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ORIGINAL RESEARCH

The Lack of Influence of Homozygous Long Allele of the 5-HTTLPR Gene on the Severity of Alcohol Craving During 6 Weeks of Rehab Hospitalisation in Comparison to Not Homozygous and Homozygous Short Alleles – Preliminary Report

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Purpose: The aim of this study was to assess changes in the severity of alcohol craving according to allelic variants of the 5-HTTLPR gene polymorphism during hospitalisation and their association with selected clinical variables in alcohol-dependent patients.

Patients and Methods: The study is exploratory. Participants were investigated at the 2nd and 6th week of alcohol-dependence therapy in the addiction treatment unit. Recruitment was conducted among alcohol-dependent patients from several Polish drug treatment centres. The total sample size was 130 persons (12 females and 118 males). Study subjects' mean age was 43.0 years. Patients were investigated twice by using the Penn Alcohol Craving Scale (PACS) and Beck Depression Inventory (BDI), and once by using Short Alcohol Dependence Data Questionnaire (SADD) and taking a swab for genetic testing. The polymorphism of the gene encoding the serotonin transporter 5-HTTLPR (SLC6A4) was determined from isolated DNA and its homozygous variants of short/short or long/long alleles and heterozygous short/long alleles were analysed.

Results: At 6th week of the follow-up, there was a decrease in the severity of alcohol craving in half of subjects with the short/short allele ($p = 0.033$) and in one-fifth of subjects with the long/short allele ($p = 0.002$) of the 5-HTTLPR gene. In subjects with long/long allele of the 5-HTTLPR gene, there was no change in the severity of alcohol craving between 2nd and 6th weeks of the study ($p = 0.242$).

Conclusion: There was no statistical influence of the homozygous long allele of the 5-HTTLPR gene on severity of alcohol craving during 6 weeks of rehab hospitalisation in comparison to not homozygous and homozygous short alleles. The s-allele was associated with decrease of alcohol craving. It may point on the potential need for differentiated rehabilitation methods depending on the genetic diversity of addicted patients and its role in the severity of alcohol craving.

Keywords: 5-HTTLPR polymorphism, alcohol craving, alcohol dependence

Introduction

One of the most significant risk factors for relapse is an intense and difficult-to-control alcohol craving.[1] The severity of alcohol craving can be assessed using a variety of psychological scales.

The findings suggest that alcohol craving is a complex phenomenon influenced by many factors.[2] Individual variables associated with alcohol craving included age, race, drinking consequences, drinking intensity, motivation to change,

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mood disorders, sleep problems and social support.[2] In multivariate analysis ($R^2 = 0.34$), alcohol craving was positively associated with mood disorders, heavy drinking, readiness to change and negatively associated with age.[2] Alcohol craving is strongly associated with drinking immediately following drug treatment and is likely to be more prevalent in those with more severe forms of addiction and deeper mood disorders.[3] In the treatment of alcohol dependence, great attention is devoted to diagnosis and education on relapse risk factors and coping skills. One measuring the intensity of alcohol craving is the Penn Alcohol Craving Scale, whose high scores indicate an increased risk of alcohol relapse.[1] The individuals with alcohol dependence are most likely to benefit from interventions designed to manage alcohol craving after drug treatment.[3]

It is noteworthy that the 2019 study indicates that changes in the severity of alcohol craving are associated with sympathetic nervous system hyperreactivity expressed as heart rate arousal among other things, which is clearly due to the intensity of alcohol consumption. Researchers suggest that normalisation of this hyperreactivity may only occur after four months of maintaining alcohol abstinence.[4,5] Thus, the reduction in the risk of relapse to drinking is slow, especially as it is often associated with autonomic hyperreactivity, mood deterioration and specifically severe alcohol craving.[5]

Addiction, alcohol craving and drinking relapse are neurobiologically determined. One role in the development of addiction is played by the neuronal pathways of the mesolimbic system, the main neurotransmitter of which is dopamine.[6] These connect the ventral tegmental area of the midbrain to the septal semicircular nucleus (nucleus accumbens) and are modulated by various neuronal systems including the serotonergic neurons from the sutural nuclei that stimulate them. Serotonergic neurons also have projections in the hippocampus, which is also involved in the development of addiction through learning processes. In it, the memory of events is consolidated and the control of drive-emotional behaviour takes place.[6]

Attempts have been made to demonstrate that the course of alcohol dependence, as well as the intensity of individual symptoms of dependence, including alcohol craving that can be genetically influenced. Studies highlight a significant association of the polymorphism of the 5-HTTLPR gene (SLC6A4), which encodes the serotonin transporter (5-HTT) protein with the development of addiction.[7,8] To date, a positive association of the 5-HTTLPR gene polymorphism of the short (S) or short/short (S/S) allele (short allele) with alcohol dependence, the greater emotional arousal, lowered mood and, in the case of the long (L) allele, a positive association with response to drug treatment in alcohol-dependent individuals has been observed.[7,8] However, other researchers do not directly link the 5-HTTLPR gene polymorphism to the risk of alcohol dependence syndrome, but to its determination.[9]

