Original article / Artykuł oryginalny

Association of oxytocin gene polymorphism with psychological distress, suicidal ideation and relapse in alcohol-dependent patients

Związek polimorfizmu genu oksytocyny z dyskomfortem psychicznym, myślami samobójczymi oraz nawrotem picia u pacjentów uzależnionych od alkoholu

Anna Mach[1], Anna Klimkiewicz[1], Sylwia Fudalej[1], Andrzej Jakubczyk[1], Maciej Kopera[1], Margit Burmeister[2,3], Kirk J. Brower[2], Marcin Wojnar[1,2]

[1]Department of Psychiatry, Medical University of Warsaw, Warsaw, Poland
[2]Department of Psychiatry, University of Michigan, Ann Arbor, MI, USA
[3]Molecular & Behavioral Neuroscience Institute, Departments of Computational Medicine & Bioinformatics and of Human Genetics, University of Michigan, Ann Arbor, MI, USA

Alcohol Drug Addict 2021; 34 (2): 91-104
DOI: https://doi.org/10.5114/ain.2021.109540

Abstract

Introduction: Oxytocin (OXT) is an important neurohormone involved in regulating the stress response with both behavioural and physiological aspects. It has anxiolytic and antidepressant properties. Studies on the mechanisms of dependence suggest that stress and psychological symptoms like anxiety and depression may increase susceptibility to alcohol use disorders (AUD) and the risk of relapse. The aim of this

Streszczenie

Wprowadzenie: Oksytocyna (OXT) jest ważnym neurohormonem biorącym udział w regulacji reakcji na stres, zarówno w aspekcie behawioralnym, jak i fizjologicznym. Ma właściwości przeciwlękowe oraz przeciwdepresyjne. Badania dotyczące mechanizmów uzależnienia sugerują, że stres i takie objawy psychiczne, jak lęk i depresja, mogą zwiększać podatność na zaburzenia związane z używaniem alkoholu (AUD) oraz ryzyko nawro-

Correspondence to/Adres do korespondencji: Anna Klimkiewicz, Department of Psychiatry, Medical University of Warsaw, 27 Nowowiejska St., 00-665 Warszawa, Poland, phone: +48 22 825 1236, fax: + 48 22 825 1315, e-mail: anna.klimkiewicz@wum.edu.pl

Authors' contribution/Wkład pracy autorów: **Study design/Koncepcja badania:** A. Klimkiewicz, A. Mach, S. Fudalej, A. Jakubczyk, M. Wojnar; **Data collection/Zebranie danych:** A. Klimkiewicz, A. Mach, S. Fudalej, A. Jakubczyk, M. Kopera, M. Wojnar; **Statistical analysis/Analiza statystyczna:** A. Klimkiewicz, A. Mach, A. Jakubczyk, M. Wojnar; **Data interpretation/Interpretacja danych:** A. Klimkiewicz, A. Mach, S. Fudalej, A. Jakubczyk, M. Kopera, M. Burmeister, K.J. Brower, M. Wojnar; **Acceptance of final manuscript version/Akceptacja ostatecznej wersji pracy:** A. Klimkiewicz, A. Mach, S. Fudalej, A. Jakubczyk, M. Kopera, M. Burmeister, K.J. Brower, M. Wojnar; **Literature search/Przygotowanie literatury:** A. Mach; **Funds collection/Pozyskanie środków (finansowania):** M. Wojnar

No ghostwriting and guest authorship declared./Nie występują zjawiska *ghostwriting* i *guest authorship*.

Submitted/Otrzymano: **15.05.2020** • **Accepted/Przyjęto do druku:** **12.01.2021**

study was to analyse the relationships between oxytocin gene (OXT) polymorphisms, psychological distress and the severity of suicidal ideation in alcohol-dependent patients.

Material and methods: The study included 288 adult patients who fulfilled a DSM-IV diagnostic criteria of alcohol dependence and participated in alcohol treatment programmes in Warsaw, Poland. The Brief Symptom Inventory (BSI) was used to assess the level of psychological distress and the Beck Scale for Suicidal Ideation (BSS) measured the severity of suicidal ideation. Four single nucleotide polymorphisms (SNPs) in the region of OXT gene were analysed (rs4813625, rs877172, rs3761248 and rs2740210).

Results: Statistical analysis of the obtained data showed a nominally significant association between the GT genotype in the rs2740210 polymorphism of the OXT gene region and symptoms of psychological distress in alcohol-dependent patients ($p = 0.0262$). After the Bonferroni correction ($p_{Bonferroni} < 0.0125$), this relationship was statistically significant in the group of patients with a family history of alcohol problems and patients who had returned to drinking after treatment. Moreover, the GT genotype was statistically significantly associated with the severity of suicidal ideation in a the post-treatment relapsing patient group.

Discussion: Our results suggest that the oxytocin system may play an important role in patients with alcohol dependence through its association with psychological distress.

Conclusions: The rs2740210 polymorphism in the region of the OXT gene should be studied further as a prognostic factor in the course and treatment of alcohol use disorders.

Keywords: Alcohol dependence, Alcohol use disorder, Genetic polymorphism, Oxytocin, Psychological distress, Suicidal ideation.

tu picia. Celem badania była ocena związku polimorfizmów w regionie genu OXT z dyskomfortem psychicznym oraz nasileniem myśli samobójczych u pacjentów uzależnionych od alkoholu.

Materiał i metody: Badaniem objęto 288 dorosłych ochotników spełniających kryteria uzależnienia od alkoholu według klasyfikacji DSM-IV, którzy uczestniczyli w programach leczenia uzależnienia od alkoholu w Warszawie. Krótki inwentarz objawów (BSI) wykorzystano do oceny cierpienia psychicznego. Nasilenie myśli samobójczych oceniono przy użyciu Skali Samobójczych Ideacji Becka (BSS). Zbadano cztery polimorfizmy pojedynczych nukleotydów (SNP) w regionie genu OXT: rs4813625, rs877172, rs3761248 i rs2740210.

Wyniki: Analiza statystyczna uzyskanych danych wykazała istotny związek między genotypem GT polimorfizmu rs2740210 genu OXT a objawami dyskomfortu psychicznego u pacjentów uzależnionych od alkoholu ($p = 0{,}0262$). Po zastosowaniu korekty Bonferroniego ($p_{Bonferroni} < 0{,}0125$) zależność była istotna statystycznie w grupie pacjentów pochodzących z rodzin z problemem alkoholowym, a także chorych, u których wystąpił nawrót picia po leczeniu. Ponadto genotyp GT był istotnie statystycznie związany z nasileniem myśli samobójczych w grupie pacjentów z nawrotem picia po leczeniu.

