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SSTR4, CHILDHOOD ADVERSITY, SELF-EFFICACY AND SUICIDE RISK IN ALCOHOLICS

Abstract

Background: Patients with alcohol dependence (AD) are known to develop poor social skills, to report a higher number of adverse childhood experiences (ACEs) and to attempt suicide more frequently than the general population. The background for the association between ACEs and a higher risk of suicide still remains understudied. *SSTR4* rs2567608 is a functional polymorphism of the gene for somatostatin receptor subtype 4, predominantly found in the CA1 hippocampus area and involved in memory formation. We hypothesize that the functional polymorphism *SSTR4* rs2567608, general self-efficacy, and adverse childhood experiences influence the risk of suicide attempt in patients with AD.

Methodology: 176 patients with AD and 127 healthy controls were interviewed regarding 13 categories of ACEs and assessed with the General Self-Efficacy Scale. Genotyping for the *SSTR4* rs2567608 polymorphism was performed according to the manufacturer's standard PCR protocol.

Results: Patients with AD and the controls did not differ significantly according to the *SSTR4* rs2567608 genotype and allele frequencies. Lower general self-efficacy, higher number of ACEs, and the *SSTR4* rs2567608 TT genotype increased the risk of suicide attempt in patients with AD, and it persisted significant only in male patients with AD. Conclusions: Our study supports previous findings on ACEs and general self-efficacy association with a risk for suicide. Additionally, we suggest that patients with AD of the *SSTR4* rs2567608 TT genotype may be more vulnerable to ACEs and at a higher risk of suicide attempt.

1. Introduction

As reported by Nock et al. (2008), lifetime prevalence of suicide ideation/suicide plan/suicide attempt (suicide behavior) across 17 countries was found in 9.2% /3.1% /2.7% of 84 850 adults of general population [1]. Individuals with mental disorders are known to show higher life-time prevalence of suicide attempt, reaching 31.9-44% in patients with alcohol dependence (AD) [2,3].

Studies on suicide, suggest adverse childhood experiences (ACEs) understood by physical, verbal, and sexual abuse, neglect, loss of attachment figures due to divorce, separation, death, exposure to domestic violence, and growing up in a household with mental illness, alcohol abuse, drug abuse, or incarceration are reported as a significant risk factor for suicide behavior/suicide in general population and in patients with AD [2,4-6].

Patients with AD are known to report a higher number of ACEs than the general population [7]. It was postulated that this may be due to having been raised in a dysfunctional household, however, the association cannot be considered absolute [8]. As reported by Hardt et al. (2008), 17% of 575 patients of psychosomatic clinic and general practitioners reported a suicide attempt in the past and in particular, two forms of early violence (i.e., sexual abuse and harsh physical punishment) were associated with an increased risk for suicide attempts [5]. As reported by Jakubczyk et al. (2014), sexual abuse in childhood overweight physical abuse itself as a risk factor of suicide attempt in patients with AD and is independent from other known factors influencing suicide risk, eg., drinking severity [2]. In our opinion, the role of ACEs as a risk

factor for suicide behavior/suicide was widely assessed in depression, posttraumatic stress disorder, and some personality traits, but is still insufficient in AD.

General self-efficacy is an important construct that may prevent individuals from committing suicide. General self-efficacy was defined by Bandura (1997) as the belief that one can successfully execute behaviors needed to produce a desired outcome [9]. Pompili et al. (2010) investigated the association of impulsivity, aggression and self-efficacy with protective factors against suicide [10]. The study population consisted of 300 Italian university students (141 males, 159 females); mean age 24.2 (SD = 3.01).Theirs results support the possibility that increasing general self-efficacy could be a useful target for interventions directed toward suicide prevention in individuals with problems of

emotional control [10]. As noted by Bandura (1986), patients who have strong self-efficacy are likely to mobilize their effort needed to successfully resist situations of high risk for alcohol drinking [11]. Bandura (1982) pointed out that there is a bimodal association between life adversities and self-efficacy [12]. Strong self-efficacy supports recovery from traumatic experiences, but self-efficacy may be impaired by previously experienced life adversities [12]. Reports in the literature indicate that ACEs may implicate adverse adaptation following traumatic experiences and self-efficacy [13-17]. Studies on AD treatment outcomes and relapse have tended to focus on self-efficacy [18-22]. We hypothesized that lower general self-efficacy is associated with a higher risk of suicide attempt in patients with AD.