It is noteworthy that the association of the 5-HTTLPR gene polymorphism shows great heterogeneity.[10] Individuals with the long/long or long allele (L or L/L, the long allele) of the 5-HTTLPR gene reveal observed increased serotonin uptake, a better response to treatment with certain antidepressants and a higher frequency of obsessive-compulsive disorder or cognitive impulsivity.[11,12] Other researchers confirm the association of the 5-HTTLPR gene polymorphism variant of the "long" allele with compulsive craving for alcohol.[11]

Note that conditioning of the response to environmental stressors by a polymorphism in the 5-HTTLPR gene is also indicated (individuals with one or two copies of the short allele of the 5-HTT promoter polymorphism had more depressive symptoms associated with stressful life events than individuals homozygous for the long allele).[13] In addition, expression of the 5-HTTLPR gene is significantly associated with addicts' temperamental traits as well as with typologies of alcohol dependence, which often have a pragmatic dimension in addiction therapy.[14,15] In addition, it is indicated that amygdala reactivity in stress reactions and their cognitive control are related on the 5-HTTLPR gene polymorphism.[16]

The above-mentioned studies on polymorphisms of the 5-HTTLPR gene with different allele variants suggest the importance of personalised therapy in alcohol-dependent patients. They show the necessity of including diagnostic, pharmacological, and psychoeducational interventions targeting, inter alia, alcohol craving, drinking intensity, or improvement of mood disorders in order to prevent relapse.

Compared to other studies, we presented longitudinally the association of the 5-HTTLPR gene polymorphism with changes in the severity of alcohol craving, which may have important clinical implications for relapse prevention during drug therapy.

The aim of this study was to assess changes in the severity of alcohol craving according to allelic variants of the 5-HTTLPR gene polymorphism during hospitalisation and their association with selected clinical variables in alcohol-dependent patients, particularly the severity of depression or alcohol dependence.

Materials and Methods

Recruitment was conducted among alcohol-dependent patients from several Polish drug treatment centres in Kujawsko-Pomorskie (3 centres), Łódzkie (1 centre) and Podlaskie (1 centre). The total sample size was 130 persons (12 females and 118 males), due to missing data of some variables, the number of analysed variables may be smaller. Study subjects' mean age was 43.0 years (24–78 years, SD=9.9). Patients were investigated twice using the same methods except for performing the SADD test and taking a swab for genetic testing. The study was longitudinally. The first examination was performed during a period of at least 2 weeks of abstinence maintenance (after the potential symptoms of the abstinence syndrome had resolved), ie, during the 2nd week of hospitalisation, and the second examination 4 weeks after the first examination (ie, up to the 6th week of hospitalisation). The study started with patients who had maintained alcohol abstinence for at least 2 weeks (considered to be week 2 of hospitalisation) in order to exclude possible abstinence symptoms interfering with the study. Thus, the study group is more clinically homogeneous. None of the subjects displayed alcohol withdrawal symptoms. It should be noted that each of the subjects participated in cognitive-behavioural psychotherapy and psychoeducation covering the issue of maintaining alcohol abstinence during hospitalisation. The therapeutic measures taken were independent methods of influencing patients outside the methodology of this study. Some of the patients studied were taking medication used in psychiatry or treatment of somatic disorders.

The inclusion criteria for the study group were age at least 18 years, no other addictions except alcohol and nicotine dependence, no symptoms of alcohol and nicotine abstinence syndrome, no metabolic diseases (eg, diabetes mellitus), ability to provide informed consent, no active viral or bacterial infections including liver dysfunction of an infectious nature.

Exclusion criteria were symptoms of abstinence syndrome, diagnosis of addiction other than alcohol and nicotine dependence, cognitive impairment preventing participation in the study, metabolic diseases and extremes of nutritional status.

The research was carried out using the face-to-face interview method. All questionnaires and measurements were carried out by specialised researchers.