Omówienie: Wyniki sugerują, że OXT może odgrywać ważną rolę u pacjentów uzależnionych od alkoholu poprzez jej związek z dyskomfortem psychicznym.

Wnioski: Polimorfizm rs2740210 w regionie genu OXT wymaga dalszych badań jako czynnik prognostyczny w leczeniu zaburzeń związanych z używaniem alkoholu.

Słowa kluczowe: uzależnienie od alkoholu, zaburzenia związane z używaniem alkoholu, polimorfizm genetyczny, oksytocyna, dyskomfort psychiczny, myśli samobójcze.

■ Introduction

Alcohol dependence is a chronic, relapsing disorder that significantly contributes to health, social and economic problems in the world. The majority of treated patients achieve only short-term remissions and frequently return to drinking. Extensive research has focused on understanding the neurobiological factors and mechanisms underlying the development and course of dependence. Among the numerous mechanisms that may be involved in the neurobiology of alcohol dependence, evidence suggests a role for oxytocin (OXT) [1].

OXT receptors are found in the nucleus accumbens, bed nucleus of the stria terminalis, ventral tegmental area, hippocampus, amygdala and

ventral pallidum. All of these regions are relevant to drug-seeking behaviour [2]. Other research has found that OXT modulates the response to alcohol through interaction with neural sites implicated in the development of substance-use disorders and craving [2-4].

OXT administered, especially centrally but also peripherally, during chronic ethanol administration in mice attenuates the development of tolerance to ethanol [5], decreases the severity of withdrawal symptoms and reduces mortality [6]. In rats, peripheral OXT prevented the development of tolerance to alcohol-induced narcosis [7]. In addition, short-term administration of OXT during early adolescence among rats reduced both generalised and social anxiety-related behaviours in late adolescence and inhibited the development of excessive alcohol consumption in adult rats [8].

The OXT system has bidirectional interactions with the stress axis, autonomic nervous system, neurotransmitter systems (e.g., dopamine, serotonin and GABA/glutamate) and the immune system. These systems play important roles in different phases of addiction [1]. Differences in OXT system functioning may be related to individual factors like age, gender and genetic variation especially in the OXT genes and receptor. They can also be influenced by environmental influences like stress or trauma [9] and social experiences [10].

Epidemiological studies have shown that alcohol use disorders (AUD) are often associated with other mental disorders like anxiety, mood and personality disorders [11]. Additionally, withdrawal from ethanol is associated with an increased release of corticotropin releasing factor (CRF) in the amygdala. Accordingly, OXT as an antagonist of CRF can block withdrawal-induced anxiety and alcohol-seeking behaviour [12, 13], and restore the rewarding value of social interaction [2].

Based on previous research focused on the neurobiology of dependence, genetic variation in OXT signalling may play an important role in modulating psychological distress [14, 15]. The motivation to consume alcohol is inextricably linked to psychological distress and social context. Individual differences in the endogenous OXT system could explain some of the individual differences in susceptibility to psychological symptoms and relapse in alcohol-dependent patients. Therefore the aim of this study was to investigate the association of four selected single nucleotide polymorphisms (SNPs) in the region of the OXT gene (rs4813625, rs877172, rs3761248, rs2740210) with psychological distress, severity of suicidal ideation (SI) and relapse in alcohol-dependent patients.

Material and methods

Participants

The participant group consisted of 288 adult patients (214 men, 74 women) with a current diagnosis of alcohol dependence, which was assessed clinically by an addiction therapist and a psychiatrist. Diagnoses were made according to DSM-IV criteria based on a clinical interview and confirmation with the Mini-International Neuropsychiatric Interview (M.I.N.I.) [16]. Patients with active alcohol withdrawal or psychotic disorder, current pharmacotherapy for comorbid mental disorders, cognitive impairment and presence of aggressive or self-harm behaviour were excluded. A more detailed description of the study sample and the treatment programmes has previously been published [17].

All subjects voluntarily entered abstinence-based alcohol treatment programmes in Warsaw. In addition, patients took part in meetings of Alcoholics Anonymous. Individuals requiring medication for mental disorders were excluded, therefore all study participants were free from any psychotropic medication.

There are no common functional polymorphisms in the region of the OXT system. We selected four SNPs located in regions just upstream or downstream to the OXT gene (rs4813625, rs877172, rs3761248, rs2740210) based on evidence of associations with psychiatric, especially mood, disorders [18-21] and their influence on human dopaminergic function [15]. The four SNPs were investigated in all study patients. The study was approved by the Medical School Institutional Review Board at the University of Michigan and the Bioethics Committee at the Medical University of Warsaw. Participation was confidential and voluntary. All subjects were informed about the overall objectives and course of the study and they signed an informed consent document prior to participation.

Measures

Participants were asked to complete a composite questionnaire that gathered data on demo-

graphic and psychosocial variables, alcohol use and severity, smoking, family history of alcohol problems and psychological symptom severity. All diagnostic instruments were used in the Polish language version. The methodology of simultaneous forward/single backward translation was used when Polish versions were unavailable [22-24]. More details of the procedure and the correctness of the translation has previously been published [17].

The level of general psychological distress was assessed with the *Brief Symptom Inventory* (BSI) [25], a self-report symptom scale consisting of 53 items covering nine primary symptom dimensions: somatisation (SOM), obsession-compulsion (OC), interpersonal sensitivity (IS), depression (DEP), anxiety (ANX), hostility (HOS), phobic anxiety (PHOB), paranoid ideation (PAR) and psychoticism (PSY). Each item measures distress on a 5-point Likert scale from 0 (not at all/no distress) to 4 (extremely/very strong distress) over a time period of the past 7 days. Our primary variable was the *Global Severity Index* (GSI) computed as a raw score (sum of all item scores divided by 53) and later transformed into age and gender-specific normative T-scores by use of a standardised reference table.

Drinking prior to admission for treatment was measured using the *Substance Abuse Outcomes Module* (SAOM) [26]. Intensive alcohol use was defined as drinking alcohol in amounts exceeding WHO levels for risky alcohol consumption (over 4 standard drinks daily for men and over 2 standard drinks daily for women).

