Article

Association of alcohol withdrawal severity with *MTNR1A (rs34532313)* and *MTNR1B (rs10830963)* genes polymorphisms

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Abstract: Alcohol withdrawal is the most threatening condition encountered in patients with alcohol use disorder. Our study aimed to investigate the association of alcohol withdrawal severity with polymorphic variants in melatonin receptor genes. **Methods**. The clinical study was carried out on the basis of the Republican Narcological Dispensary №1 in Ufa and the Republican Narcological Dispensary №2 in Sterlitamak. Genetic analysis was performed at the Department of Personalised Psychiatry and Neurology at the V.M. Bekhterev Research Centre, Saint Petersburg. The final sample consisted of 307 subjects. **Results**. Carriers of the TT genotype of the *MTNR1A* gene (rs34532313) were found to have less hypertension during alcohol withdrawal than carriers of the other genotypes. In comparison, carriers of the GG genotype of the *MTNR1B* gene (rs10830963) experienced more symptoms than other genotypes: paroxysmal sweating, visual hallucinations, anxiety, and overall CIWA-Ar score. **Conclusions**. Thus, it can be concluded that the TT genotype of *MTNR1A* gene (rs34532313) is associated with a lower risk of hypertension during alcohol withdrawal compared to carriers of other gene genotypes. The GG genotype of *MTNR1B* gene (rs10830963) is associated with severe withdrawal. In general, it can be concluded that melatonin receptors are involved in the pathogenesis of alcohol withdrawal and the severe of some of its symptoms.

Keywords: alcoholism; alcohol withdrawal syndrome; melatonin; MTNR1A; MTNR1B

Introduction

Alcohol use problems remain a matter of concern to modern public health. At the end of 2018, according to the Federal State Statistics Service, 1,208,600 people (823.4 per 100,000) in the Russian Federation were reported to be on treatment and prevention programmes for alcohol abuse and alcohol-induced psychoses. Alcohol misuse causes severe somatic [1,2,3]; and mental disorders [4,5]. Alcohol withdrawal, an acute clinical condition that develops due to the abrupt cessation of alcohol intake or a marked decrease in consumption, is one of the dangerous conditions that occur in patients with alcoholism. This syndrome develops 6 to 24 hours after alcohol withdrawal due to prolonged alcohol consumption. Alcohol withdrawal syndrome is characterised by vegetative and mental disorders, which can be complicated by alcohol delirium, seizures and lead to disability and death [6]. However, the severity of alcohol withdrawal can be predicted by analysing anamnestic, clinical and laboratory data. For instance, male gender,

young age; severe trauma; history of mental illness, alcohol delirium; low potassium, chlorine, platelet levels; the presence of hallucinations, disorientation, seizures, systolic blood pressure above 140 mmHg; high CIWA-Ar, AUDIT scores are predictors of severe alcohol withdrawal syndrome [7, 8, 9,10]. The genetic associations of severe alcohol withdrawal are of particular interest. A study by Smith A. H. et al. revealed that the *SORCS2* gene, which encodes a multiligand receptor, is associated with the severity of alcohol withdrawal, and the stress hormones and ethanol enhance the expression of this gene in vitro [11]. Earlier, we identified an association between the severity of alcohol withdrawal and insomnia in the post-holiday period [12], suggesting the involvement of melatonin metabolism in these disturbances [13, 14]. However, we have not found any published studies investigating the relationship between the severity of alcohol withdrawal and melatonin receptor genes.

Objective

Purpose was the study of the association alcohol withdrawal syndrome severity with polymorphisms of the *MTNR1A* (rs34532313) and *MTNR1B* (rs10830963) genes.