During the interview with patients, information characterising the clinical situation (eg, duration of alcohol dependence, antiepileptic, antidepressant, anti-anxiety and antipsychotic medication taken) was collected. The following questionnaires were used in the study:

Assessment of Alcohol Dependance Severity

Short Alcohol Dependence Data Questionnaire (SADD), which is used to assess the depth of alcohol dependence. The scale consists of 15 questions. Four response options are possible. The successive response variants correspond to degrees of depth of addiction and are therefore scored incrementally from 0 to 3 points. Depth of addiction is assessed on the total score obtained. The following criteria are adopted 1–9 points - mild depth of addiction; 10–19 points - moderate; 20–45 points - deep. The scale achieved good psychometric properties in studies - Cronbach's alpha was 0.87.[17,18]

Assessment of Alcohol Craving

Penn Alcohol Craving Scale (PACS) - in a Polish adaptation by Chodkiewicz et al, 2016. This scale consists of five test items. Three questions address the frequency, intensity and duration of craving, one measures the ability to resist temptation when drinking is possible, and another estimates the degree of overall alcohol craving over the past week. Responses are recorded on a scale of 0–6. The higher the score, the stronger the severity of craving. For example, to question 1 (How often have you thought about drinking or about how good a drink would make you feel during this period?), the possible answers are: 0-never, 1- rarely, 2- occasionally, 3- sometimes, 4 often, 5- most of the time, 6- nearly all of the time. The scale has good psychometric properties and is very often used in studies of alcohol craving. Furthermore, it can predict, better than other methods, the risk of relapse during treatment.[1] The score range 0–3 indicates low severity of alcohol craving, 4–9 average, 10 and above, high severity of alcohol craving. In the present study, Cronbach's alpha was 0.86.[1,19]

Assessment of Depressive Disorders

Beck Depression Inventory (BDI) - a questionnaire used to assess depressive symptoms. The questionnaire consists of twenty-one items describing the severity of depressive symptoms, on a scale of 0–3. When summed up, the scores are

read according to criteria: 0–9 points ie, no depressive symptoms, 10–19 are mild depressive symptoms, while 20–25 points suggest moderate depressive symptoms, 25 and more deep depressive symptoms. In alcohol-dependent individuals, scores of 13 and above may suggest depressive disorders. The questionnaire was adapted to Polish conditions by Parnowski and Jernajczyk in 1977.[20,21]

Genetic Analysis

Determination of Gene Polymorphisms

DNA isolation from oral mucosal swabs was carried out using professional disposable swabs. Sampling took place approximately 2 hours after a meal or while fasting. In addition, the test subjects were not allowed to smoke cigarettes or chew gum for approximately 2 hours as well.

The polymorphism of the gene encoding the serotonin transporter 5-HTTLPR (SLC6A4) was determined from isolated DNA. Its homozygous variants of short/short or long/long alleles and heterozygous short/long alleles were analysed in the study.

DNA from buccal swabs was extracted by GeneMATRIX Bio-Trace DNA Purification Kit (EurX) according to the manufacturer's instructions. Genotyping of 5-HTT LPR was performed utilizing PCR-based method following by fragment analysis in a capillary electrophoresis sequencer ABI3130 (Thermo Fisher Scientific). The PCR reactions consisted of 15 ng of total DNA, 15 pmol of each primer (forward: 5'-6FAM-ATGCCAGCACCTAACCCCTAATGT-3' and reverse: 5'-GGACCGCAAGGTGGGCGGGA-3'), 1 U of Go Taq Flexi polymerase, 1x PCR Buffer, 0,2 mM dNTP, 1,5 mM $MgCl_2$ (Promega) and milli-Q water to a total volume of 20μL. The thermal profile composed of: 10 min at 94°C and 35 cycles of the following steps: denaturation 94°C, 1 min, annealing 66°C, 1 min, and extension 72°C, 1 min. The results of genotyping were analysed using GeneMapper v3.2 software.

Method of Elaboration of the Results and Statistical Analysis

For the analysed variables, the conformity of their distribution with the normal distribution was checked. To assess the distribution, the Shapiro–Wilk test was applied, which showed that the analysed variables deviated from the normal distribution. It was decided to use non-parametric Mann–Whitney *U*-test, Wilcoxon test, Kruskal–Wallis test and post hoc ANOVA test (Games-Howell post hoc test), linear regression test in the statistical analysis. Tests from the Statistica programme and the IMAGO6 statistical package (IBM SPSS 27) were used in the study. It was decided to use the median(s) and the minimum and maximum values (min-max) of the data obtained in the statistical descriptions. The level of statistical significance was taken as $p<0.05$.

Ethics

Bioethical supervision of the study was exercised by the Bioethics Committee of the Collegium Medicum (consent number KB 692/2017). The study complies with the Declaration of Helsinki. During recruitment prior to the study prior to procedures being performed, patients were provided with information about the rules of participation and signed a consent form including a statement to agree to the collection of biological material, its use in the study and the investigators' use of the subject's personal and clinical data.

Results

The first stage of analyses compared the association of allelic variants in the 5-HTTLPR gene polymorphism with clinical and psychological variables.