The severity of alcohol dependence and related problems were assessed applying the Polish version of the *Michigan Alcoholism Screening Test* (MAST) [27, 28] and a modified version of the SAOM [26].

The *Alcohol Time-Line Follow-Back Interview* (TLFB) [29, 30] was used to evaluate post-treatment *relapse* defined as any drinking in the follow-up period (no/yes). Patients who were lost to follow-up 6 to 12 months after discharge from treatment were considered to have relapsed ($n = 74$).

Questions about demographics and family history of alcohol problems were obtained using the *University of Arkansas Substance Abuse Outcomes Module* (SAOM), which is a self-administered questionnaire [26].

The severity of suicidal ideation (SI) was assessed applying the *Beck Scale for Suicidal Ideation* (BSS) [31]. The BSS is a 24-item self-report scale that evaluates a patient's thoughts, intent and plans to commit suicide. All items are rated on a three-point scale (0 to 2). Total scores range from 0 to 38 with higher scores reflecting a greater severity of suicidal thoughts. The BSS was completed by all patients at the time of study entry. If current suicidal tendencies were revealed during the examination of the patient, a management plan was developed consisting of immediate referral to a psychiatrist for examination, direct patient support and referral or transport to a psychiatric hospital. There was no need to follow these procedures throughout the study.

Genotyping

DNA was extracted from participants' white blood cells using the Gentra Puregene Blood Kit (Qiagen). The SNPs (rs4813625, rs877172, rs3761248, rs2740210) have been identified according to the previously described design method [15, 32]. We examined four SNPs (rs4813625, rs877172, rs3761248, rs2740210) located in genomic region containing sequence 5 kb upstream or 1 kb downstream of OXT. All polymorphisms were analysed using the Illumina GoldenGate Platform at the University of Michigan.

Statistical analysis

The statistical analysis was performed using Statistica Software, version 12.0. Data are presented as arithmetic means and standard deviations (± SD) for parametric variables. For non-parametric variables, data are presented as median and quartiles.

The primary analysis focused on examining associations between the genetic polymorphisms in the region of the OXT gene and global psychological distress as measured by the GSI. Taking into account the four SNPs, a Bonferroni-corrected value of $p < 0.0125$ was considered statistically significant. All SNPs were chosen not to be in high linkage disequilibrium (LD) ($r^2 < 0.7$) although there is some moderate LD ($r^2 \sim 0.4$) between rs3761248 and rs877172.

We analysed the associations first in all subjects and then in selected subgroups categorised by family history of alcohol problems, post-treatment drinking course and nicotine use/dependence. We also examined associations between the genetic polymorphisms in the region of the OXT gene and severity of SI (suicidal ideation) in all patients.

Table I. Sociodemographic and clinical characteristics of study participants

Characteristics	*N* = 288
Age (years), mean (SD)	44 (10)
Gender (males), n (%)	214 (74)
Married, n (%)	130 (45)
Age of onset of drinking problem (years), mean (SD)	24 (9)
Duration of intensive alcohol use (year), median (IQR)	18 (12; 28)
Family history of alcohol dependence, n (%)	179 (62)
Severity of alcohol dependence (MAST; T-score), mean (SD)	34.4 (9.5)
Severity of suicidal ideation (BSS; T-score), median (IQR)	3 (0; 1)
No history of SA and baseline SI, n (%)	186 (65)
Severity of psychopathology (GSI; T-score), median (IQR)	46 (39; 56)
Relapsed (confirm and suspected), n (%)	191 (66)

MAST – Michigan Alcoholism Screening Test, SD – standard deviation, IQR – interquartile range, GSI – General Severity Index derived from Brief Symptom Inventory, BSS – Beck Scale for Suicidal Ideation, SI – suicidal ideation, SA – suicide attempt.

When comparing the means (normally distributed variables) or medians (non-normal distributions) of two groups, differences were tested with either Student's *t*-test or Mann-Whitney *U* test respectively. When the means or medians of more than two groups were compared, analyses of variance or Kruskal-Wallis tests were used. When comparing categorical variables, the χ^2 test was used.

The Epsilon-squared ($\mathcal{E}^2$) for the Kruskal-Wallis H-test was used to estimate of effect size [33].

Results

Demographic and clinical characteristics

The sample consisted of 288 patients (74 females, 214 males) with a mean of 44 ± 10 years of age (43 ± 9 in females; 44 ± 10 in males). Sociodemographic and clinical description of the sample is provided in Table I. The scores from the BSI are presented in Table II. The median score on the GSI was 46 and 35 respondents (12%) had a GSI T-score > 63 indicating clinically relevant psychological distress.

Genetic analysis

The distribution of analysed genotypes and allele frequencies are presented in Table III. The genotype frequencies for all 4 SNPs were in Hardy-Weinberg equilibrium.

After applying the Bonferroni correction, there were no significant associations between any of the four SNPs (rs2740210, rs4813625, rs877172, rs3761248) and psychological distress as measured by the GSI in the full sample of patients. However, there was a nominally significant association between the rs2740210 polymorphism and psychological distress (p = 0.0262; Table IV). Visual inspection revealed a difference between the heterozygous (GT) and homozygous patients (GG/TT). A comparison of those two groups revealed a statistically significant relationship with GSI (p = 0.0071). Thus we conducted analyses between this SNP and our four *a priori* defined subgroups of study patients using a Bonferroni correction factor of 0.0125.

The association between psychological distress and the rs2740210 polymorphism was significant for patients who reported alcohol problems

Table II. BSI T-scores in alcohol-dependent patients (*N* = 288)

GSI and BSI subscales	T-score	
	Median (quartiles)	Range
Global Severity Index (GSI)	46 (39; 57)	12-78
Somatization	49 (44; 58)	33-78
Obsessive-compulsiveness	47 (42; 57)	26-80
Interpersonal sensitivity	47 (42; 55)	4-73
Depression	45 (39; 53)	27-73
Anxiety	44 (36; 53)	26-78
Hostility	48 (41; 53)	30-78
Phobic anxiety	46 (37; 55)	36-80
Paranoid ideation	51 (45; 58)	32-80
Psychoticism	49 (43; 56)	41-75

BSI – Brief Symptom Inventory.