ACEs have been shown to moderate both, neurotransmission and brain structures in developing brain. Lower volume of both, hippocampus and amygdala, in children experienced with ACEs, was considered as a structural warrant of further behavioral problems following ACEs [23,24]. As summarized by Hanson et al. (2015), the hippocampus is involved in learning, memory, and neuroendocrine response to stress; the amygdala is important for emotions and social information processing [24]. Hanson et al. (2015) collected MRI images for 128 children of three subgroups of experienced ACEs: early caregiving neglect while living in institutions for orphaned or abandoned children, living in household of low socioeconomic status, and who suffered physical abuse [24]. When compared with 41 children with no history of maltreatment, children who suffered early neglect and children raised in low socioeconomic status households had smaller left amygdala; children from low socioeconomic status households had smaller left and right hippocampi; and children who experienced physical abuse had smaller right hippocampi [24]. Approximately 60-90% of people experience at least one category of a traumatic event during their lifespan, but many fewer develop prolonged psychological consequences of events in this category [25]. The neuroendocrine hypothesis of posttraumatic stress disorder (PTSD) posits that trauma-vulnerable subjects exhibit more effective consolidation and retention of memories of emotionally arousing events [26]. When compared with norepinephrine's and glucocorticoid's role in memorizing stressors, somatostatin's role in memory formation and retention still remains to be determined [27]. The sympathetic system and the hypothalamic-pituitary-adrenal axis (HPA) act together to provide the organism with sufficient supplementation of oxygen and glucose to support the "fight or flight" response to a stressor and to memorize the stressor for future proper reaction, which is crucial for the evolutionary process of the human species. Immediate, i.e. within seconds, epinephrine/norepinephrine release after exposition to a stressor, increased heart rate, force of heart contractions, peripheral vasoconstriction, blood pressure and energy mobilization. Slower, i.e. within minutes, is the HPA axis response which initiates a slow-rising release of cortisol that persists over the course of several hours. This cortisol release results in both metabolic (i.e. glucose metabolism) and immune (i.e. cytokine expression) actions. However, a biological imbalance in norepinephrine/cortisol release, which may appear in genetically/epigenetically predisposed subjects, while a sudden stressor or a chronic stressor acts, may result in stress-related disorders and depression [26,28,29]. HPA axis hyperactivity may result in lower levels of brain neurotrophic factors and poor neuroplasticity. Serum levels of cortisol and the brain-derived neurotrophic factor were found to be inversely associated in female suicide attempters [30]. A meta-analysis conducted by Lopez-Duran, Kovacs, and George (2009) confirmed that the HPA-axis system was dysregulated in depressed youth, as evidenced by atypical responses to the dexamethasone test, higher baseline cortisol values, and an overactive response to psychological stressors [31]. Somatostatin is a peptide hormone that, in addition to exo- and endocrine suppression, also acts as a neurotransmitter and neuromodulator of other neurotransmitters [32]. Intra-cerebroventricular, intra-amygdalar and intra-septal microinfusions of somatostatin demonstrated its anxiolytic effect in the rat brain [33,34]. The mice model of memorizing fear as studied by Kluge et al. (2008) suggests that the somatostatin system plays a critical role in the acquisition of contextual fear memory but not for tone fear learning, and further highlights the role of hippocampal synaptic plasticity for information processing concerning contextual information [27]. Somatostatin-positive interneurons of the dentate gyrus were found to control the size of the cellular engram and the stability of contextual fear memory [35]. Somatostatin acts via G protein-coupled receptors; one of them, somatostatin receptor subtype 4, is mainly located in the adult human lung and brain, especially in area CA1 of the hippocampus [36,37]. Animal studies by Gastambide et al. (2009, 2010) found that intra-hippocampal injections of the somatostatin receptor subtype 4 agonist (L-803.087) in mice dramatically impaired place memory formation in a dose-dependent manner, yet agonists of somatostatin receptor subtypes 1, 2, or 3 had no effect [38,39]. Lin and Sibile (2015) showed that mice cortical somatostatin neurons displayed selective vulnerability to chronic stress when compared with pyramidal neurons [40]. As reviewed by Lin and Sibille (2013), low somatostatin levels were found in cerebrospinal fluid, dorsolateral prefrontal cortex, anterior cingulate cortex, and amygdala in patients with major depressive disorder; in cerebrospinal fluid, dorsolateral prefrontal cortex, hippocampus, caudal entorhinal cortex and parasubiculum in patients with schizophrenia; in caudal entorhinal cortex, parasubiculum, hippocampus and dorsolateral prefrontal cortex in patients with bipolar disorder; in cerebrospinal fluid, temporal and frontal cortex, hippocampus and parahippocampal cortex in patients with Alzheimer's disease; in cerebrospinal fluid and frontal cortex in Parkinson's disease [41]. The clinical output of this finding is still being investigated [41]. NNC-26-9100, a selective somatostatin receptor subtype 4 agonist, has been shown to reduce soluble Aβ brain levels and to improve learning and memory following chronic administration in mice, thus suggesting that somatostatin receptor subtype 4 agonists may provide a beneficial therapeutic approach for the treatment of Alzheimer's disease and cognitive impairment [42,43].

Here, we hypothesize that the functional polymorphism in the gene for somatostatin receptor subtype 4, *SSTR4* rs2567608, general self-efficacy, and adverse childhood experiences influence the risk of suicide attempt in patients with AD. Also the role of epigenetic modification of gene expression has been shown to be involved in suicide behavior. An example that will be further discussed are microRNAs (miRNAs), a non-coding RNA transcripts. As reviewed by Serafini et al. (2014), several studies have identified miRNAs as a fundamental class of gene expression regulators involved in the development, physiology, and diseases of the central nervous system [44]. Moreover, the expression of miRNAs in frontal cortex, amygdala and hippocampus were found to be influenced by acute and chronic stress [44]. This is the first study to assess the somatostatin receptor subtype 4 gene (*SSTR4*) functional polymorphism, rs2567608, in patients with AD and generally, in patients with mental disorders. Our main aim is to: 1. Evaluate the difference in affection with childhood adversities between patients with AD and healthy non-clinical subjects; 2. Evaluate the difference in *SSTR4* rs2567608 allele and genotype frequencies between patients with AD and healthy non-clinical subjects; 3. Assess if *SSTR4* rs2567608 genotypes/alleles, ACEs' history, general self-efficacy influence the risk of suicide attempt in patients with AD.

