PPN

Personalized Psychiatry and Neurology

Article

Genetic associations of clinical manifestations of alcohol withdrawal syndrome among patients with and without insomniac disorders in the post-abstinence period

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Citation Efremov, I.S..; Ashurov, Z.Sh.; Tulbaeva, N.R.; Islamova E.D.; Abdrakhmanova A.E.; Asadullin A.R. Genetic associations of clinical manifestations of alcohol with-drawal syndrome among patients with and without insomniac disorders in the post-abstinence period. *Personalized Psychiatry and Neurology* **2022**, *x*, x. https://doi.org/10.52667/xxxxx

Chief Editor: Nikolaj G. Neznanov, D Med Sci, Professor

Received: date
Accepted: date
Published: date

Publisher's Note: V.M. Bekhterev NMRC PN stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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Abstract: Our study aimed to determine clinical and genetic associations between severity of alcohol withdrawal syndrome and polymorphic variants of genes HTR2A (rs6313), MTNR1A (rs34532313), MTNR1B (rs10830963), CLOCK (rs1801260), DRD2 (rs1800497) among patients with alcohol dependence syndrome, with and without insomniac disorders in the post-abstinence period. Methods. 306 adults were examined. 2 groups of patients were identified: the main group – patients with insomniac disorders in the post-abstinent period, the comparison group – patients without insomniac disorders in the post-abstinent period. Results. The following associations were identified for the analyzed group with insomniac disorders: the GG genotype of the MTNR1B gene (rs10830963) with paroxysmal sweating and disorientation on the CIWA-Ar scale; the TT genotype of the HTR2A gene (rs6313) and paroxysmal sweating; the TT genotype of the CLOCK gene (rs1801260) and convulsive seizures in the structure of alcohol withdrawal syndrome; the TT genotype of the MTNR1A gene (rs34532313) and auditory hallucinations, tachycardia and arterial hypertension. For patients without insomniac disorders: the homozygous genotype of the HTR2A gene (rs6313) is associated with anxiety, visual and auditory hallucinations; the GG genotype of the MTNR1B gene (rs10830963) is associated with anxiety and tachycardia; the CC genotype of the CLOCK gene (rs1801260) is associated with arousal; the CC genotype of the DRD2 gene (rs1800497) is associated with headache.

Keywords: *alcoholism; alcohol withdrawal syndrome; MTNR1A; MTNR1B; HTR2A; CLOCK; DRD2.*

Introduction

Alcohol withdrawal syndrome is a condition that occurs after a sudden reduction in ethanol use and is characterized by a diversity of clinical manifestations, including deadly conditions such as alcoholic delirium [1,2]. One of the criteria for the severity of alcohol withdrawal syndrome is insomniac disorders, which also continue in the post-abstinence period (2-3 weeks after the cessation of alcohol withdrawal syndrome) and can be used as a marker for predicting the severity of the withdrawal syndrome in the future [3]. An important field of study of alcohol withdrawal syndrome is the search for clinical and genetic associations [4]. Knowing the importance of insomniac disorders, it can be assumed that single-nucleotide polymorphic variants of genes that are involved in the regulation of circadian rhythm may be associated with the specifies of the clinical picture of alcohol withdrawal syndrome.

Objective

To determine clinical and genetic associations between severity of alcohol withdrawal syndrome and polymorphic variants of genes HTR2A (rs6313), MTNR1A (rs34532313), MTNR1B (rs10830963), CLOCK (rs1801260), DRD2 (rs1800497) among patients with alcohol dependence syndrome, with and without insomniac disorders in the post-abstinence period.

Materials and Methods

It was held a comparative cross-sectional study of patients with alcohol dependence syndrome, with and without insomniac disorders in the post-abstinence period. The study was approved by the local ethics committee of the Bashkir State Medical University of the Ministry of Health of Russia (Protocol of July 8, 2020 No. 7).