The study patients (Table 1), irrespective of the allele variant in the 5-HTTLPR gene polymorphism, were characterised by similar age, similar gender distribution, similar results of duration of dependence in years (11.5 vs 15.0 vs 11.5 years), depth of alcohol dependence (SADD score, 22.5 vs 21.0 vs 24.0) and gamma glutamyl transferase activity (GGT, 73.0 vs 68.5 vs 67.0 U/l) and similar frequency of psychopharmacotherapy, mental disorders and therein depression (it were the responses of patients).

Table 2A shows the results of measuring the intensity of alcohol craving at the start of treatment and after 6 weeks (using the dependent samples test).

Table 1 Comparison of Selected Clinical and Psychological Variables in Relation to Allelic Variants in the 5-HTTLPR Gene Polymorphism

Variables	(1) 5-HTTLPR Gene Polymorphism (Short/Short or Short Allele) n=18	(2) 5-HTTLPR Gene Polymorphism (Long/Short Allele) n=62	(3) 5-HTTLPR Gene Polymorphism (Long/Long or Long Allele) n=50	P
	Median (min-max) or number (%)	Median (min-max) or number (%)	Median (min-max) or number (%)	
Age (years)	43.5(27.0–57.0)	44.0 (27.0–67.0)	40.5(24.0–78.0)	0.115
Sex				
Women	1(5.6%)	5(8.1%)	6(12.0%)	0.654
Men	17(94.4%)	57(91.9%)	44(88.0%)	
Length of alcohol dependence (years)	11.5(3.0–30.0)	15.0(2.0–40.0)	11.5(1.0–30.0)	0.140
SADD (score)	22.5(6.0–35.0)	21.0(7.0–37.0)	24.0(0.0–38.0)	0.331
BDI (score)	14.5(2.0–31.0)	17.0(0.0–51.0)	15.0(0.0–47.0)	0.733
GGT (U/l)	73.00 (25.0–227.0)	68.50 (10.0–611.0)	67.00 (19.0–1495.0)	0.998
Psychopharmacotherapy				
Antiepileptics*	2(20.0%)	2(9.5%)	2(15.4%)	0.712
Antidepressants*	2(20.0%)	2(9.5%)	5(38.5%)	0.127
Antianxiety medications*	3(30.0%)	1(4.8%)	3(23.1%)	0.140
Antipsychotics medications*	4(40.0%)	6(28.6%)	5(38.5%)	0.759
Mental disorders (response of patients)	5(29.4%)	17(27.4%)	23(46.0%)	0.107
Depression (response of patients)	3(17.6%)	5(8.1%)	11(22.0%)	0.110

Notes: Chi^2 test and Kruskal–Wallis test and Games-Howell post hoc test, *Group size for frequency of pharmacotherapy - columns: (1) n=10, (2) n=21, (3) n=13.

It was shown (Table 2A) that patients with variants of the 5-HTTLPR short/short and short/long gene polymorphisms experienced a decrease in alcohol craving severity during the four weeks of hospitalisation. And in patients with the 5-HTTLPR long/long gene polymorphism variant, on the contrary, there was no significant statistical difference in alcohol craving severity scale scores between weeks 2 and 6 of hospitalisation.

As the data analysis was applied to 130 individuals and the Wilcoxon test only shows data where changes in data values occurred, detailed analyses had to be performed.

For all subjects (Table 2B), a statistically significant difference between measurement one and measurement two occurred for 58 people.

For the polymorphism of the 5-HTTLPR gene (in people with short/short or short alleles), a significant difference occurred for 10 people. For 9 people the result improved, for 1 person it increased.

For the 5-HTTLPR gene polymorphism (in individuals with short/long alleles) difference significant for 30 individuals. For 22 people the result improved, for 8 people it increased.

For the 5-HTTLPR gene polymorphism (in people with long/long or long alleles) there was no statistical significance. Although the result was statistically non-significant in these individuals, it is worth noting that changes occurred in 18 cases. In 10 there was an improvement ie, a decrease in PACS scores, and in eight there was no change.

The results of Figure 1 showed that in the subgroup of subjects with short/short or short alleles, at the second measurement of alcohol craving severity, there is a significant increase in the proportion of subjects with PACS scores of 0–3 (with weak alcohol craving) and a concomitant absence of subjects with PACS scores of 10 or more (with strong alcohol craving). A similar trend was observed in the subgroup of subjects with long/short alleles, where an increasing proportion of subjects with PACS scores of 0–3 and a decreasing proportion of subjects with PACS scores of 10 or more points were shown in the second measurement. In contrast, in the subgroup of subjects with long/long or long alleles, no significant change was seen in the percentages of subjects with PACS 0–3, PACS 4–9 or even PACS 10 and above scores between the first and second measurement of alcohol craving severity.