2. Subjects and Methods

This is a study based on retrospective and self-reported data, performed in Poland between the years 2013 and 2015.

2.1. Subjects

A total of 209 consecutive patients with AD who were admitted to psychiatry wards for a course of AD psychotherapy or a treatment of alcohol withdrawal syndrome and gave informed consent were involved in the study. Patients were informed in the study informed consent that they have the right to withdraw the consent at any step of the study without giving any reason. Of 209 patients, 33 did not undergo further analysis because of

incomplete data (giving the questionnaire back without all the answers completed) or consent's withdrawal during the study (mainly, when finding the questions too personal/intimate or deciding not to undergo buccal smear). The study analyzed 176 inpatients with AD (134 males and 42 females) aged 43.4±10.5 (mean ± SD years). Each patient received a consensus diagnosis of alcohol dependence by 2 psychiatrists according to the ICD-10 (F10.2) [45]. The period from the most recent alcohol intake was at least one week. Patients with AD scored 27.2±7.5 (mean ± SD points) out of the possible 40 points on the AUDIT interview (Alcohol Use Disorders Identification Test) [46]. The exclusion criteria were: 1. age < 18; 2. a history of a significant psychiatric comorbidity according to the ICD-10 [44]; 3. ever having received chemotherapy consisting of drugs that influence DNA methylation, i.e., 5-azacytidin and decitabine (the group of patients with AD was also introduced to another study on genome methylation).

The controls were initially 140 healthy volunteers who gave informed consent. Controls were informed in the study informed consent that they have the right to withdraw the consent at any step of the study without giving any reason. Of them, 13 did not go further analysis because of incomplete data (giving back the questionnaire with incomplete data) or meeting any from below listed exclusion criteria. The study analyzed 127 healthy volunteers (96 males and 31 females) aged ≥18 [39.4±12.0 (mean ± SD years]. Exclusion criteria for controls were: 1. ever been diagnosed with a mental disorder according to the ICD-10 [46] in their lifetimes; 2. ever attempted suicide or self-mutilated; 3. reaching the AUDIT scoring [46] indicating alcohol abuse (F10.1 according to the ICD-10) [44] or possible AD (F10.2 according to the ICD-10) [45]; 4. ever having received chemotherapy consisting of drugs that influence DNA methylation, i.e., 5-azacytidin and decitabine (the controls were also introduced to another study on genome methylation). Controls were introduced to the study to assess the difference between non-clinical subjects and patients with AD according to the history of ACEs' and *SSTR4* rs2567608 allele and genotype frequency.

Patients with AD and the controls were sex matched. Female and male patients with AD were significantly older than the control female and male subjects (P=0.012 and P=0.039, respectively). Patients with AD and the controls were native, unrelated inhabitants of Central Poland.

2.2. Data collection

This study used a structured self-reported questionnaire that had been designed for the study to measure the sociodemographic and clinical characteristics of the study participants. The study participants were ensured confidentiality of the obtained data. The researcher remained present during the completion of the questionnaires in order, to address the participants' questions and to make sure the respondents understood all of the items.

Study participants were asked about the life time prevalence of at least one suicide attempt with a question: "Have you ever tried to commit suicide during your life-time?"

The Alcohol Use Disorders Identification Test (AUDIT) [46] with a Cronbach's alpha index of 0.85 was applied to characterize alcohol intake severity during the past year in patients with AD and to exclude healthy volunteers with alcohol abuse (F10.1 according to the ICD-10) [44]or suspected AD (F10.2 according to the ICD-10) [45].

The ACEs were measured with a tool designed for this study that was named the ACE (13) Score. The first 10 questions were developed by Kaiser Permanente and the Centers for Disease Control and Prevention and evaluated exposure to abuse and family dysfunction occurring during the first 18 years of life (ACE Study Score) [47]. These 10 questions focus on chronic physical, verbal, and sexual abuse; neglect; the loss of one or both parents for any reason (i.e., divorce, separation, or death); exposure to domestic violence; and growing up in a household with mental illness, alcohol abuse, drug abuse, or incarceration. The 3 additional questions concern events that also took place in one's life under the age of 18 and included: witnessing a family member's suicide attempt; witnessing a family member's death due to any cause; and witnessing a stranger's

death due to any cause (e.g., traffic accident). The details of our statistical analysis, allow for our results to still be still comparable with studies based on the ACE Study Score.