The study was carried out on the basis of the republican narcological dispensary № 1 in Ufa (Republic of Bashkortostan), the republican narcological dispensary № 2 in Sterlitamak (Republic of Bashkortostan). Molecular genetic studies were carried out on the basis of the Institute of Personalized Psychiatry and Neurology ("The St. Petersburg V. M. Bekhterev National Medical Research Center for Psychiatry and Neurology" of the Ministry of Health of the Russian Federation).

Inclusion, non-inclusion and exclusion criteria have been developed to form a selection. Inclusion criteria:

1. Verified diagnosis F10.2 "Mental and behavioral disorders due to use of alcohol. Dependence syndrome";
2. The period of observation in the narcological dispensary which lasted more than a year;
3. Signed voluntary informed consent;
4. The age of the subjects is from 18 to 55 years;
5. From 7 to 14 days must have passed since the hospitalization day;
6. No use of psychotropic drugs for 3 days before the examination;

Non-inclusion criteria:

1. Alcohol withdrawal syndrome at the time of the study;
2. Addiction to another psychoactive substance except alcohol and nicotine;
3. Objective reasons which are making verbal contact difficult;
4. Comorbid mental pathology: schizophrenia, schizotypal states, delusional disorders (F20-F29), affective disorders (F30-F31), dementia in Alzheimer's disease (F00-F03), mental retardation (F70-F79), somatic pathology in the decompensation stage;
5. Fact of use of psychotropic drugs during 3 days before the examination;
6. Convulsive attacks due to other reasons than alcohol withdrawal syndrome in patient's anamnesis.

Exclusion criteria: refusal to participate in the study after its start; finding non-inclusion criteria in the process of clinical interviewing.

The examination of patients took place from February 2019 to September 2020. A continuous screening of patients with alcohol dependence syndrome who underwent inpatient treatment at a narcological dispensary was conducted on the 7th-14th day of stay (post-withdrawal period). All patients were diagnosed with alcohol dependence syndrome of the middle stage. All patients had no alcohol withdrawal syndrome at the time of inclusion in the study. At the time of the study patients did not take psychotropic drugs. All patients underwent neurological examination, no severe neurological pathology was detected.

325 patients were screened, 19 were not included in the study according to the criteria of non-inclusion. The final selection included 306 patients. The average age of patients was 41.92±7.9 years. Among those included in the study: 21% (64/306) are women, 79% (242/306) are men, which 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 insomniac disorders, 2 groups of patients were identified: the main group – patients with insomniac disorders in the post-abstinent period, the comparison group – patients without insomniac disorders in the post-abstinent period.

To estimate the presence and severity of insomnia disorders, the Insomnia Severity Index was used. The Insomnia Severity Index (ISI, Bastien C.H. et al., 2001) is a brief instrument that was designed to assess the severity of both nighttime and daytime components of insomnia. ISI is a fast and reliable clinical method for examining the presence of sleep problems, widely used in science (Suleiman K.H. et al., 2011; Fernandez-Mendoza J. et al., 2011; Lahan V. et al., 2011; Yazdi Z. et al., 2012; Gerber M. et al., 2016). The index contains 7 questions, which are used to estimate the severity of insomnia, the presence and severity of insomnia disorders: difficulty falling asleep, sleep interruptions, early awakenings.

As a cut-off point for diagnosing the presence of insomnia, the authors of the technique suggest 10 points, at which subclinical insomnia is defined. The sensitivity of the method is 86.1%, the specificity is 87.7%. The technique is also used to rank the severity of insomnia from subclinical to severe according to a score scale: 10-14 – subclinical insomnia, 15-21 - moderate clinical insomnia, 22-28 – severe clinical insomnia.

