	191,5	212,9	5,452140	0,0655
Reported sadness	176,38	192,64	211,49	4,401985	0,1107
Inner tension	189,21	192,99	209,9	2,796534	0,2470
Reduced sleep	189,86	196,31	207,04	1,394133	0,4980
Reduced appetite	171,5	191,49	212,95	12,26514	0,0022*
Concentration difficulties	213,88	185,29	213,87	8,288142	0,0159*
Lassitude	192,21	184	217,15	11,97251	0,0025*
Inability to feel	186,67	193,23	209,95	3,544900	0,1699
Pessimistic thoughts	176,05	198,37	206,71	1,794693	0,4077
Suicidal thoughts	206,43	199,23	202,9	0,3264508	0,8494
Total MADRS score	168,45	190,32	214,25	5,877384	0,05*

Note. * – p⩽0,05.

REFERENCES

1. McHugh RK, Weiss RD. Alcohol use disorder and depressive disorders. *Alcohol Res.* 2019 Jan 1;40(1):arcr.v40.1.01. doi: 10.35946/arcr.v40.1.01. eCollection 2019 Oct 21.

2. Leclercq S, Le Roy T, Furgiuele S, et al. Gut microbiota-induced changes in β-Hydroxybutyrate metabolism are linked to altered sociability and depression in alcohol use disorder. *Cell Rep.* 2020 Oct 13;33(2):108238. doi: 10.1016/j.celrep.2020.108238

3. Choi KW, Na EJ, Hong JP, et al. Alcohol-induced disinhibition is associated with impulsivity, depression, and suicide attempt: a nationwide community sample of Korean adults. *J Affect Disord.* 2018 Feb;227:323-9. doi: 10.1016/j.jad.2017.11.001. Epub 2017 Nov 3.

4. Ефремов ИС, Асадуллин АР, Добродеева ВС и др. Ассоциация полиморфных вариантов генов *(HTR2A, MTNR1A, MTNR1B, CLOCK, DRD2)* и бессонницы при синдроме зависимости от алкоголя. *Неврология, нейропсихиатрия, психосоматика.* 2021;13(5):34-9. doi: 10.14412/2074-2711-2021-5-34-39 [Efremov IS, Asadullin AR, Dobrodeeva VS, et al. Association of polymorphic variants of genes (*HTR2A, MTNR1A, MTNR1B, CLOCK, DRD2)* and insomnia in alcohol dependence syndrome. *Nevrologiya, neiropsikhiatriya, psikhosomatika = Neurology, Neuropsychiatry, Psychosomatics.* 2021;13(5):34-9. doi: 10.14412/2074-2711-2021-5-34-39 (In Russ.)].

5. McClung CA, Sidiropoulou K, Vitaterna M, et al. Regulation of dopaminergic transmission and cocaine reward by the *Clock* gene. *Proc Natl Acad Sci U S A.* 2005 Jun 28;102(26):9377-81. doi: 10.1073/pnas.0503584102. Epub 2005 Jun 20.

6. Umemura Y, Koike N, Tsuchiya Y, et al. Circadian key component CLOCK/BMAL1 interferes with segmentation clock in mouse embryonic organoids. *Proc Natl Acad Sci U S A.* 2022 Jan 4;119(1):e2114083119. doi: 10.1073/pnas.2114083119

7. Roenneberg T, Wirz-Justice A, Merrow M. Life between clocks: daily temporal patterns of human chronotypes. *J Biol Rhythms.* 2003 Feb;18(1):80-90. doi: 10.1177/0748730402239679

8. Mendoza J, Vanotti G. Circadian neurogenetics of mood disorders. *Cell Tissue Res.* 2019 Jul;377(1):81-94. doi: 10.1007/s00441-019-03033-7. Epub 2019 May 10.

9. Gyorik D, Eszlari N, Gal Z, et al. Every Night and Every Morn: Effect of Variation in CLOCK Gene on Depression Depends on Exposure to Early and Recent Stress. *Front Psychiatry.* 2021 Aug 26;12:687487. doi: 10.3389/fpsyt.2021.687487. eCollection 2021.

10. Davidson J, Turnbull CD, Strickland R, et al. The Montgomery-Asberg Depression Scale: reliability and validity. *Acta Psychiatr Scand.* 1986 May;73(5):544-8. doi: 10.1111/j.1600-0447.1986.tb02723.x

11. Ferrer A, Costas J, Gratacos M, et al. *Clock* gene polygenic risk score and seasonality in major depressive disorder and bipolar disorder. *Genes Brain Behav.* 2020 Nov;19(8):e12683. doi: 10.1111/gbb.12683. Epub 2020 Jul 26.

12. Webb IC. Circadian rhythms and substance abuse: chronobiological considerations for the treatment of addiction. *Curr Psychiatry Rep.* 2017 Feb;19(2):12. doi: 10.1007/s11920-017-0764-z

13. Hisler GC, Rothenberger SD, Clark DB, et al. Is there a 24-hour rhythm in alcohol craving and does it vary by sleep/circadian timing? *Chronobiol Int.* 2021 Jan;38(1):109-21. doi: 10.1080/07420528.2020.1838532. Epub 2020 Nov 9.

14. Partonen T. Clock genes in human alcohol abuse and comorbid conditions. *Alcohol.* 2015 Jun;49(4):359-65. doi: 10.1016/j.alcohol.2014.08.013. Epub 2015 Jan 16.

15. Forsyth CB, Voigt RM, Burgess HJ, et al. Circadian rhythms, alcohol and gut interactions. *Alcohol.* 2015 Jun;49(4):389-98. doi: 10.1016/j.alcohol.2014.07.021. Epub 2014 Nov 14.

16. McCarthy MJ, Nievergelt CM, Kelsoe JR, et al. A survey of genomic studies supports association of circadian clock genes with bipolar disorder spectrum illnesses and lithium response. *PLoS One.* 2012;7(2):e32091. doi: 10.1371/journal.pone.0032091. Epub 2012 Feb 22.

17. Gonda X, Petschner P, Eszlari N, et al. Genetic variants in major depressive disorder: From pathophysiology to therapy. *Pharmacol Ther.* 2019 Feb;194:22-43. doi: 10.1016/j.pharmthera.2018.09.002. Epub 2018 Sep 4.

18. Ma HY, Liu ZF, Xu YF, et al. The association study of *CLOCK* gene polymorphisms with antidepressant effect in Chinese with major depressive disorder. *Per Med.* 2019 Mar;16(2):115-22. doi: 10.2217/pme-2018-0123. Epub 2018 Dec 20.

19. Garriock HA, Kraft JB, Shyn SI, et al. A genomewide association study of citalopram response in major depressive disorder. *Biol Psychiatry.* 2010 Jan 15;67(2):133-8. doi: 10.1016/j.biopsych.2009.08.029

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Conflict of Interest Statement

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