Self-efficacy was measured with the Polish version of the Generalized Self-Efficacy Scale (GSES) by Schwarzer, Jerusalem and Juczyński, a 10-item psychometric scale, was used to assess optimistic self-beliefs into coping with a variety of difficult demands in life [48]. The GSES of internal reliability measured by the Cronbach's alphas equal to 0.85, for internal reliability, was created to assess the general sense of perceived self-efficacy and to predict coping with daily hassles as well as adaptation after experiencing all kinds of stressful life events [48]. Responses are made on a 4-point scale for each item' total scoring ranges from 10 to 40. The higher the score, the greater is the individual's generalized sense of self-efficacy. The scoring of ≤ 24 points is interpreted as low;, between 25 to 29 points – as medium;, and ≥ 30 points – as a high outcome [48]. In order to address the possible bias connected with the participant's intentional attempt to present him or herself in either a better or worse mental and general condition, the researcher who remained present during completion of the questionnaires listed above was not involved in the patients' therapy. The recall bias was still possible during ACE (13) Score completion, which was listed among the limitations of the study.

2.3. Ethics
Patients with AD and the controls gave written informed consent for their participation in the study. The study was approved by a Local Bioethics Committee: Nos. RNN/467/13/KB and KB/843/13/P. The study was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

2.4. Laboratory testing
Laboratory work was carried out at the Central Scientific Laboratory of the Medical University in Lodz. Buccal smears were obtained by rubbing the buccal mucosal with a sterile, DNA-free set of forensic swabs (Sarstedt). The buccal smears were obtained by trained personnel and then stored in accordance with the manufacturer's instructions until laboratory analysis. The buccal smears were obtained at least 2 hours after eating, tooth brushing, cigarette smoking, or gum chewing.

Genomic DNA was isolated from the buccal swabs using a High Pure PCR Template Preparation Kit (Roche) according to the manufacturer's protocol. DNA was eluted in 100 µl Elution Buffer and quantified using a Picodrop spectrophotometer (Picodrop Limited). The quality of the DNA samples was analyzed by measuring the ratio of absorption at 260/280 nm. Purified total DNA was immediately used for PCR reactions or stored at −20 °C.

SSTR4 rs2567608 was analyzed using a commercially available Pre-made TaqMan SNP Genotyping Assay (Applied Biosystems, ID: C_3206279_1). The assay consisted of PCR primers and reporter probes that were labeled with a quencher (MGB) and either 6-carboxyfluorescein (FAM) or VIC (Applied Biosystems proprietary dye with λex = 488 nm and λem = 552 nm). Amplification of the probe-specific product causes cleavage of the probe, thus generating an increase in reporter fluorescence.

Amplification was performed according to the manufacturer's standard PCR protocol. Briefly, 10 ng total DNA was mixed with 10 µl TaqMan Genotyping PCR Master Mix and 0.5 µl TaqMan Assay to a final volume of 20 µl. PCR thermal cycling was as follows: initial denaturing at 95 °C for 10 min; 40 cycles of 92 °C for 15 sec; and 60 °C for 1 min. Thermal cycling was performed using a GeneAmp PCR System 9700 (Applied Biosystems). Each 96-well plate contained 92 test samples and 4 reaction mixtures without the DNA template (no-template control).

The end-point fluorescence intensities of each probe were monitored using the ABI7900HT Real-Time PCR System (Applied Biosystems). The genotypes were determined automatically and then visually verified based on the dye component's fluorescence emission data depicted in the X-Y scatter-plot of Sequence Detection System 2.3 Software.

2.5. Statistical analysis
Between-group comparisons were made by the Mann-Whitney U test for independent samples (differences between means) and contingency tables were compared by the chi-square test. Bonferroni correction for multiple testing was applied. Changes in the odds ratio (risk) of suicide attempt and AD with an increased history of 13 categories of ACEs (also with additional variables such as age, general self-efficacy or the presence of specific alleles or genotypes) were assessed with logistic regression. The level of statistical significance was set at P values of ≤0.05. Normality of data distribution was evaluated with the Shapiro-Wilk test. Parameters with normal distribution (age and HBI) were presented as an average and standard deviation (SD). If distribution was other than normal, the median and range (min-max) were provided. Calculations were performed using the Statistical Package for Social Scientists version 22 (IBM SPSS v. 22) and GRETL packages. SNPs were evaluated for deviation from the Hardy–Weinberg equilibrium using Michael H. Court's (2005–2008) online calculator (http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls).

3. Results

3.1. Frequency of *SSTR4* rs2567608 alleles and genotypes in patients with AD and the controls
The genotype and allele frequencies of *SSTR4* rs2567608 in patients with AD and the controls are shown in Table 1. For the studied polymorphism, the distribution of genotypes within patients with AD and the controls was in the Hardy–Weinberg equilibrium (Chi2=3.629, P=0.056 for patients with AD; Chi2=0.044, P=0.833 for the controls). There were no significant differences in genotype and allele frequencies between patients with AD and the controls (Table 1) nor between males and females in each group (P>0.1).