Table III. Distribution of OXT genotypes and allele frequencies in male and female alcohol-dependent patients (N = 288)

OXT SNPs	Genotypes *n* (%)			Allele frequency		Hardy-Weinberg test
rs4813625	**G/G**	**G/C**	**C/C**	**G**	**C**	
Men	67 (31.3)	107 (50.0)	40 (18.7)	0.56	0.44	
Women	20 (27.0)	41 (55.4)	13 (17.6)	0.55	0.45	
Total	87 (30.2)	148 (51.4)	53 (18.4)	0.56	0.44	χ^2 = 0.52 p = 0.56
rs877172	**A/A**	**A/C**	**CC**	**A**	**C**	
Men	94 (43.9)	105 (49.1)	15 (7.0)	0.69	0.31	
Women	39 (52.7)	28 (37.8)	7 (9.5)	0.72	0.28	
Total	133 (46.2)	133 (46.2)	22 (7.6)	0.69	0.31	χ^2 = 2.07 p = 0.69
rs3761248	**T/T**	**C/T**	**CC**	**T**	**C**	
Men	148 (69.1)	62 (29.0)	4 (1.9)	0.84	0.16	
Women	53 (71.6)	18 (24.3)	3 (4.1)	0.84	0.16	
Total	201 (69.8)	80 (27.8)	7 (2.4)	0.84	0.16	χ^2 = 0.08 p = 0.84
rs2740210	**G/G**	**G/T**	**T/T**	**G**	**T**	
Men	85 (39.7)	111 (51.9)	18 (8.4)	0.66	0.34	
Women	33 (44.6)	33 (44.6)	8 (10.8)	0.67	0.33	
Total	118 (41.0)	144 (50.0)	26 (9.0)	0.66	0.34	χ^2 = 3.72 p = 0.66

SNP – single nucleotide polymorphism.

in the family (p = 0.0029) and for those who experienced a relapse during a 1-year follow-up (p = 0.0098; Table IV).

There were no significant associations between the other studied SNPs (rs4813625, rs877172, rs3761248) and psychological symptoms in the subgroups with a family history or relapse (data not shown).

The second objective of the study was to determine the association between polymorphisms in the region of the OXT gene and suicidal ideation. The statistical analysis did not show a significant association between the rs2740210 and the severity of SI (p = 0.0415). However, those patients who relapsed after treatment and were carriers of the heterozygous genotype GT of rs2740210 had significantly higher levels of SI as compared to either type of homozygote (p = 0.0048). In the group of patients without relapse, the association between rs2740210 and SI was not significant (p = 0.3991).

There were no significant associations between SI and the other studied SNPs in the total sample or the study subgroups.

Discussion

The study's major finding was the significant association between the rs2740210 polymorphism in the region of the OXT gene and symptoms of psychological distress in subgroups of alcohol-dependent patients. The GT genotype of the rs2740210 OXT gene region was significantly associated with the severity of psychological symptoms. The results showed that in rs2740210 heterozygotes, factors like alcohol problems in the family might predispose to psychological distress and intensity of psychological symptoms. Moreover, this association was significant in patients who experienced a relapse during a 1-year follow-up. These findings suggest that the rs2740210 heterozygote polymorphism in the OXT gene region can be involved in mechanisms responsible for relapse to drinking potentially acting through the severity of psychological symptoms.

Patients with family history of alcohol problem have a potentially increased genetic risk of alcohol dependence. We hypothesised that in these patients, the influence of the OXT gene region

Table IV. Associations between rs2740210 genotypes in the OXT gene region and psychological distress (GSI scores) in alcohol-dependent patients with selected characteristics

	GSI scores						GSI scores			
	N	**TT median (quartiles)**	**GT median (quartiles)**	**GG median (quartiles)**	***p***	$\mathcal{E}^2$	**GT median (quartiles)**	**GG/TT median (quartiles)**	***p***	$\mathcal{E}^2$
All patients	288	44 (36; 54)	47 (39; 59)	44 (38; 51)	0.0262	0.025	47 (39; 59)	44 (38; 51)	**0.0071**	0.026
Men	214	41 (36; 53)	48 (39; 60)	44 (39; 51)	0.0572	0.026	48 (39; 60)	43 (38; 51)	0.0212	0.025
Women	74	50 (38; 58)	46 (42; 57)	45 (38; 53)	0.2924	0.034	46 (42; 57)	45 (38; 56)	0.2065	0.022
Positive family history	179	45 (39; 57)	53 (43; 61)	43 (38; 52)	**0.0029**	0.065	53 (43; 61)	43 (38; 53)	**0.0008**	0.063
Relapsed at follow-up	191	42 (36; 54)	49 (42; 60)	43 (35; 50)	**0.0098**	0.049	49 (42; 60)	43 (36; 51)	**0.0024**	0.049

GSI – Global Severity Index of the Brief Symptom Inventory; $\mathcal{E}^2$-Epsilon square.

Variables are presented as median and quartiles of T-scores and analysed by Kruskal-Wallis test. Bonferroni-corrected value of $p < 0.0125$ are bolded.

Table V. Associations between rs2740210 genotypes in the OXT gene region and suicide ideation (BSS *T*-scores) in alcohol-dependent patients with selected characteristics

BSS (T-scores)				
	All patients ***N* = 286**	**Men** ***n* = 212**	**Women** ***n* = 74**	**Relapsed at follow-up** ***n* = 190**
TT v GT v GG	$p = 0.0375$ $\varepsilon^2 = 0.023$	$p = 0.0129$ $\varepsilon^2 = 0.041$	$p = 0.4158$ $\varepsilon^2 = 0.024$	$p =$ **0.0048** $\varepsilon^2 = 0.056$
GT v TT/GG	$p =$ **0.0123** $\varepsilon^2 = 0.022$	$p =$ **0.0103** $\varepsilon^2 = 0.031$	$p = 0.5361$ $\varepsilon^2 = 0.005$	$p =$ **0.0011** $\varepsilon^2 = 0.056$

BSS – Beck Scale for Suicidal Ideation.

Variables are presented as median and quartiles of T-scores and analysed by Kruskal-Wallis test. Bonferroni-corrected value of $p < 0.0125$ are bolded.

rs2740210 polymorphism could be more apparent on the severity of psychological distress as a genetic factor. Although studies of genetic vulnerability in predicting relapse in alcohol-dependent patients are inconclusive, some researchers suggest that genetic factors may be associated with relapse [34-36]. Based on these reports, we extend this study to explore whether associations between the rs2740210 polymorphism of the OXT gene region and GSI would be more significant in the patients who had relapsed. In both analysed groups, the associations were statistically significant and more pronounced.