Table 2 The Comparison of the Intensity of Alcohol Craving During 4 Weeks of Hospitalisation of Alcohol-Dependent Patients and the Gene Polymorphisms (2A). The Changes of the Intensity Alcohol Craving Between the First and Second Study According Gene Polymorphism – Detailed Analysis (2B)

(A) Comparison of the intensity of alcohol craving measured with the PACS scale during hospitalisation of alcohol-dependent patients according to allelic variants of the 5-HTTLPR gene polymorphism.				
Variables		**Severity of alcohol craving in the first 2 weeks of hospitalisation PACS score Median (min-max)**	**Severity of alcohol craving at week 6 of hospitalisation PACS score Median (min-max)**	**p**
	N	AFTER 4 WEEKS → AFTER 4 WEEKS		
Patients with 5-HTTLPR gene polymorphism (short/short or short allele)	18	5.0(0–15)	2.5(0–7)	0.033
Patients with 5-HTTLPR gene polymorphism (long/short allele)	62	5.0(0–24)	4.0(0–15)	0.002
Patients with 5-HTTLPR gene polymorphism (long/long or long allele)	50	5.0(0–24)	4.5(0–19)	0.242
(B) Differences in PACS results between "study I" and "study II" (detailing the results of Table 2A)				
5-HTTLPR gene polymorphism	**N**	**T**	**Z**	**p**
Total	58	532.500	2.501	0.012
Short/short or short allele	10	5.000	2.293	0.022
Long/short allele	30	130.000	2.108	0.035
Long/long or long allele	18	82.000	0.152	0.879

Notes: Wilcoxon test; N - number of subjects; p - probability level p; T - sum of ranks; Z - Z function of the U statistic; out of 130 subjects analysed, the result for 58 was statistically significant. These should be interpreted as follows according to the rule - "for this many people the result improved and for this many the result worsened", ie for the short/short polymorphism, for 9 people the result improved and it worsened for 1 (% of total). For short/long, there was an improvement for 22 and for 8 a worsening (% of total). For long/long no change with an improvement for 10 and no improvement for 8 (% of total).

Table 3A presents an attempt to assess whether, in addition to the 5-HTTLPR gene polymorphism, other factors were associated with changes in the severity of alcohol craving between weeks 2 and 6 of treatment. (improvement in terms of reduction in alcohol craving severity during four weeks hospitalisation) was based on the PACS scale score. Single variables were analysed in the regression model.

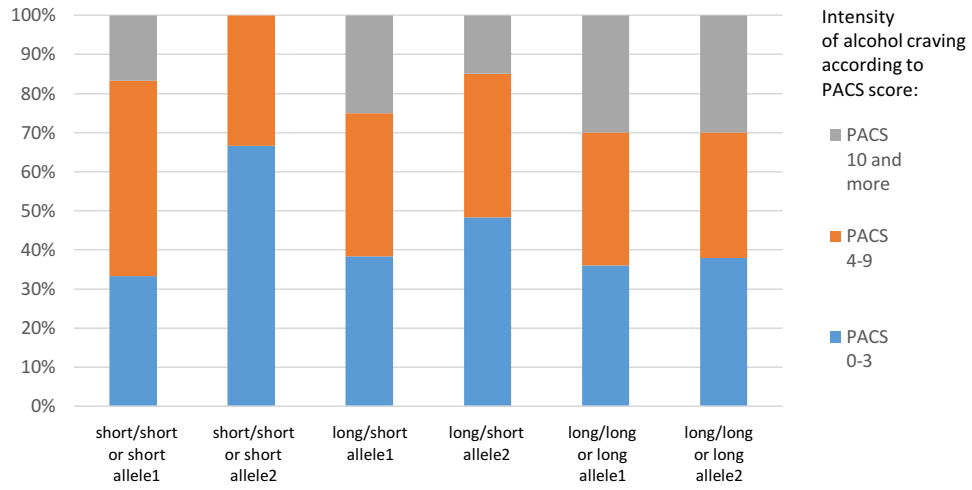

Figure 1 Shows the percentages of people with mild, moderate and severe alcohol craving (according to the PACS scale) divided into three subgroups with respect to the 5-HTTLPR polymorphism. 1 Measurement of severity of alcohol craving at the start of treatment (in the first 2 weeks of hospitalisation); 2- measuring the severity of alcohol craving 4 weeks after the first examination. Illustration of the percentage incidence of PACS results in the three subgroups by descriptive statistics - no statistical tests were used here.