3.2. Self-reporting of ACEs' categories in patients with AD and the controls
The number of each self-reported ACE in the group of patients with AD and in the controls is depicted in Figure 1. Only 26 (14.8%) of patients with AD reported no ACE vs. 85

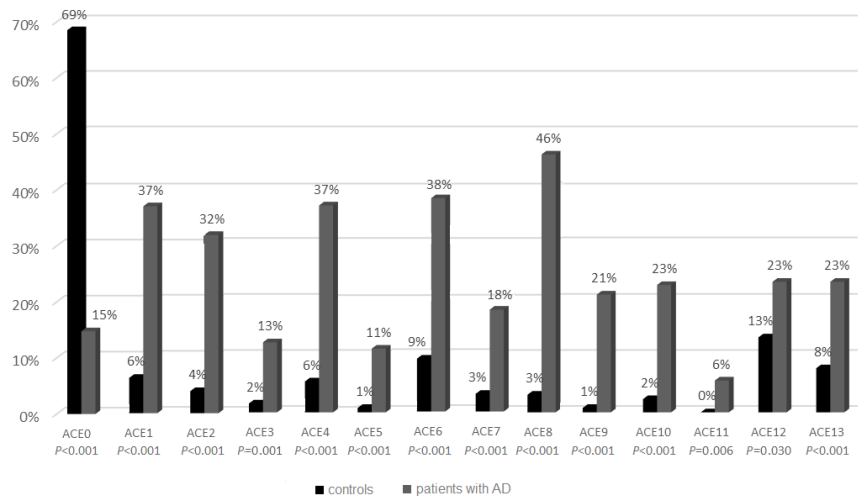Table 1. The frequency of *SSTR4* rs2567608 alleles and genotypes in patients with AD (n=176) and the controls (n=127)

		Patients with AD (n=176)	controls (n=127)	Chi²	P-value
		n (%)	n (%)		
SSTR4 rs2567608 genotypes	CC	31 (17.6)	28 (22.1)	2.1126	0.348[1]
	CT	100 (56.8)	62 (48.8)		
	TT	45 (25.6)	37 (29.1)		
SSTR4 rs2567608 alleles	T (wild)	190 (54.0)	141 (54.23)	0.0039	0.950[1]
	C (variant)	162 (46.0)	119 (45.8)		

[1] The Chi² test
P-level of statistical significance
AD – alcohol dependence
SD – standard deviation

(66.9%) of the controls (*P*<0.001). Patients with AD reported each ACE category significantly more frequently than the controls (Figure 1). Female patients with AD reported sexual abuse (P=0.045), witnessing physical abuse towards their mother or stepmother (P=0.046), and problem drinking/alcohol dependence of a household member (related or not) (P=0.044) significantly more frequently than male patients with AD.

3.3. Association between life-time suicide attempt prevalence and selected demographic and clinical characteristics of patients with AD

Of 176 patients with AD, 68 attempted suicide at least once during the life time. The mean number of suicide attempts was 2.6 (SD 2.7). Patients with AD who reported ever attempting suicide were significantly younger (P=0.007, Table 2), had significantly lower general self-efficacy (scored significantly lower on the GSES) (P=0.002, Table 2), and reported a significantly higher number of ACEs (both on the ACE Study Score and ACE 13 Score) (P<0.001, Table 2). Characteristics that differed significantly between patients with AD who reported ever attempting suicide and patients with AD who have never attempted suicide were further analyzed for chance risk of suicide attempt in male and female patients with AD. Both subgroups of patients with AD did not differ significantly according to place of living, educational level, or marital and occupational level (Table 2). Male and female patients with AD did not differ significantly according to place of

ACE0. No ACE reported
ACE1. Psychological abuse
ACE2. Physical abuse
ACE3. Sexual abuse
ACE4. Emotional neglect
ACE5. Physical neglect
ACE6. Contact loss with one or both parents due to separation, divorce, or other reason
ACE7. Witnessing physical abuse towards one's mother or stepmother

ACE8. Problem drinking/alcoholic/street drug use of a household member
ACE9. Mental illness or suicide attempt of a household member
ACE10. Incarceration of a household member
ACE11. Witnessed a family member's suicide attempt
ACE12. Witnessed a family member's death of any cause
ACE13. Witnessed a stranger's death of any cause (i.e. a traffic accident)

P – level of statistical significance (Chi² test)
ACE – adverse childhood experience
AD – alcohol dependence
Mixed numbers of % values were rounded to the nearest whole number to increase visibility in the figure.

Fig. 1. Prevalence of self-reporting for each ACE category on the ACE 13 Score in patients with AD (n=176) and controls (n=127)

living (P=0.377), educational level (P=0.852), and occupational status (P=0.248). There was a significant difference between male and female patients with AD according to marital status (P=0.004) as there were more single male patients than female patients and a

higher number of divorced and widowed female patients. The female patients were significantly older than the male patients (43.5 (11.20) vs 42.5 (10.1), P=0.042). Both male and female patients with AD scored equally on the GSES (P=0.129).