Clinical Institute Withdrawal Assessment for Alcohol scale (CIWA-Ar) is a clinical method used to estimate the somatic and mental symptoms of alcohol withdrawal syndrome, their presence and severity (Sullivan J.T. et al., 1989; Stuppaeck C.H. et al., 1994; Corniello A. et al., 2012; Knight E. et al., 2017; Melkonian A. et al., 2019). The method is recommended for use among patients with alcohol withdrawal syndrome in the clinical recommendations of the Ministry of Health of the Russian Federation (2019). The evaluation according to the scale was made on the basis of an objective anamnesis and subjective information received during the interviewing procedure. Also, in addition to the option presented in the recommendations, information about coordination disorders, pulse and blood pressure was recorded. The technique allows to evaluate the following symptoms of alcohol withdrawal syndrome: nausea and vomiting, tremor, paroxysmal sweating, visual hallucinations, agitation, tactile disturbances, tachycardia, arterial hypertension, movement coordination disorders, orientation/clarity of consciousness, auditory hallucinations, anxiety, headache.

Venous blood samples of 10 ml were taken from the subjects using Vacutainer vacuum systems for molecular genetic research, the samples were frozen (-20 ° C) and transported to the Department of Personalized Psychiatry and Neurology ("The St. Petersburg V. M. Bekhterev National Medical Research Center for Psychiatry and Neurology" of the Ministry of Health of the Russian Federation), where the research was continued. Sample preparation of blood samples for DNA extraction was carried out with a

reagent for pretreatment of peripheral and umbilical cord blood "Hemolytic" (AmpliSens®). DNA extraction was carried out with a set of "RIBO-prep" (AmpliSens®). HTR2A (rs6313), MTNR1A (rs34532313), MTNR1B (rs10830963), CLOCK (rs1801260), DRD2 (rs1800497) genes were genotyped using a real-time polymerase chain reaction (RT-PCR) on a RotorGene 6000 amplifier (Quigen, Germany) using a set of reagents manufactured by Syntol (Moscow). Genetic examination of two groups of patients was carried out: the first group - patients with alcohol dependence syndrome (F10.2); the second group consists of patients with alcohol dependence syndrome and insomniac disorders.

Statistical processing was conducted using STATISTICA 10 software packages (Stat. Soft, США, Serial number AXXR902E261711FAN4), Microsoft Excel, IBM SPSS Statistics 26. The Shapiro-Wilk test was used as a method for determining the normality of the distribution of quantitative variables. During the frequency analysis, the criterion $\chi 2$ was used (Pearson's chi-squared test).

Results

In the group of patients with insomniac disorders, statistically significant differences were found during the Kraskel-Wallis test of the severity of clinical manifestations of alcohol withdrawal syndrome, its severity in owners of various genotypes of the studied genes, these differences are presented in Table 1.

Table 1. Severity of scores obtained using the Clinical Institute Withdrawal Assessment for Alcohol scale among patients with insomniac disorders and different genotypes

№	Gene (OHB)	The symptom (question of the CIWA-Ar scale)	Genotypes; Mean Rank			H	p-value
			CC	CT	TT		
1.	HTR2A rs6313	Paroxysmal sweating	56,6	73,13	81,1	7,107165	0,0286*
2.		Agitation	52,6	75,3	59,6	7,550592	0,0229*
			CC	CT	TT		
3.	MTNR1A	Auditory hallucinations	70,7	68,2	42	6,318427	0,0425*
4.	rs34532313	Pulse	65,3	75,6	37,1	9,448369	0,0089*
5.		Blood pressure	66,8	73,3	33,7	9,386682	0,0092*
			CC	CG	GG		
6.	MTNR1B	Paroxysmal sweating	60,2	64,1	87	9,862143	0,0072*
7.	rs10830963	Clarity of consciousness	60	67	82	6,447435	0,0398*
			CC	CT	TT		
8.	CLOCK	Tremor	72,7	75,2	56,8	7,480141	0,0238*
9.	rs1801260	Blood pressure	78,3	73,5	57,1	6,693501	0,0352*
			CC	CT	TT		
10.	DRD2 rs1800497	Discoordination	55	55,1	79,3	6,385093	0,0411*

* - p-value < 0,05 In the group of patients with insomniac disorders, statistically significant differences in the occurrence of convulsive seizures in the structure of alcohol withdrawal were found among owners of different genotypes of the rs1801260 gene CLOCK (Table 2). But it was not possible to construct a statistically significant regression model that includes that

predictor. The results received during the analysis of a group of patients without insomniac disorders are presented in Table 3.

