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Article

Association of Single Nucleotide Variants rs34532313 of the *MTNR1A* Gene and rs10830963 of the *MTNR1B* Gene with Suicidal Risk in Alcohol Dependence Syndrome and Insomnia

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Abstract: Background: Suicidal behaviour is the leading cause of mortality from external causes at all ages worldwide. More than a million people commit completed suicide each year. According to the World Health Organisation, 25-50% of suicide victims suffered from alcohol and other substance use disorders, 22% of all suicide deaths were attributable to alcohol use (WHO, 2014). Several papers have suggested potential associations of insomnia and increased suicide risk in patients with alcoholism. We hypothesise that mutations in melatonin receptor genes may be associated with suicide risk in patients with alcoholism. Methods. The Insomnia Severity Index (ISI) was used as a tool to assess the presence and severity of insomnia. The Columbia Suicide Severity Rating Scale (C-SSRS) was used as a method to examine suicidal behavior. Genotyping of *MTNR1A* (rs34532313), *MTNR1B* (rs10830963) genes was performed using real-time polymerase chain reaction (RT-PCR). A comparative genetic study of two groups of patients was carried out: the first group, patients with alcohol dependence syndrome (F10.2); the second group, patients with alcohol dependence syndrome (F10.2) and insomnia, which persisted 7-14 days after starting alcohol withdrawal therapy. Results. Suicidal thoughts and a history of auto-aggressive behaviour were more common in subjects with insomnia in the post-withdrawal period. Carriers of the TT genotype of the *MTNR1A* gene (rs34532313) were more likely to have suicidal thoughts and a history of suicide attempts in a genetic study of patients with insomnia. Conclusions. Our study found that the TT genotype of the *MTNR1A* gene (rs34532313) is a genetic marker of suicidal behaviour risk in patients with insomnia in the post-withdrawal period. However, the same pattern was not observed in patients without insomnia.

Keywords: *insomnia; single nucleotide variants; suicidal risk; alcohol use disorder; melatonin.*

Introduction

In modern psychiatry, the problem of suicidal behavior is still one of the most relevant. [1,2,3]. According to the World Health Organization, 25-50% of suicide victims suffered from disorders associated with the use of alcohol and other psychoactive substances,

while of all deaths from suicide 22% can be attributed to alcohol use [4]. In the cohort study conducted by Dambrauskiene K. and co-authors, 2/3 of the men and half of the women who committed suicide had problems with alcohol, the risk of suicide was also increased in alcohol abusers compared with drinkers within normal limits [5]. Similar results could be seen in population studies, while it is indicated that with the apparent linearity of the similarity, the relationship between such phenomena is more complex [6]. However, not all patients with chronic alcohol dependence are at risk of suicide, which makes the attribution of additional factors that could serve as tools for preventive impact on suicide very relevant. A number of authors indicate insomnia as a clinically significant correlate of suicidal behavior in patients with alcohol dependence [7,8,9,10]. There is data on the relationship of insomnia and the risk of relapse of active alcohol consumption with the risk of suicidal thoughts [11]. A sample of adolescents with burdened alcohol history (from families of alcoholics) showed that sleep problems were a strong predictor of subsequent suicidal thoughts and behavior as discovered by Maria M. Wong et al [12]. Michael R. Nadorff et al. indicate that symptoms of insomnia mediate a link between suicide risk and alcohol consumption, concluding that insomnia is an important factor, explaining the mechanism by which alcohol consumption increases the risk of suicide [13]. An important link in the pathogenesis in the development of insomnia disorders are melatonin system and its precursors [14,15]. We hypothesise that mutations in melatonin receptor genes may be associated with suicide risk in patients with alcoholism.

Materials and Methods

A comparative cross-sectional study of patients with alcohol dependence syndrome (F10.2) and insomnia disorders in the post-withdrawal period and without the aforementioned was carried out. All patients signed informed voluntary consent, consent of personal data processing. The study was approved by the local ethics committee of the Federal State Budgetary Educational Institution of Higher Education BSMU of the Ministry of Health of Russia (Protocol of 07/08/2020 No. 7). The study was carried out at the Republican Narcological Dispensary No. 1 of the city of Ufa (Republic of Bashkortostan), the Republican Narcological Dispensary No. 2 of the city of Sterlitamak (Republic of Bashkortostan). Molecular genetic studies were carried out at the Center of Personalized Psychiatry and Neurology, V.M. Bekhterev, National Medical Research Centre for Psychiatry and Neurology (St. Petersburg).