It is known that alcohol dependence is a complex mental disorder, which in addition to alcohol seeking and loss of control over intake, is characterised by the emergence of negative emotions during withdrawal [37]. Based on previous studies, there is general agreement that stress and psychiatric symptoms like anxiety and depression may lead to relapse and require attention when treating alcohol dependence [38-40]. On one hand, stress is among the critical risk factors of relapse [41] while on the other, early abstinence from alcohol activates stress mechanisms [42].

Brain OXT has generally been described as an important regulator of the stress response with both behavioural and physiological aspects [43]. Research has suggested that OXT can reduce reactivity of the hypothalamic-pituitary-adrenal (HPA) axis after stress [44] and inhibit activation of the amygdala, which has a key function in the acute potentiation of drugs of abuse [45]. It also may decrease levels of adrenocorticotropic hormone [46] and cortisol [47]. Previous studies have shown that OXT may have both anxiolytic [48] and antidepressant [49] properties. In our study, we used the BSI self-report inventory as

a measure of psychological distress and individual coping with stress. OXT has been shown to be a hormone whose release increases during times of stress, thereby reducing the stress response in human [50]. According to McQuaid *et al.* oxytocin receptor (OXTR) SNPs are associated with increased stress responses [51].Therefore we aimed to test whether the individual psychological distress in alcohol-dependent patients is moderated by OXT region SNPs. The significant effects of the genetic variant in the region of OXT gene as found in our study might affect psychological symptoms and relapse through such mechanisms.

The severity of psychiatric symptoms in patients dependent on alcohol is associated with a more difficult course and longer treatment [52, 53]. Our study results showed that a higher level of psychiatric symptoms at the time of treatment entry was associated with relapse during a 6-12 month follow-up period in patients with a particular OXT region polymorphism. The potential ability of endogenous OXT to regulate anxiety or depression-related behaviour by reducing stress, decreasing autonomic arousal, and dampening HPA axis reactivity may improve the course of treatment [54, 55]. This suggests a potential role for OXT administration during treatment for psychologically distressed patients with this polymorphism. Although speculative, other studies have demonstrated the potential therapeutic benefits of OXT administration without regard to genotype [40, 56-58].

The correlation between the rs2740210 polymorphism and psychological symptoms in alcohol-dependent patients with family alcohol problems might be expected given the polygenic inheritance of AUD. Besides other genes implicated in alcohol dependence, such as BDNF [34], HTR2A [59], GABRA2 [60] and SCL6A4 [36], genetic variation in the OXT system may also play an important role. Individual differences in the endogenous OXT system might modulate psychiatric symptoms and influence the course of alcohol dependence including post-treatment relapse. Previous studies have reported that alcohol increase HPA axis activity in humans [41, 61, 62]. This modification may lead to increased negative emotions and by modifying the stress hormone systems may influence alcohol use [63].

Love *et al.* first showed a moderating effect of the OXT genotype (rs2740210) on the relationship between social support and psychological distress in alcohol-dependent patients [14]. They noted that heterozygotes GT exhibited significantly higher overall GSI scores compared to homozygotes (GG). Our study is in line with this result in relation to psychological distress. We additionally extend this report by showing associations between the rs2740210 polymorphism and psychological distress among different subgroups of patients with alcohol dependence including those with a family history of alcoholism and post-treatment relapse.

The association is unusual in that the relationship between genotype and these traits is not linear but rather U-shaped; the heterozygous individuals are under more stress and have higher levels of SI than those with either type of homozygote. While U-shaped relationships have been occasionally observed (e.g. COMT catechol-o-methyltransferase), usually heterozygotes are the most well adapted in cases of this kind with either extreme being inferior [64-66]. Moreover, Love *et al.* and our research are the first studies showing the effect of rs2740210 in group of alcohol-dependent patients. Despite the small groups in both studies, the results point to a similar direction indicate on heterozygotes (GT) more stressed. It is unclear why heterozygotes relative to homozygotes seems to be more exposed to psychological distress. Previous studies on the significance of this polymorphism in other aspects (postpartum depression, breastfeeding duration [18], childhood-onset mood disorders [67], schizophrenia [68]) were inconsistent but in terms of direction were not in line with ours.

The significant association between the rs2740210 variant and the severity of suicidal ideation in this study was found only in post-treatment relapse patients. Some studies have reported significantly lower plasma [69] and CSF [70] OXT levels in those patients who had attempted suicide. Additionally, most researchers have documented that genes modulating brain functions may be important in the aetiology of suicidal behaviour [71]. Our results suggest that polymorphic variants in the region of OXT gene may be associated with neuromodulation of emotional processes associated with suicidal thoughts.

We examined four SNPs (rs4813625, rs877172, rs3761248, rs2740210) located in noncoding regions just upstream or downstream to the OXT

gene in chromosome 20. Evolutionary analyses show that many non-coding sequences are conserved to the same extent as protein-coding sequences, and thus are also likely to be functionally important for gene expression [72]. Despite the lack of conclusive evidence of the functional significance of these SNPs, they have been previously associated with substance use disorders, psychiatric symptomatology and stress responsivity in alcohol-dependent patients [14, 15, 18, 32, 73]. Additionally, SNP rs2740210 which was significant in our study, is located on the 3'flanking region of the OXT gene. Some researchers speculate that this SNP could be in linkage disequilibrium with a variant that has a functional impact on the expression or regulation of OXT gene [14].

The main limitation of this study is its small sample size, which is not fully representative of all individuals with alcohol dependence. Moreover, the data were obtained by self-administered questionnaires and not confirmed by an objective assessment. Another limitation is that we focused only on polymorphisms in the region of OXT gene, the functional significance of which is not fully known. Together with other stress-related genes that we did not study, additional factors, like legal or financial problems, that may influence psychological distress in alcohol-dependent patients were not analysed. The statistically significant relationships revealed by the Kruskal-Wallis test had a relatively weak-to-moderate effect [74].This confirms that the OXT system is one of the many factors influencing on psychological distress and severity of SI. Therefore the obtained results should be treated with due deliberation and caution.

Conclusions

Our results show that variant rs2740210 in the region of the OXT gene is one of the factors that may influence on psychological distress and the severity of SI in alcohol-dependent patients. In this context, future studies are needed to assess the potential clinical impact of OXT region genetic variants.