Table 2. Comparison of demographic and clinical characteristics between patients with AD (n=176) who have attempted suicide at least once during the lifetime (n=68) and who have never attempted suicide (n=108)

Characteristic		Suicide attempt (n=68)	No suicide attempt (n=108)	P-value
Age (Mean ± SD) [years]		40.8 (10.5)	45 (10.2)	**0.007**[1]
Gender [Number (%)]	Female	22 (55.4)	20 (47.6)	0.036[2]
	Male	46 (34.3)	88 (65.7)	
GSES (Mean ± SD points)		26.4 (6.6)	29.4 (6.0)	**0.002**[1]
ACE Study Score (Mean ± SD points)		4.6 (3.0)	2.5 (2.4)	**0.000**[1]
ACE 13 Score (Mean ± SD points)		3.8 (2.6)	2.1 (2.1)	**0.000**[1]
Place of living [Number (%)]	village	6 (54.6)	5 (45.5)	0.520[2]
	small Town	7 (41.2)	10 (58.8)	
	big Town	55 (37.4	92 (62.6)	
Educational level [Number (%)]	basic	18 (38.3)	29 (61.7)	0.979[2]
	vocational	18 (40.9)	26 (59.1)	
	secondary	24 (36.9)	41 (63.1)	
	higher	8 (40.0)	12 (60.0)	
Marital status [Number (%)]	single	27 (39.1)	42 (60.9)	0.590[2]
	married	12 (31.6)	26 (68.4)	
	divorced	23 (45.1)	28 (54.9)	
	widowed	6 (33.3)	12 (66.7)	
Occupational status [Number (%)]	employed	20 (48.8)	21 (51.2)	0.222[2]
	unemployed	41 (37.6)	68 (62.4)	
	retired or survivor sickness	7 (28.0)	18 (72.0)	

[1] the U Mann-Whitney test, [2] the Chi[2] test - bold values mean a statistical significance
P-level of statistical significance that according to Bonferroni correction was 0.005
ACE – adverse childhood experience
AD – alcohol dependence
SD – standard deviation

3.4. Changes in the odds ratio (OR, risk) of suicide attempt according to the history of ACE, the *SSTR4* rs2567608 genotype, general self-efficacy, and age – logit model estimation in patients with AD (n=176)

In the group of patients with AD as a whole, every ACE reported with the ACE 13 Score significantly raised the risk of suicide attempt with OR 1.24 (CI 95%). In males this relation was more pronounced, increasing with OR 1.37 (CI 95%). On the other hand, every ACE reported with the ACE Study Score significantly raised the risk of suicide attempt only in males and with OR 1.28 (CI 95%) (Table 3).

With increasing general self-efficacy (every point in the GSES), the risk of suicidal attempt was lowered with OR 0.82 (CI 95%) in the whole group of patients with AD and with OR 0.87 (CI 95%) in male patients with AD (Table 3).

For female patients with AD, the risk of suicide attempt decreased significantly with ageing [with OR 0.84 (CI 95%) with every subsequent year] (Table 3).

The *SSTR4* rs2567608 TT genotype increased the risk of suicidal attempt with OR 1.23 (CI 95%) in the whole group of patients with AD and with OR 2.07 (CI 95%) in male patients with AD (Table 3).

4. Discussion

The *SSTR4* rs2567608 TT genotype, higher number of ACEs reported with the ACE 13 Score, and lower general self-efficacy significantly raised the risk of suicide attempt and it persisted significant in male patients with AD. This is the first study on *SSTR4* rs2567608 in clinical subjects with AD and, generally, with mental disorder, that proposes *SSTR4* rs2567608 TT as a molecular background for vulnerability to ACEs. It has to be underlined that alcohol use and suicide are both complex phenomena coming from a multitude of factors. Alcohol consumption may lead to suicidality through disinhibition, impulsive behavior and impaired judgment, but it may also be used as a means to ease distress by committing the act of suicide [10]. Thus, alcohol dependence may be a direct cause of attempted suicide independently from environmental inequities, from childhood, or from any other lifetime period. As studied by Kaplan et al. (2014), postmortem blood alcohol content positivity was detected in nearly 36% of males and 28% of females that committed suicide between years 2003 and 2011 in the USA [49].

Table 3. Changes in the odds ratio (risk) of suicide attempt according to the history of ACEs, *SSTR4* rs2567608 genotype, general self-efficacy and age – logit model estimation in patients with AD (*n*=176)

		Patients with AD (*n*=176)	Female Patients with AD (*n*=42)	Male Patients with AD (*n*=134)
ACE 13 Score	*OR*	1.24	1.08	1.37
	P	**<0.001**	0.167	**<0.001**
	95% CI	1.11-1.42	0.81-1.30	1.16-1.55
ACE Study Score	*OR*	1.10	1.06	1.28
	P	0.098	0.325	0.045
	95% CI	0.95-1.32	0.92-1.65	1.02-1.52
GSES	*OR*	0.82	0.96	0.87
	P	**<0.001**	0.142	**0.003**
	95% CI	0.71-0.93	0.87-1.36	0.81-0.96
SSTR4 rs2567608 Genotype	*OR*	1.23	1.10	2.07
	P	**<0.001**	0.242	**<0.001**
	95% CI	1.02-1.61	0.95-1.32	1.51-2.44
Age	*OR*	0.91	0.84	0.89
	P	0.072	**0.034**	0.067
	95% CI	0.72-1.03	0.62-0.95	0.73-1.02