Criteria for inclusion, non-inclusion, and exclusion were developed for sampling. Inclusion criteria: 1) The presence of a verified diagnosis F10.2 "Alcohol dependence syndrome"; 2) Age between 18 and 55 years; 3) Between 7 and 14 days from the date of hospitalization; 4) No intake of psychotropic drugs within 3 days prior to the examination. Non-inclusion criteria: 1) Alcohol withdrawal syndrome at time of examination; 2) Dependence on a different psychoactive substance, except for alcohol and nicotine; 3) Objective reasons impeding verbal communication; 4) Comorbid mental pathology: schizophrenia, schizotypal conditions, delusional disorders (F20-F29), affective disorders (F30-F31), dementia (F00-F03), mental retardation (F70-F79), somatic pathology at the decompensation stage; 5) Intake of psychotropic drugs within 3 days prior to the examination. Exclusion criteria: refusal to participate in the study after its commencement, discovery of non-inclusion criteria during the course of clinical interviewing.

Patient examination took place from February 2019 to September 2020. A continuous screening of patients with alcohol dependence syndrome undergoing inpatient treatment in a narcological dispensary was carried out between the 7th and 14th day of stay (post-withdrawal period). All patients were diagnosed with intermediate stage alcohol dependence syndrome. All patients had no alcohol withdrawal syndrome at the time of inclusion in the study. At the time of the study, patients were not receiving psychotropic drugs. All patients underwent a neurological examination; no severe neurological pathology was revealed. 325 patients were screened, 19 were not included in the study in accordance with

the exclusion criteria. The final sample included 306 patients. The average age of the patients was 41.92 ± 7.9 years. Among those included in the study: 21% (64/306) - women, 79% (242/306) - men, which, in general, corresponds to the distribution by gender in the general population among those suffering from alcohol dependence. The sample can be considered representative of the surveyed population group. In terms of the presence of insomnia disorders, 2 groups of patients were identified: the main group - patients with insomnia disorders in the post-withdrawal period, the comparison group - patients without insomnia disorders in the post-withdrawal period (Figure 1).