Table 3 An Attempt to Assess Whether, in Addition to the 5-HTTLPR Gene Polymorphism, Other Factors Were Associated with Changes in the Severity of Alcohol Craving (3A) and the Model of the Intercorrelation of These Variables (3B)

(A) Univariate significance tests for the variable occurrence of PACS performance improvement (parameterisation with sigma-constraints; effective hypothesis decomposition)

Effect	SS	*df*	MS	F	p
Free expression	0.870	1	0.870	3.854	0.052
5-HTTLPR Polymorphism	1.176	1	1.176	5.212	0.024
Sex	0.001	1	0.001	0.005	0.944
Age	0.434	1	0.434	1.923	0.168
SADD (score)	0.946	1	0.946	4.190	0.043
BDI (score)	0.186	1	0.186	0.823	0.366
Error	27.536	122	0.226	-	-

(B) SS test for full model against SS for residuals (sum) - improvement

Dependent variable	Multiple R	Multiple R^2	Corrected R^2	SS Model	*df* Model	MS Model	SS Rest	*df* Rest	MS Rest	F	p
Improvement	0.349	0.122	0.086	3.831	5	0.766	27.536	122	0.226	3.395	0.007

Notes: (Part A) The one-dimensional significance tests and (Part B) Multiple linear regression test; legend: SS - sum of squares of effects; df - number of degrees of freedom; MS - mean sum of squares of effects; F- F-test value; p- probability level p; R- coefficient of multiple correlation; R^2 - coefficient of multiple determination; SS Rest - residual sum of squares; MS Rest - residual mean of squares.

The data in Table 3A, showed that there were two significant predictors of craving, ie, 5-HTTLPR polymorphism (p=0.024) and severity of alcohol dependence (SADD; p=0.043).

Further analyses were based on the above variables (Table 3B).

Table 3B shows the intercorrelation of the variables in the model of Table 3A. The table data confirm that the model was well chosen.

The multiple coefficient of determination (multiple R^2) (Table 3B) indicated an explanation of approximately 12% of the dependent variable (improvement). The entire model was found to be statistically significant. The total correlation of all variables (multiple R), with the overall variable improvement in the mean correlation (0.349).

The data in Figure 2 indicated that the highest rate of improvement (ie, a reduction in the severity of alcohol craving ie, PACS scale scores) was observed in 5-HTTLPR gene polymorphism "0" (in subjects with short/short or

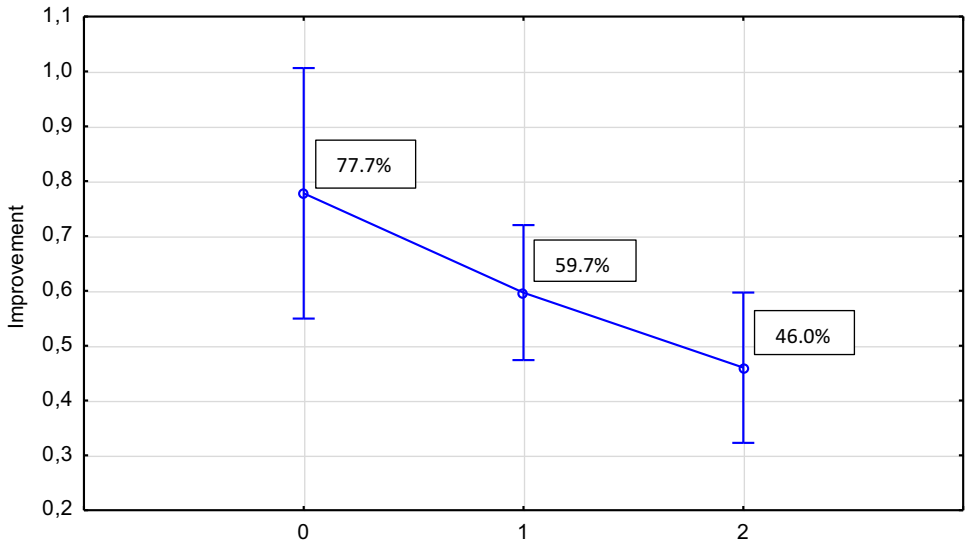

Figure 2 Alcohol craving severity scores (PACS scale scores) in depending on the type of polymorphism of the 5-HTTLPR gene ("0" - subjects with the short/short or short alleles; "1" - individuals with alleles of short/long; "2"- subjects with long/long or long alleles) in alcohol-dependent patients tested (improvement means low alcohol craving severity scores; the decrease in PACS scores obtained by the subjects after 4 weeks of treatment compared to the first test of severity of alcohol craving with the PACS scale is presented as an improvement; the decrease in scores is included as a percentage of the decrease in relation to the original PACS values and compared between patient groups. 5-HTTLPR polymorphism; Expected marginal averages. Current effect: F (2, 127)=2.9747, p=0.05463. Decomposition of effective hypotheses. Vertical bars indicate 0.95 confidence intervals.