Materials and Methods

A cross-sectional study of patients with alcohol dependence syndrome (F10.2) was conducted at the Republican Narcological Dispensary No 1 in Ufa and the Republican Narcological Dispensary No 2 in Sterlitamak in the Republic of Bashkortostan. Genetic screening was carried out in the Department of Personalised Psychiatry and Neurology at the V.M. Bekhterev National Medical Research Centre for Psychiatry and Neurology, Saint Petersburg. All patients signed consent forms. The study was approved by the Local Ethical Committee of the Federal State Budgetary Educational Institution of Higher Professional Education, Bashkir State Medical University, Ministry of Health of the Russian Federation (protocol no. 2 of 27.02.2019; protocol no. 7 of 08.07.2020).

Inclusion criteria were the presence of a confirmed F10.2 diagnosis of Alcohol Dependence Syndrome; age between 18 and 55 years inclusive. Exclusion criteria included: refusal to participate in the study and presence of comorbid psychiatric pathology (schizophrenia, schizotypal conditions, delusional disorders (F20-F29), dementia (F00 - F03), mental retardation (F70 - F79) with severe somatic pathology of cardiovascular, respiratory, endocrine, gastrointestinal and urinary systems). Subsequent exclusion criteria were refusal to participate in the study after it had begun and identification of exclusion criteria during the clinical interviews.

The final sample consisted of 307 subjects with a mean age of 42.5 ± 7.8. 21% (65/307) of the subjects were female, and 79% (242/307) were male. The Cochran formula was used to determine the sample size. Thus, the sample can be considered representative of the studied population. To investigate the genotypes' consistency with Hardy-Weinberg's law, a population group with similar inclusion criteria was recruited but subsequently withdrawn from the study due to identifying exclusion criteria. The genotypes were allocated according to Hardy-Weinberg law.

Clinical-psychopathological, psychometric, molecular-genetic and statistical methods have been used.

The clinical-psychopathological method involved clinical interviewing of patients and obtaining medical history and identification of clinical symptoms and syndromes. The severity of alcohol withdrawal was assessed using the Clinical Institute Withdrawal Assessment Alcohol Scale (CIWA-Ar, revised scale, Saitz R. et al., 1994). Evaluation of the severity of alcohol withdrawal in patients included the presence of a history of seizures after alcohol withdrawal and the presence of one or more episodes of alcohol delirium.

A venous blood sample of 10 ml was collected from all subjects using Vacutainer vacuum systems for molecular genetics and biochemical analysis. Venous blood samples for biochemical tests were obtained in the morning on an empty stomach following a 10-12 hour fast. Venous blood samples for molecular genetic testing were frozen (-20ºC) and transferred to the Centre of Personalised Psychiatry and Neurology at the V.M. Bekhterev National Medical Research Centre for Psychiatry and Neurology, where the research was continued.

Molecular genetic analysis. For desoxyribonucleic acid (DNA) extraction, blood samples were pre-treated with Hemolytic (AmpliSense®) whole peripheral blood and umbilical cord blood pre-treatment reagent. DNA extraction was carried out with a Ribo-PREP kit (AmpliSense®). Genotyping of the *MTNR1A* (rs34532313) and *MTNR1B* (rs10830963) genes by single nucleotide variants (SNVs) was performed using real-time polymerase chain reaction (RT-PCR) on a RotorGene 6000 amplifier (Quigen, Germany) with a reagent kit manufactured by Syntol (Moscow, Russia).

Statistical data were analysed using the software packages STATISTICA 6.1 (Stat. Soft, USA, Serial number AXXR902E261711FAN4), Microsoft Excel, IBM SPSS Statistics 22. The Shapiro-Wilk test determined the normality of the distribution of the quantitative variables. Frequency analysis was performed using the χ^2 criterion (Pearson Chi-square). The Mann-Whitney non-parametric U-test was used to compare quantitative variables in the two independent groups, and the Kruskal-Wallis test was used to compare quantitative variables in several independent groups. The choice of non-parametric methods was due to the difference from a normal distribution of some of the quantitative variables in the sample. Statistical analyses of the genetic data were performed using IBM SPSS 20.0. Differences in the frequencies of genetic variables were detected using Pearson's test (x2), with $p<0.05$ being considered statistically significant. Consistency of the distribution of genotype frequencies of the studied loci according to Hardy-Weinberg equilibrium was assessed using Fisher's exact test.