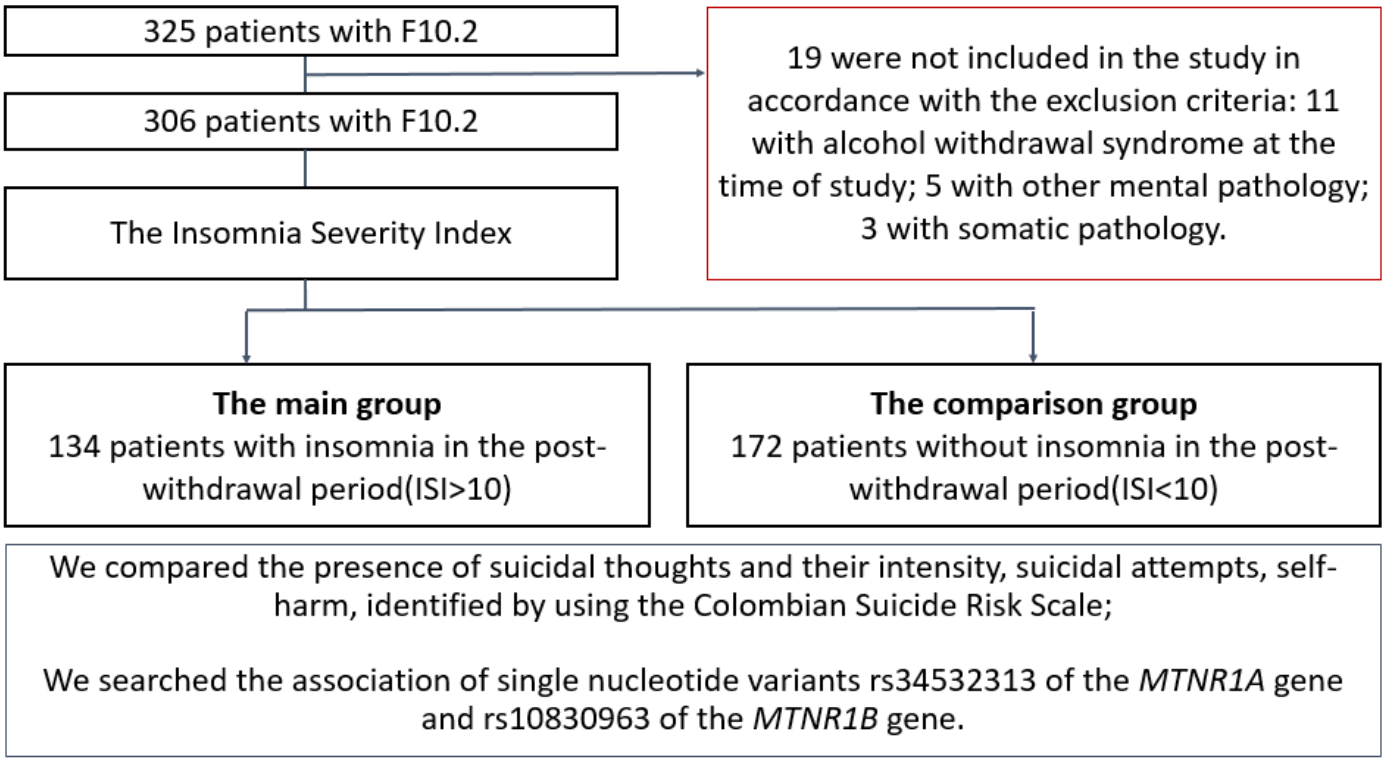

Figure 1. Study design

The Insomnia Severity Index was used as a tool to assess the presence and severity of insomnia. The insomnia severity index (Insomnia Severity Index, ISI, Bastien CH et al., 2001) is a quick and reliable clinical method for investigating the presence of sleep problems, the most common in the world of science [16].

As a method of studying suicidal behavior, the Columbia Suicide Severity Rating Scale (C-SSRS, Columbia Suicide Severity Rating Scale; Posner K. et al., 2007) was used, which makes it possible to assess the presence and intensity of suicidal ideas arising in a patient throughout his/her life [17].

All subjects gave samples of venous blood in an amount of 10 ml using the vacuum system Vacutainer for molecular genetic research, the samples were frozen (-20° C) and transported to the The Center of Personalized Psychiatry and Neurology, V.M. Bekhterev National Medical Research Centre for Psychiatry and Neurology, where research was continued. Sample preparation of blood samples for DNA isolation was carried out with a reagent for pretreatment of whole peripheral and umbilical cord blood "Hemolytic" (AmpliSens®). DNA extraction was carried out with the Ribot-PREP kit (AmpliSens®). A single-nucleotide variant (SNV) genotyping of genes MTNR1A (rs34532313), MTNR1B (rs10830963) was performed using real-time polymerase chain reaction (RT-PCR) on a RotorGene 6000 amplifier (Quigen, Germany) using a kit of reagents manufactured by Syntol (Moscow). A genetic examination was carried out in two groups of patients.

A genetic examination of two groups of patients was held: the first group - patients with the syndrome of alcohol dependence (F10.2); the second group - patients with alcohol dependence and insomnia.

Genotypes distribution compliance with the Hardy-Weinberg law was assessed using Fisher's exact test using the Munich Institute of Human Genetics portal (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl), as well as using Pearson's Chi-square test using Microsoft Excel.

Statistical processing was carried out using the software packages STATISTICA 10 (Stat. Soft, USA, Serial number AXXR902E261711FAN4), Microsoft Excel, IBM SPSS Statistics 26. Frequency analysis was performed using the χ^2 test (Pearson's Chi-square).

Results

We considered the presence of suicidal thoughts and their intensity, suicidal attempts, self-harm, identified by using the Colombian Suicide Risk Scale. Frequency of occurrence in patients with and without insomnia was assessed using the Chi-square test. The results are presented in the table 1.

Table 1. Comparison of the frequency of suicidal thoughts, suicidal attempts and self-harm in patients with insomnia and without the aforementioned.

№	Attribute	Sample group	%	Control group	%	Chi-square	p-value
1.	Suicidal ideation in patient history	46/134	34	29/172	17	12,421	0,000[1]
2.	Suicidal attempts in patient history	15/134	11	10/172	6	2,906	0,088
3.	Self-harm in patient history	27/134	20	19/172	11	4,886	0,027[1]

[1] – $p<0,05$

The intensity of suicidal thoughts, if any, in the main group and the comparison group was assessed using the Mann-Whitney U-test. In the group with insomnia disorders in the post-withdrawal period, the intensity of suicidal ideas is higher than in the group without them ($p = 0.002$).

Next, a search was carried out for genetic associations in suicidal behavior in a group of patients with insomnia during the post-withdrawal period and without. For the main group, there were statistically significant differences in the carriage of the TT genotype of the MTNR1A gene (rs34532313). No statistically significant differences were found for carriers of different genotypes of the MTNR1B gene (rs10830963).The results are shown in the table 2.

Table 2. Association of single nucleotide variants rs34532313 of the *MTNR1A* gene and rs10830963 of the *MTNR1B* gene with suicidal risk caused by alcohol dependence syndrome and insomnia

№	Gene (SNV)	Attribute	Genotype; n (%)			Chi-square	p-value
			CC	CT	TT		
1.	*MTNR1A* (rs34532313)	Suicidal ideation in patient history	26/68 (38%)	13/56 (23%)	7/10 (70%)	9,173	0,01[1]

2.		Suicidal attempts in patient history	8/68 (12%)	3/56 (5%)	4/10 (40%)	10,289	0,006[1]
3.		Self-harm in patient history	13/68 (19%)	10/56 (18%)	4/10 (40%)	2,677	0,262
			CC	CG	GG		
4.	*MTNR1B* (rs10830963)	Suicidal ideation in patient history	19/52 (37%)	19/53 (36%)	8/29 (28%)	0,752	0,687
5.		Suicidal attempts in patient history	3/52 (6%)	8/53 (15%)	4/29 (14%)	2,547	0,28
6.		Self-harm in patient history	9/52 (17%)	10/53 (19%)	8/29 (28%)	1,312	0,519

[1] – p<0,05

For the comparison group (without insomnia), no statistically significant differences in suicidal risk in carriers of different genotypes of the studied genes were found. The results are shown in the table 3.