Acknowledgements/Podziękowania

We thank all members of the research team in Poland (especially Katarzyna Kopera, MS; Julia Pupek, MD; Piotr Serafin, MD; Izabela Nowosad, MD) as well as the medical staff and patients at Kolska Alcohol Addiction Treatment Centre in Warsaw for their support of this research.

Dziękujemy wszystkim członkom polskiej grupy badawczej (szczególnie mgr Katarzynie Koperze, dr Julii Pupek, dr. Piotrowi Serafinowi i dr Izabeli Nowosad), jak również pracownikom i pacjentom Ośrodka Leczenia Osób Uzależnionych od Alkoholu przy ulicy Kolskiej w Warszawie za ich pomoc w przeprowadzeniu badania.

Conflict of interest/Konflikt interesów

None declared./Nie występuje.

Financial support/Finansowanie

This study was supported by/Badanie zostało sfinansowane przez the Polish Ministry of Science and Higher Education, grant NN405357239/, and the Fogarty International Center/National Institute on Drug Abuse International Substance Abuse Research Program grant D43-TW05818, the Fogarty International Center/National Institute on Alcohol Abuse and Alcoholism International Collaborative Alcohol & Injury Research Training Program grant D43-TW007569, NIAAA grant R21 AA016104 and the National Science Center grant 2012/07/B/HS6/02370.

Ethics/Etyka

The work described in this article has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) on medical research

involving human subjects, Uniform Requirements for manuscripts submitted to biomedical journals and the ethical principles defined in the Farmington Consensus of 1997.

Treści przedstawione w pracy są zgodne z zasadami Deklaracji Helsińskiej odnoszącymi się do badań z udziałem ludzi, ujednoliconymi wymaganiami dla czasopism biomedycznych oraz z zasadami etycznymi określonymi w Porozumieniu z Farmington w 1997 roku.

References/Piśmiennictwo

1. Buisman-Pijlman FT, Sumracki NM, Gordon JJ, Hull PR, Carter CS, Tops M. Individual differences underlying susceptibility to addiction: Role for the endogenous oxytocin system. *Pharmacol Biochem Behav* 2014; 119: 22-38.
2. McGregor IS, Callaghan PD, Hunt GE. From ultrasocial to antisocial: a role for oxytocin in the acute reinforcing effects and long-term adverse consequences of drug use? *Br J Pharmacol* 2008; 154(2): 358-68.
3. Carson DS, Hunt GE, Guastella AJ, Barber L, Cornish JL, Arnold JC, et al. Systemically administered oxytocin decreases methamphetamine activation of the subthalamic nucleus and accumbens core and stimulates oxytocinergic neurons in the hypothalamus. *Addict Biol* 2010; 15(4): 448-63.
4. Kovacs GL, Sarnyai Z, Szabo G. Oxytocin and addiction: a review. *Psychoneuroendocrinology* 1998; 23(8): 945-62.
5. Jodogne C, Tirelli E, Klingbiel P, Legros JJ. Oxytocin attenuates tolerance not only to the hypothermic but also to the myorelaxant and akinesic effects of ethanol in mice. *Pharmacol Biochem Behav* 1991; 40(2): 261-5.
6. Szabo G, Kovacs GL, Telegdy G. Effects of neurohypophyseal peptide hormones on alcohol dependence and withdrawal. *Alcohol Alcohol* 1987; 22(1): 71-4.
7. Pucilowski O, Kostowski W, Trzaskowska E. The effect of oxytocin and fragment (MIF-I) on the development of tolerance to hypothermic and hypnotic action of ethanol in the rat. *Peptides* 1985; 6(1): 7-10.
8. Bowen MT, Carson DS, Spiro A, Arnold JC, McGregor IS. Adolescent Oxytocin Exposure Causes Persistent Reductions in Anxiety and Alcohol Consumption and Enhances Sociability in Rats. *PLoS ONE* 2011; 6(11): 1-11.
9. Opacka-Juffry J, Mohiyeddini C. Experience of stress in childhood negatively correlates with plasma oxytocin concentration in adult men. *Stress* 2012; 15(1): 1-10.
10. Feldman R, Zagoory-Sharon O, Weisman O, Schneiderman I, Gordon I, Maoz R, et al. Sensitive parenting is associated with plasma oxytocin and polymorphisms in the OXTR and CD38 genes. *Biol Psychiatry* 2012; 72(3): 175-81.
11. Harford TC, Yi HY, Chen CM, Grant BF. Psychiatric Symptom Clusters as Risk Factors for Alcohol Use Disorders in Adolescence: A National Study. *Alcohol Clin Exp Res* 2015; 39(7): 1174-85.
12. George O, Ghozland S, Azar MR, Cottone P, Zorrilla EP, Parsons LH, et al. CRF-CRF1 system activation mediates withdrawal-induced increases in nicotine self-administration in nicotine-dependent rats. *Proc Natl Acad Sci USA* 2007; 104(43): 17198-203.
13. Heilig M, Koob GF. A key role for corticotropin-releasing factor in alcohol dependence. *Trends Neurosci* 2007; 30(8): 399-406.
14. Love TM, Cranford JA, Burmeister M, Wojnar M, Zucker RA, Brower KJ. Oxytocin Genotype Moderates the Impact of Social Support on Psychiatric Distress in Alcohol-Dependent Patients. *Alcohol Alcohol* 2018; 53(1): 57-63.
15. Love TM, Enoch MA, Hodgkinson CA, Pecina M, Mickey B, Koeppe RA, et al. Oxytocin gene polymorphisms influence human dopaminergic function in a sex-dependent manner. *Biol Psychiatry* 2012; 72(3): 198-206.
16. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 1998; 59(20): 22-33.