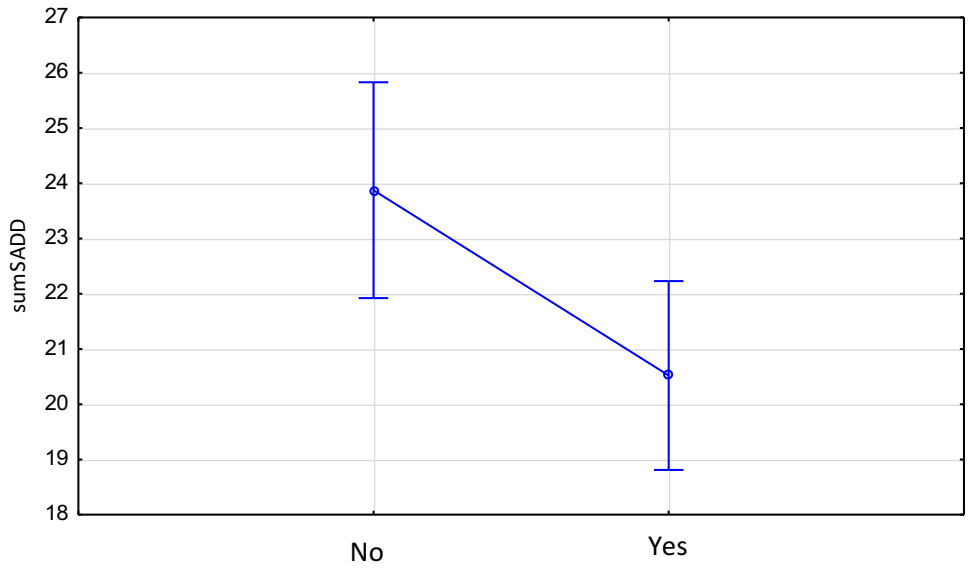

Figure 3 Alcohol craving severity scores (PACS scale score) in relation to severity of alcohol dependence (SADD scale score) in surveyed alcohol-dependent patients (improvement indicates low alcohol craving severity scores). Improvement; Expected marginal averages. Current effect F (1, 127)=6.5383, p=0.01173. Decomposition of effective hypotheses. Vertical bars indicate 0.95 confidence intervals. Improvement (Yes) - is a decrease in PACS value. No improvement (No) - no decrease or increase in PACS value.

short alleles) 77.7%, and the lowest in 5-HTTLPR gene polymorphism "2" (in subjects with long/long or long alleles) 46.0%. In contrast, in the 5-HTTLPR "1" gene polymorphism (in individuals with short/long alleles) was 59.7%.

Figure 3 data showed that improvement (reduction in severity of alcohol craving ie, PACS scale scores) occurred to a greater extent among subjects with lower SADD scores (ie, lower severity of alcohol dependence).

Discussion

In this study, we showed that in alcohol-dependent patients with the long/long allele variant of the polymorphism in the serotonin transporter gene 5-HTTLPR, alcohol craving persists at a similar level both at baseline and after four weeks of inpatient rehab. As mentioned in the "study subjects" section of this article, each of the study participants participated in cognitive behavioural therapy and psychoeducation. These interventions included coping with alcohol craving, abstinence maintenance or treatment methods. However, in patients with polymorphisms in the 5-HTTLPR gene, short/short and long/short variants, the severity of alcohol craving decreased statistically significantly during therapy. Relevant to the success of therapy is the fact that increased alcohol craving is a risk factor for abstinence fracture. Thus, research may suggest that individuals with the long/long allele variant of the serotonin transporter gene 5-HTTLPR polymorphism will be at particular risk of relapse to alcohol.[10] Previous studies have shown that in the Hispanic population, individuals with long/long or long/short alleles of the 5-HTT gene, had higher severity of alcohol craving than those with short/short alleles.[22,23] Bleich et al, indicated an association of the long variant with compulsive rather than obsessive craving for alcohol.[10] In contrast, scores on a questionnaire measuring obsessive-compulsive drinking (OCDS) appear to be sensitive to severity of addiction and changes during abstinence and relapse.[24]

In the present study, the PACS scale, which does not measure compulsive and obsessive aspects of alcohol craving, was applied to assess alcohol craving.[1] Hence, we conclude that not only compulsivity, but also other characteristics of alcohol craving, such as frequency, intensity or degree of resisting temptation may be conditioned by the homozygous variant of the long allele of the 5-HTTLPR gene polymorphism. This is confirmed by the results presented in Table 2A and B, that the severity of alcohol craving was at a similar level, both at the beginning of drug treatment and four weeks after the first craving test in patients with the variant of the long/long polymorphism. Patients with the other allelic variants of the 5-HTTLPR gene polymorphism (short/short and long/short) showed that differences in alcohol craving severity and the proportion of subjects with low alcohol craving severity between measurement points were more