Table 1. Association of genotypes of rs34532313 of the *MTNR1A* gene with severity of alcohol withdrawal symptoms in humans.

№	Symptom	Genotypes *MTNR1A* (rs34532313) Mean Rank			H	p-value
		CC	CT	TT		
1.	Nausea/vomiting	151,05	153	158,3	0,20	0,9
2.	Tremor	155,48	146,28	158,81	0,95	0,62
3.	Paroxysmal sweats	159,55	144,89	142,97	2,32	0,31
4.	Visual disturbances	149,5	159,04	145,17	1,69	0,43
5.	Agitation	154,69	152,3	142,08	0,62	0,74
6.	Tactile disturbances	150,57	154,95	153,84	0,23	0,89
7.	Anxiety	151,17	156,36	145,95	0,44	0,80
8.	Headache/fullness in head	147,73	158,76	155,13	1,18	0,55
9.	Auditory disturbances	153,58	156,07	134,78	2,17	0,34
10.	Orientation/clouding of sensorium	153,62	149,85	149,85	0,19	0,9
11.	Movement coordination disorders	159,22	143,98	147,75	2,32	0,31
12.	Pulse	149,46	162,4	133,89	3,25	0,2
13.	Blood pressure	154,14	160,06	113,73	7,56	0,02*
14.	Total points (CIWA-Ar)	152,58	157,15	145,67	0,45	0,8

Table 2. Association of genotype rs10830963 of the *MTNR1B* gene with severity of alcohol withdrawal symptoms in humans.

№	Симптом	Genotypes *MTNR1B* (rs10830963) Mean Rank			H	p-value
		CC	CG	GG		
1	Nausea/vomiting	156,3	146,8	152,8	0,77	0,68
2	Tremor	146,3	149,2	173,9	4,57	0,10
3	Paroxysmal sweats	145,5	146,2	180,9	7,75	0,02*
4	Visual disturbances	146,96	147,34	175,25	7,96	0,02*
5	Agitation	147,72	151,77	165,79	1,97	0,37
6	Tactile disturbances	150,21	155,55	153,01	0,30	0,86
7	Anxiety	139,07	153,86	183,97	11,23	< 0,01*
8	Headache/fullness in head	154,45	157,76	138,52	2,11	0,35
9	Auditory disturbances	147,51	151,73	166,39	2,77	0,25
10	Orientation/clouding of sensorium	147,41	149,73	170,09	3,16	0,21
11	Movement coordination disorders	148,9	158,05	151,94	0,72	0,7
12	Pulse	156,74	136,91	168,72	5,88	0,05
13	Blood pressure	148,12	153,46	159,37	0,76	0,68
14	Total points (CIWA-Ar)	143,45	149,8	185,4	9,62	< 0,01*

Results

Data on the relationship of genotypes of the MTNR1A (rs34532313) and MTNR1B (rs10830963) genes with the severe of withdrawal are presented in Table 1, Table 2.

The severity of alcohol withdrawal syndrome was assessed using the CIWA Ar scale to analyse the association between alcohol withdrawal severity and the SNVs *MTNR1A* (rs34532313) and *MTNR1B* (rs10830963) genotypes. In addition, the severity of individual symptoms on responses to this scale was assessed using the Kruskal-Wallis test.

It was observed that carriers of the TT genotype of the *MTNR1A* gene (rs34532313) had a lower incidence of hypertension in the alcohol withdrawal syndrome than carriers of other genotypes. In contrast, carriers of the GG genotype of the *MTNR1B* gene (rs10830963) had the following symptoms: paroxysmal sweating, visual hallucinations, anxiety and overall CIWA-Ar score (Figure 1) to a greater extent than other genotypes.