17. Wojnar M, Ilgen MA, Jakubczyk A, Wnorowska A, Klimkiewicz A, Brower KJ. Impulsive suicide attempts predict post-treatment relapse in alcohol-dependent patients. *Drug Alcohol Depend* 2008; 97(3): 268-75.
18. Jonas W, Mileva-Seitz V, Girard AW, Bisceglia R, Kennedy JL, Sokolowski M, et al. Genetic variation in oxytocin rs2740210 and early adversity associated with postpartum depression and breastfeeding duration. *Genes Brain Behav* 2013; 12(7): 681-94.
19. Souza RP, Ismail P, Meltzer HY, Kennedy JL. Variants in the oxytocin gene and risk for schizophrenia. *Schizophr Res* 2010; 121(1-3): 279-80.
20. Malik AI, Zai CC, Abu Z, Nowrouzi B, Beitchman JH. The role of oxytocin and oxytocin receptor gene variants in childhood-onset aggression. *Genes Brain Behav* 2012; 11(5): 545-51.
21. Couto TC, Brancaglion MY, Alvim-Soares A, Moreira L, Garcia FD, Nicolato R, et al. Postpartum depression: A systematic review of the genetics involved. *World J Psychiatry* 2015; 5(1): 103-11.
22. Cella DF, Lloyd SR, Wright BD. Cross-cultural instrument equating: current research and future directions. In: Spilker B (ed.). *Quality of life and pharmacoeconomics in clinical trials*. 2nd ed. Philadelphia: Lippincott-Raven Publishers; 1996, p. 707-15.
23. Guillemin F, Bombardier C, Beaton D. Cross-cultural adaptation of health-related quality of life measures: Literature review and proposed guidelines. *J Clin Epidemiol* 1993; 46(12): 1417-32.
24. Hunt SM. *A methodology for the translation of health measures.* Manchester: Galen Research and Consultancy; 1988, p. 1-8.
25. Derogatis LR, Melisaratos N. The Brief Symptom Inventory: an introductory report. *Psychol Med* 1983; 13(3): 595-605.
26. Smith GR, Ross RL, Rost KM. Psychiatric outcomes module: Substance abuse outcomes module (SAOM). In: Sederer LI, Dickey B (eds.). *Outcome Assessment in Clinical Practice*. Baltimore: Wiliams and Wilkins; 1996, p. 85-8.
27. Habrat B (ed.). *Szkody zdrowotne spowodowane alkoholem: przewodnik lekarza praktyka*. Warszawa: Springer PWN; 1996.
28. Selzer ML. The Michigan alcoholism screening test: the quest for a new diagnostic instrument. *Am J Psychiatry* 1971; 127(12): 1653-8.
29. Sobell LC, Maisto SA, Sobell MB, Cooper AM. Reliability of alcohol abusers' self-reports of drinking behavior. *Behav Res Ther* 1979; 17(2): 157-60.
30. Sobell LC, Sobell MB, Leo GI, Cancilla A. Reliability of a Timeline Method: assessing normal drinkers' reports of recent drinking and a comparative evaluation across several populations. *Br J Addict* 1988; 83(4): 393-402.
31. Beck AT, Steer RA (eds.). *Manual for Beck Scale for Suicidal Ideation*. New York: Psychological Corporation; 1991.
32. Hodgkinson CA, Yuan Q, Xu K, Shen PH, Heinz E, Lobos EA, et al. Addictions biology: haplotype-based analysis for 130 candidate genes on a single array. *Alcohol Alcohol* 2008; 43(5): 505-15.
33. Tomczak M, Tomczak E. The need to report effect size estimates revisited. An overview of some recommended measures of effect size. *Trends Sport Sci* 2014; 21: 19-25.
34. Wojnar M, Brower KJ, Strobbe S, Ilgen M, Matsumoto H, Nowosad I, et al. Association between Val66Met brain-derived neurotrophic factor (BDNF) gene polymorphism and post-treatment relapse in alcohol dependence. *Alcohol Clin Exp Res* 2009; 33(4): 693-702.
35. Wiesbeck GA, Weijers HG, Wodarz N, Herrmann MJ, Johann M, Keller HK, et al. Dopamine D2 (DAD2) and dopamine D3 (DAD3) receptor gene polymorphisms and treatment outcome in alcohol dependence. *J Neural Transm (Vienna)* 2003; 110(7): 813-20.
36. Pinto E, Reggers J, Gorwood P, Boni C, Scantamburlo G, Pitchot W, et al. The short allele of the serotonin transporter promoter polymorphism influences relapse in alcohol dependence. *Alcohol Alcohol* 2008; 43(4): 398-400.
37. Koob GF, Volkow ND. Neurocircuitry of addiction. *Neuropsychopharmacol* 2010; 35(1): 217-38.

38. Shalev U, Erb S, Shaham Y. Role of CRF and other neuropeptides in stress-induced reinstatement of drug seeking. *Brain Res* 2010; 1314: 15-28.
39. Sinha R, Shaham Y, Heilig M. Translational and reverse translational research on the role of stress in drug craving and relapse. *Psychopharmacology (Berl)* 2011; 218(1): 69-82.
40. Faehrmann T, Zernig G, Mechtcheriakov S. Oxytocin and the mechanisms of alcohol dependence. *Neuropsychiatr* 2018; 32(1): 1-8.
41. Sinha R. Chronic stress, drug use, and vulnerability to addiction. *Ann NY Acad Sci* 2008; 1141: 105-30.
42. Roberto M, Cruz MT, Gilpin NW, Sabino V, Schweitzer P, Bajo M, et al. Corticotropin releasing factor-induced amygdala gamma-aminobutyric acid release plays a key role in alcohol dependence. *Biol Psychiatry* 2010; 67(9): 831-9.
43. Engelmann M, Landgraf R, Wotjak CT. The hypothalamic-neurohypophysial system regulates the hypothalamic-pituitary-adrenal axis under stress: an old concept revisited. *Front Neuroendocrinol* 2004; 25(3-4): 132-49.
44. Dabrowska J, Hazra R, Ahern TH, Guo JD, McDonald AJ, Mascagni F, et al. Neuroanatomical evidence for reciprocal regulation of the corticotrophin-releasing factor and oxytocin systems in the hypothalamus and the bed nucleus of the stria terminalis of the rat: Implications for balancing stress and affect. *Psychoneuroendocrinology* 2011; 36(9): 1312-26.
45. Riem MM, Bakermans-Kranenburg MJ, Pieper S, Tops M, Boksem MA, Vermeiren RR, et al. Oxytocin modulates amygdala, insula, and inferior frontal gyrus responses to infant crying: a randomized controlled trial. *Biol Psychiatry* 2011; 70(3): 291-7.
46. Parker KJ, Buckmaster CL, Schatzberg AF, Lyons DM. Intranasal oxytocin administration attenuates the ACTH stress response in monkeys. *Psychoneuroendocrinology* 2005; 30(9): 924-9.
47. Ditzen B, Schaer M, Gabriel B, Bodenmann G, Ehlert U, Heinrichs M. Intranasal oxytocin increases positive communication and reduces cortisol levels during couple conflict. *Biol Psychiatry* 2009; 65(9): 728-31.
48. Morales-Rivera A, Hernandez-Burgos MM, Martinez-Rivera A, Perez-Colon J, Rivera R, Montalvo J, et al. Anxiolytic effects of oxytocin in cue-induced cocaine seeking behavior in rats. *Psychopharmacology (Berl)* 2014; 231(21): 4145-55.
49. Slattery DA, Neumann ID. Oxytocin and Major Depressive Disorder: Experimental and Clinical Evidence for Links to Aetiology and Possible Treatment. *Pharmaceuticals* 2010; 3(3): 702-24.
50. Matsushita H, Latt HM, Koga Y, Nishiki T, Matsui H. Oxytocin and Stress: Neural Mechanisms, Stress-Related Disorders, and Therapeutic Approaches. *Neuroscience* 2019; 417: 1-10.
51. McQuaid RJ, McInnis OA, Stead JD, Matheson K, Anisman H. A paradoxical association of an oxytocin receptor gene polymorphism: early-life adversity and vulnerability to depression. *Front Neurosci* 2013; 7: 128.
52. Rounsaville BJ, Dolinsky ZS, Babor TF, Meyer RE. Psychopathology as a predictor of treatment outcome in alcoholics. *Arch Gen Psychiatry* 1987; 44(6): 505-13.
53. Wetterling T, Junghanns K. Psychopathology of alcoholics during withdrawal and early abstinence. *Eur Psychiatry* 2000; 15(8): 483-8.
54. Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biol Psychiatry* 2003; 54(12): 1389-98.
55. Cardoso C, Ellenbogen MA, Orlando MA, Bacon SL, Joober R. Intranasal oxytocin attenuates the cortisol response to physical stress: a dose-response study. *Psychoneuroendocrinology* 2013; 38(3): 399-407.
56. Naja WJ, Aoun MP. Oxytocin and Anxiety Disorders: Translational and Therapeutic Aspects. *Curr Psychiatry Rep* 2017; 19(10): 67.
57. Sippel LM, Allington CE, Pietrzak RH, Harpaz-Rotem I, Mayes LC, Olff M. Oxytocin and Stress-related Disorders: Neurobiological Mechanisms and Treatment Opportunities. *Chronic Stress* 2017; 1: 1-15.