Table 2. Occurrence of convulsive seizures in the structure of alcohol withdrawal among owners of different genotypes of the OHB rs1801260 gene CLOCK in the group of patients with insomniac disorders

№	The symptom (question of the CIWA-Ar scale)	Gene (OHB)	Genotypes; n (%)			Pearson's chi-squared test	p-value
			CC	CT	TT		
1.	Convulsive seizures	CLOCK (rs1801260)	1/9(11 %)	17/70 (24%)	28/55 (51%)	11,991	0,002*

* - p-value < 0,05

Table 3. Severity of scores obtained using the Clinical Institute Withdrawal Assessment for Alcohol scale among owners of various genotypes of the studied genes among patients without insomniac disorders

№	Gene (OHB)	The symptom (question of the CIWA-Ar scale)	Genotypes; Mean Rank			H	p-value
			CC	CT	TT		
1.	HTR2A rs6313	Visual hallucinations	92,9	79,1	96,6	6,734207	0,0345*
2.		Anxiety	95,9	77,6	95,7	6,047801	0,0486*
3.		Auditory hallucinations	95,8	79,7	84,8	5,861878	0,0533*
			CC	CG	GG		
4.	MTNR1B rs10830963	Anxiety	78,8	88,3	102,9	5,793143	0,0552*
5.		Pulse	87,4	72,9	99,9	6,164151	0,0459*
			CC	CT	TT		
6.	CLOCK rs1801260	Agitation	128,9	79,2	86,6	8,237668	0,0163*
			CC	CT	TT		
7.	DRD2 rs1800497	Headache	86,4	74,5	60,5	8,614721	0,0135*

* - p-value < 0,05

There were no statistically significant associations between the frequency of occurrence of alcoholic delirium, seizures after alcohol withdrawal and owning of the studied genotypes in the group of patients without insomniac disorders.

Therefore, clinical and genetic associations characteristic of patients with alcohol dependence syndrome and insomniac disorders and without them in the post-abstinence period were found.

Conclusions

The differences of clinical and genetic associations of alcohol withdrawal syndrome among patients with insomniac disorders and without them were determined. The following associations were identified for the analyzed group with insomniac disorders: the GG genotype of the MTNR1B gene (rs10830963) with paroxysmal sweating and disori-

entation on the CIWA-Ar scale; the TT genotype of the HTR2A gene (rs6313) and paroxysmal sweating; the TT genotype of the CLOCK gene (rs1801260) and convulsive seizures in the structure of alcohol withdrawal syndrome; the TT genotype of the MTNR1A gene (rs34532313) and auditory hallucinations, tachycardia and arterial hypertension. For patients without insomniac disorders: the homozygous genotype of the HTR2A gene (rs6313) is associated with anxiety, visual and auditory hallucinations; the GG genotype of the MTNR1B gene (rs10830963) is associated with anxiety and tachycardia; the CC genotype of the CLOCK gene (rs1801260) is associated with arousal; the CC genotype of the DRD2 gene (rs1800497) is associated with headache.

Author Contributions: conceptualization I. S. E.; methodology I. S. E., A. R. A.; software, I. S. E., E. D. I.; formal analysis I. S. E., A. E. A., N. R. T.; investigation I. S. E., E. D. I., A. E. A.; resources I. S. E., N. R. T., A. R. A.; data curation I. S. E., E. D. I., Z. Sh. A., N. R. T.; writing—original draft preparation I. S. E., A. E. A., Z. Sh. A.; writing—review and editing I. S. E., E. D. I., N. R. T., A. E. A.; supervision I. S. E., Z. Sh. A., A. R. A.; project administration I. S. E., Z. Sh. A, A. R. A.; All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Bashkir State Medical University, Ministry of Health of the Russian Federation (protocol no. 7 of 08.07.2020)

Informed Consent Statement: Informed consent was obtained from all subjects in-volved in the study.

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

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