pronounced than in subjects with the long/long variant (Table 2B, Figure 1). A 2014 study by other authors found no association of the 5-HTTLPR polymorphism with alcohol craving. However, these were exploratory studies, our study is longitudinal, which changes the perspective on changes in the severity of alcohol craving per unit time (shows the risk of relapse that an addicted patient may experience during treatment).[25] The ambiguity of the results of the study may emphasise that the polymorphism in the 5-HTTLPR gene does not have a simple translation into the experience of alcohol craving and rather determines factors such as temperamental traits or reactions to difficult situations that sustain the experience of alcohol craving.[10,26] In contrast, the authors of previous studies, despite inconclusive results on the association of the 5-HTTLPR polymorphism with alcohol craving, indicated the clinical significance of the above polymorphism in the expression of alcohol craving.[10,26] It is worth adding that there are results available which highlight the importance of the 5-HTTLPR polymorphism in the treatment of addiction eg, in the context of the interaction of this polymorphism with cognitive behavioural therapy or in relation to drug treatment. It has been shown that alcohol-dependent individuals with this polymorphism (genotype short/short) show a better response to naltrexone treatment and to psychosocial interventions.[27] Patients with the short/short genotype consumed more drinks than those with the long/long or short/long genotype, and this was independent of the treatment administered.[27]

Psychopharmacotherapy, including the individual patient's response to the medication, may be a factor that may influence the degree to which alcohol craving is experienced. Serretti et al, and Muhonen et al, showed that depressed alcohol-dependent patients with the long allele may be more likely to respond to serotonin reuptake group antidepressants.[8,28] Other studies have shown that patients with long/long alleles, and a history of early-onset dependence, who simultaneously experienced intense anxiety consumed more alcohol during treatment with sertraline.[29] And on the other hand, patients with long/long alleles were found to significantly reduce their alcohol drinking when receiving placebo.[29] Studies on the effect of 5-HTTLPR gene polymorphism on psychopharmacological effect are inconclusive and still need to be continued.

In the present study, there were no differences in the frequency of psychopharmacotherapy use and BDI scale scores between subgroups of subjects with different allele variants of the 5-HTTLPR gene polymorphism. This means that patients, irrespective of their 5-HTTLPR gene allele variant, experienced depressive disorders to the same extent and did not differ in the frequency of antidepressant, antiepileptic, anti-anxiety or antipsychotic medication.

Although the present analyses did not confirm the relationship between decreases in alcohol craving during hospitalisation and depressive symptoms in study participants, studies by other authors, confirm the associations between depressive symptoms and increased alcohol craving. Research by other authors suggests that alcohol-abusing young adults who are experiencing stress or depression may have problems with alcohol, in part due to increased alcohol cravings and demands and less sensitivity to the effects of drinking in the future.[30]

Kavanagh et al conclude that depression and negative mood were associated with a more frequent, stronger desire to drink alcohol.[31]

From the regression model of the present study, it is known that predictors of improvement in PACS scale scores were the 5-HTTLPR polymorphism and severity of alcohol dependence (according to the SADD scale), which explained up to 12% of the variance.

Constant et al, suggested that severity of addiction, as measured by the Alcohol Urge Questionnaire scale, showed a strong correlation with severity of alcohol craving. However, the same scale moderately measured severity of addiction itself, drinking behaviour and mental health.[32]

Other studies have found that greater severity of alcohol dependence is associated with greater severity of alcohol craving.[4,33]

Of great interest that begs further research into its usefulness in addiction treatment, is our study finding that improvement (reduction in alcohol craving severity, ie, PACS scale scores) occurred to a greater extent among subjects with lower SADD scores (ie, lower severity of alcohol dependence). Our results suggest that training in coping with alcohol craving should be targeted first at those with deeper dependence as they reveal slower severity of alcohol craving reduction over time.

Limitations of the Study

Limitations of the study include the low size of the study subjects and the small number of women in the study population. The authors also encountered a limitation in assessing the type of medication taken by all study subjects - The study only focused on a small group of patients.

Nevertheless, our research sheds important light on the neurobiological basis of alcohol craving and indicates the need to look for new therapeutic avenues, including pharmacotherapy for individuals, especially those whose perception of the severity of alcohol craving is determined by biological factors such as the 5-HTTLPR polymorphism.

Conclusions

1. During one month of follow-up (between weeks 2 and 6 of inpatient treatment for):
   (a) in subjects with the 5-HTTLPR short/short or short allele, there was a decrease in the severity of alcohol craving in half of them,
   (b) in those with the 5-HTTLPR long/short allele, one-fifth of them experienced a decrease in the severity of alcohol craving,
   (c) in subjects with the 5-HTTLPR long/long allele, there was virtually no decrease in the severity of alcohol craving.
2. A decrease in the severity of alcohol craving occurred to a greater extent in subjects with lower SADD scores (ie, less severity of alcohol dependence) than in those with stronger alcohol dependence.
3. Changes in craving intensity during follow-up were not found to be influenced by the subjects' gender or age.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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