58. Zanos P, Georgiou P, Weber C, Robinson F, Kouimtsidis C, Niforooshan R, et al. Oxytocin and opioid addiction revisited: old drug, new applications. *Br J Pharmacol* 2018; 175(14): 2809-24.
59. Jakubczyk A, Klimkiewicz A, Kopera M, Krasowska A, Wrzosek M, Matsumoto H, et al. The CC genotype in the T102C HTR2A polymorphism predicts relapse in individuals after alcohol treatment. *J Psychiatr Res* 2013; 47(4): 527-33.
60. Bauer LO, Covault J, Gelernter J. GABRA2 and KIBRA genotypes predict early relapse to substance use. *Drug Alcohol Depend* 2012; 123(1-3): 154-9.
61. al'Absi M. Hypothalamic-pituitary-adrenocortical responses to psychological stress and risk for smoking relapse. *Int J Psychophysiol* 2006; 59(3): 218-27.
62. Mendelson JH, Sholar MB, Goletiani N, Siegel AJ, Mello NK. Effects of low- and high-nicotine cigarette smoking on mood states and the HPA axis in men. *Neuropsychopharmacol* 2005; 30(9): 1751-63.
63. Funk D, Marinelli PW, Le AD. Biological processes underlying co-use of alcohol and nicotine: neuronal mechanisms, cross-tolerance, and genetic factors. *Alcohol Res Health* 2006; 29(3): 186-92.
64. Fallon SJ, Smulders K, Esselink RA, van de Warrenburg BP, Bloem BR, Cools R. Differential optimal dopamine levels for set-shifting and working memory in Parkinson's disease. *Neuropsychologia* 2015; 77: 42-51.
65. Arnold C, Gispert S, Bonig H, von Wegner F, Somasundaram S, Kell CA. Dopaminergic Modulation of Cognitive Preparation for Overt Reading: Evidence from the Study of Genetic Polymorphisms. *Cereb Cortex* 2016; 26(4): 1539-57.
66. Meyer-Lindenberg A, Weinberger DR. Intermediate phenotypes and genetic mechanisms of psychiatric disorders. *Nat Rev Neurosci* 2006; 7(10): 818-27.
67. Strauss JS, Freeman NL, Shaikh SA, Vetró A, Kiss E, Kapornai K, et al. No association between oxytocin or prolactin gene variants and childhood-onset mood disorders. *Psychoneuroendocrinol* 2010; 35(9): 1422-8.
68. Montag C, Brockmann E-M, Bayerl M, Rujescu D, Müller DJ, Gallinat J. Oxytocin and oxytocin receptor gene polymorphisms and risk for schizophrenia: a case-control study. *World J Biol Psychiatry* 2013; 14(7): 500-8.
69. Chatzittofis A, Nordstrom P, Uvnas-Moberg K, Asberg M, Jokinen J. CSF and plasma oxytocin levels in suicide attempters, the role of childhood trauma and revictimization. *Neuro Endocrinol Lett* 2014; 35(3): 213-7.
70. Jokinen J, Chatzittofis A, Hellstrom C, Nordstrom P, Uvnas-Moberg K, Asberg M. Low CSF oxytocin reflects high intent in suicide attempters. *Psychoneuroendocrinol* 2012; 37(4): 482-90.
71. Mandelli L, Serretti A. Gene environment interaction studies in depression and suicidal behavior: An update. *Neurosci Biobehav Rev* 2013; 37(10 Pt 1): 2375-97.
72. Xu Y, Xue Y, Asan, Daly A, Wu L, Tyler-Smith C. Variation of the oxytocin/neurophysin I (OXT) gene in four human populations. *J Hum Genet* 2008; 53(7): 637-43.
73. Mileva-Seitz V, Steiner M, Atkinson L, Meaney MJ, Levitan R, Kennedy JL, et al. Interaction between oxytocin genotypes and early experience predicts quality of mothering and postpartum mood. *PloS one* 2013; 8(4): e61443.
74. Budzicz Ł. The interpretation of the statistical data in scientific papers – advices for practitioners. *Psychologiczne Zeszyty Naukowe* 2017; 1: 143-58.