Complications with seizures after alcohol withdrawal and the development of alcohol delirium are one of the criteria for the severity of alcohol withdrawal syndrome. In the study sample, 83/307 (27.03%) had a history of seizures after alcohol withdrawal syndrome 119/188 (63.29%) had a history of alcohol delirium. Frequency analysis using the χ^2 criterion demonstrated that the incidence of alcohol delirium and seizures in a patient history of alcohol withdrawal was not different among carriers of different the *MTNR1A* (rs34532313) and *MTNR1B* (rs10830963) genotypes.
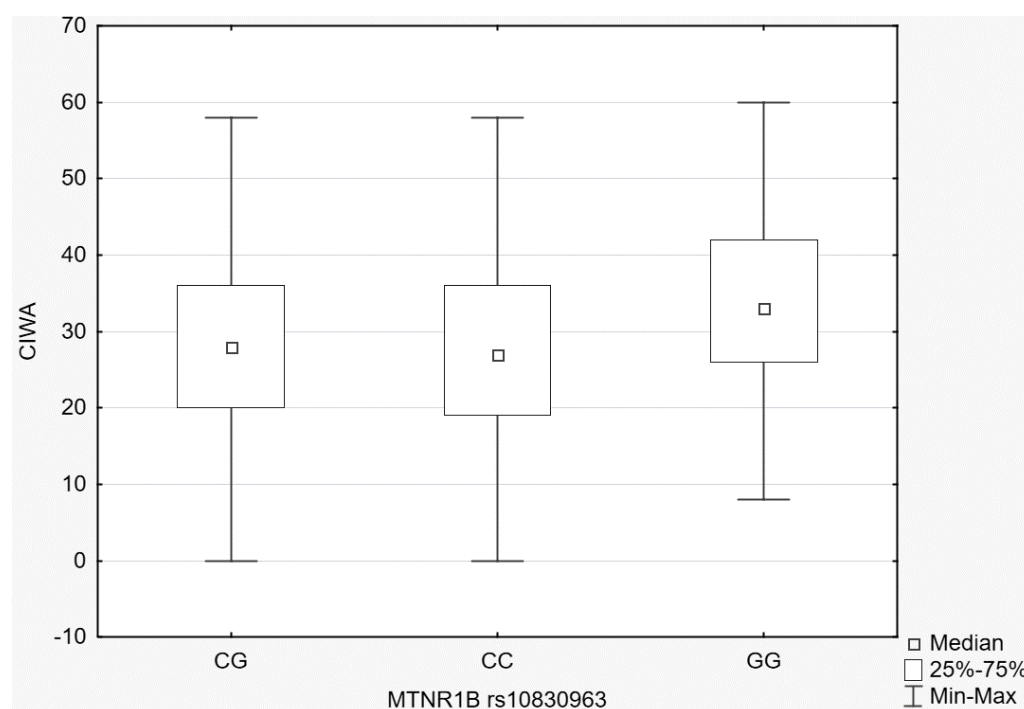
Figure 1. The severity of alcohol withdrawal syndrome according to the results of CIWA-Ar in carriers of different genotypes of the *MTNR1B* gene

Conclusions

Thus, it can be concluded that the TT genotype of the *MTNR1A* gene (rs34532313) is associated with a lower risk of arterial hypertension during alcohol withdrawal compared to carriers of other gene genotypes. The GG genotype of the *MTNR1B* gene (rs10830963) is associated with severe withdrawal. Not association of these genes with the risk of seizures in alcohol withdrawal and alcohol delirium was observed.

However, carriers of the GG genotype of the *MTNR1B* gene (rs10830963) had a greater incidence of light sensitivity or visual hallucinations in alcohol withdrawal, which requires further investigation and follow-up.

In general, it can be concluded that melatonin receptors are involved in the pathogenesis of alcohol withdrawal and the severe of some of its symptoms.

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

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

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