Bold values indicate a statistical significance
OR - odds ratio
P – significance level of the odds ratio
95%CI- 95% confidence interval for the odds ratio
SA-suicide attempt
ACE – adverse childhood experience
GSES-General Self-Efficacy Scale

Patients with AD and the controls did not differ significantly according to the *SSTR4* rs2567608 genotype and allele frequencies. However, we cannot conclude that a lack of this difference mirrors that there are no differences in memory formation granted by the somatostatin receptor subtype 4 gene in these groups. Since the limitation of gene polymorphism studies is that they rely on a dominant model of inheritance, they adequately provide the reader with very careful conclusions. Many binary phenotypes do not follow the classical Mendelian inheritance pattern. Interaction between genetic and environmental factors is thought to contribute to incomplete penetrance phenomena that are often observed in these complex binary traits [52]. Moreover, epigenetic modifications, both inherited and resulting from gene–environment interaction, modify gene expression independently of the genotype and result in different clinical outcomes in subjects of the same genotype [53,54]. miRNAs are a class of gene expression regulators involved in the development, physiology, and diseases of the central nervous system [44]. They may reduce the final protein synthesis, first on transcriptional and second, on translational levels (2014). As reviewed by Serafini et al. (2014) , both acute and chronic stress, may influence the level of miRNAs in certain area of central nervous system in animal studies and miRNA expression was found to be globally downregulated in the prefrontal cortex of depressed suicide victims when compared to that of nonpsychiatric controls who died from other causes [44]. miRNAs have been considered to be involved in neuroplasticity, hypothesized as connected with mood disorders and suicidal behavior [55].Thus, epigenetic modification of gene expression is undoubtedly one confounder of single nucleotide studies, including ours.

A number of studies have investigated the role of genes connected with neurotransmission, memory formation, and neuroplasticity in patients with mental disorders or general population, i.e., the brain-derived neurotrophic factor gene (*BDNF*) [56,57] and the serotonin transporter gene (*SLC6A4*) [58-62]. Only three studies have been conducted regarding *SSTR4* rs2567608 on human sample [63-65], but none in the field of memory formation. Two of the three studies indicated *SSTR4* rs2567608 as an additional tool for forensic individual identification[63,65]. The third study, conducted by Kim et al. (2010), suggested that *SSTR4* rs2567608 was connected with human somatostatin receptor subtype 4 activity [64]. Patients with colorectal cancer carrying the *SSTR4* rs2567608 TT genotype showed lower disease

control rates in response to chemotherapy consisting of somatostatin receptor subtype 4 ligands (irinotecan and oxaliplatin) than patients carrying the *SSTR4* rs2567608 C allele [64]. However, to our knowledge, there have been no studies supporting data that *SSTR4* rs2567608 TT genotype carriers display lower somatostatin receptor subtype 4 activity in the brain. Thus, our study presents very exploratory results which needs further investigation.

Our patients with AD reported each ACE category assessed with the ACE 13 Score significantly more frequently than healthy controls. Two national surveys of the general population were conducted with the ACE Study Score in the USA and GB [46,66]. In the ACE Study by Felitti et al. (1998), at least 1 ACE was reported by 64% of respondents [45]. In a study by Bellis et al. (2015), a significantly lower number of ACEs was reported. In the latter study, 46.4% of respondents reported ≥1 ACE and 8.3% reported ≥4 ACEs [66]. Our patients with AD reported a higher number of ACE categories, probably due to the study inclusion criteria. Patients with AD are not representative of the general population and were found to report higher number of ACEs' categories than the general population [7,46]. We expanded the ACE Study Score with three additional questions concerning events that took place before the age of 18 and included: witnessing a family member's attempted suicide (ACE 11); witnessing a family member's death due to any cause (ACE 12); and witnessing a stranger's death due to any cause (i.e., a traffic accident) (ACE 13). All three were reported by patients with AD significantly more frequently than by the controls (Figure 1).They mirror both household and wider environmental inequities in an individual's place of living, but witnessing an individual's death or act of violence is an accidental and acute stressor which was shown in the USA National Survey of Adolescents to be of lower significance in creating risk for PTSD than personal victimizations [67]. We found that every ACE reported with the ACE 13 Score significantly raised the risk of suicide attempt by 24% in the whole group of patients with AD [68]. This relation persisted significant in male patients with AD and was even more

pronounced, increasing the risk by 37%. Every chronic ACE reported with the ACE Study Score significantly raised the risk of suicide attempt only in males and by 28%. Although female patients with AD reported a significantly higher number of chronic but not sudden ACEs [due to the higher frequency of sexual abuse (ACE 3), witnessing physical abuse towards their mother or stepmother (ACE 7), and problem drinking/alcohol dependence of a household member (ACE 8)], ACEs significantly raised suicide attempt risk in male, not female patients with AD. Thus, here suicide risk appears to be more related to ACEs in male patients with AD. Our hypothesis that subjects with the *SSTR4* rs2567608 TT genotype may be more vulnerable to childhood trauma was supported by the finding that the *SSTR4* rs2567608 TT genotype significantly increased the risk of suicide attempt in male, but not female, patients with AD. This is the first study hypothesizing higher vulnerability to ACEs in subjects with the *SSTR4* rs2567608 TT genotype and proposing the possible role of *SSTR4* rs2567608 in suicide risk. The results of the study should be interpreted carefully due to its exploratory and preliminary character. However, they also encourage further studies on *SSTR4* rs2567608 in clinical subjects.