Table 3. Association of single nucleotide variants rs34532313 of the *MTNR1A* gene and rs10830963 of the *MTNR1B* gene with suicidal risk of alcohol dependence syndrome and without insomnia

№	Gene (SNV)	Attribute	Genotype; n (%)			Chi-square	p-value
			CC	CT	TT		
1.	*MTNR1A* (rs34532313)	Suicidal ideation in patient history	16/96 (17%)	9/54 (17%)	4/22 (18%)	0,031	0,984
2.		Suicidal attempts in patient history	5/96 (5%)	3/54 (6%)	2/22 (9%)	0,502	0,778
3.		Self-harm in patient history	10/96 (10%)	7/54 (13%)	2/22 (9%)	0,326	0,849
			CC	CG	GG		
4.	*MTNR1B* (rs10830963)	Suicidal ideation in patient history	13/95 (14%)	9/48 (19%)	7/29 (24%)	1,902	0,386
5.		Suicidal attempts in patient history	6/95 (6%)	1/48 (2%)	3/29 (10%)	2,351	0,309
6.		Self-harm in patient history	12/95 (13%)	4/48 (8%)	3/29 (10%)	0,617	0,735

It was established for the first time that genetic markers of the risk of suicidal behavior in patients with insomnia in post-withdrawal period, in contrast to patients without insomnia disorders, has the TT genotype of MTNR1A gene (rs34532313).

When comparing the severity of the intensity of suicidal ideas in their presence in carriers of different genotypes of the studied genes using the Kruskal - Wallis test, similar results were found. In the group with insomnia, the intensity of suicidal ideation was statistically significantly higher in the group of patients with insomnia (MTNR1A (rs34532313). No statistically significant differences were found in the group of patients without insomnia. The comparison results in the group with insomnia are presented in table 4.

Table 4. Comparison of the intensity of suicide ideas in carriers of different genotypes rs34532313 of the MTNR1A gene and rs10830963 of the MTNR1B gene in alcohol dependence syndrome and insomnia

№	Gene (SNV)	Attribute	Genotype, Mean Rank			H	p-value
			CC	CT	TT		
1.	*MTNR1A* (rs34532313)	The intensity of suicidal thoughts	68,9	59,2	96,8	11,55	0,0031[1]
			CC	CG	GG		
2.	*MTNR1B* (rs10830963)	The intensity of suicidal thoughts	68,08	67,99	63,29	0,47	0,7900

[1] – p<0,05

The results of comparing the intensity of suicidal ideation in the group of patients without insomnia are presented in table 5.

Table 5. Comparison of the intensity of suicide ideas in carriers of different genotypes rs34532313 of the MTNR1A gene and rs10830963 of the MTNR1B gene in alcohol dependence syndrome and without insomnia

№	Gene (SNV)	Attribute	Genotype, Mean Rank			H	p-value
			CC	CT	TT		
1.	MTNR1A (rs34532313)	The intensity of suicidal thoughts	86,4	86,78	86,23	0,006	0,997
			CC	CG	GG		
2.	MTNR1B (rs10830963)	The intensity of suicidal thoughts	83,32	88,66	93,36	2,29	0,32

Thus, we can find that the TT genotype of the MTNR1A gene (rs34532313) is characterized by a high frequency of suicidal attempts in history, as well as the frequency of occurrence of suicidal thoughts and their intensity if present. For patients without insomnia, the described associations were not found. No associations of the MTNR1B gene (rs10830963) were found in both groups.

Discussion.

The relationships found correlate with previously discovered clinical results [18]. Despite the description in literature of some genetic associations of melatonin receptor genes [19,20,21,22], the connections found by us are described for the first time. The results obtained indirectly confirm our hypothesis about the connection between suicidal behavior and insomnia disorders through genetic variants of melatonin receptor genes. At the same time, it is impossible to point out the pathophysiological mechanism at the present stage of the study; it can only be assumed that suicidal behavior and insomnia disorders in patients with alcohol dependence may be associated with the metabolism of melatonin and its precursors, as well as the molecules involved in this. Further work in this area may contribute to the development of personalized approaches in early diagnosis and correction of suicidal behavior in alcohol-dependent patients with and without sleep disorders, which is relevant in modern psychiatry [23].

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Conflicts of Interest: The authors declare no conflict of interest.

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