Patients with AD who reported at least one suicide attempt had significantly lower general self-efficacy. The risk of suicide attempt was significantly lowered along with increasing general self-efficacy, but it persisted significant only in male patients with AD. As was pointed out by Bandura (1982), general self-efficacy may be impaired by previously experienced life adversities [12]. Although our female patients reported a higher number of ACEs than male patients with AD, both male and female patients assessed their general self-efficacy equally. Also, the sociodemographic status of both male and female patients was comparable (no difference in place of living, educational level, and occupational status) despite the differences in marital status, as the male patients were more frequently single but the female patients were more frequently divorced or widowed. This may again suggest the higher vulnerability of our male patients to ACEs. General self-efficacy was referred to as

global confidence in one's coping with novel situations, it warrants dealing with a variety of stressful situations and characterizes one's social skills [28]. Our study replicates other research studies that pointed lower general self-efficacy as related with higher risk of suicide ideation, but assess it in patients with AD [69, 70].

4.1. Limitations of the study

Due to the methodological limitations of the research presented here, our conclusions need to be formulated carefully.

The data were retrospective and self-reported, and recall bias is still a possibility in retrospective reports of childhood adversities. The cross-sectional design precludes both causal inference (as event reporting may be confounded by current psychological condition and age) and longitudinal analysis of adjustment trajectories [77].

The results of the study should be interpreted cautiously due to the relatively small study sample.

The study was based on a Caucasian population of Central Poland. Samples/populations of other origins may vary according to *SSTR4* rs2567608 allele and genotype frequencies. Thus the conclusions of our study do not consider samples/populations other than Caucasian.

As we listed in the Subjects and Methods section, our patients had no significant psychiatric comorbidity, but we neither assessed nor excluded patients with personality disorders. Borderline personality disorder, for example, is associated with suicide attempts. Our study cannot account for this possible confounding factor.

5. Conclusions

1. Patients with alcohol dependence are severely affected by adverse childhood experiences which can arise from adversities both within the household and in the wider place of living.
2. Adverse childhood experiences should be targeted as a primary preventive strategy for suicide attempt in patients with alcohol dependence.

3. The ACE 13 Score should be considered as an additional tool for suicide risk evaluation in patients with alcohol dependence.

4. The *SSTR4* rs2567608 TT genotype in male patients with AD may be a molecular underpinning for higher vulnerability to childhood adversities.

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Authors' Disclosures

Regarding research work described in the paper, each one of our co-authors declares that there is no conflict of interest, and conformed to the Helsinki Declaration concerning human rights and informed consent, and followed correct procedures concerning treatment of humans in research.

Authors' Contribution

DB designed and coordinated the study, qualified the patients and controls for entry into the study, analyzed and interpreted the results, and wrote the manuscript.
GE critically revised the manuscript.
MP performed the statistical analysis and interpreted the results.
EK performed the statistical analysis.
BSz performed the laboratory testing.
DK-W performed the laboratory testing.
MM qualified the controls for entry into the study.
ZP coordinated and performed the laboratory testing.
All of the authors approved the final version of this manuscript.
All the authors gave agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

[1] Nock M.K., Borges G., Bromet E.J., Alonso J., Angermeyer M., Beautrais A., et al., Cross-national prevalence and risk factors for suicidal ideation, plans and attempts, Br. J. Psychiatry, 2008, 192(2), 98-105

[2] Jakubczyk A., Klimkiewicz A., Krasowska A., Kopera M., Sławińska-Ceran A., Brower K.J., et al., History of sexual abuse and suicide attempts in alcohol-dependent patients, Child Abuse Negl., 2014, 38(9), 1560-1568

[3] Wojnar M., Ilgen M.A., Czyz E., Strobbe S., Klimkiewicz A., Jakubczyk A., et al., Impulsive and non-impulsive suicide attempts in patients treated for alcohol dependence, J. Affect. Disord., 2009,115(1-2), 131-139

[4] Johnson J.G., Cohen P., Gould M.S., Kasen S., Brown J., Brook J.S., Childhood adversities, interpersonal difficulties, and risk for suicide attempts during late adolescence and early adulthood, Arch. Gen. Psychiatry, 2002, 59(8), 741-749

[5] Hardt J., Sidor A., Nickel R., Kappis B., Petrak F., Egle U.T.,et al., Childhood Adversities and Suicide Attempts: A Retrospective Study, J. Fam. Viol., 2008, 23, 713-718

[6] Bruffaerts R., Demyttenaere K., Borges G., Haro J.M., Chiu W.T., Hwang I., et al., Childhood adversities as risk factors for onset and persistence of suicidal behavior, Br. J. Psychiatry, 2010, 197(1), 20-27

[7] Felitti V.J., The relationship of adverse childhood experiences to adult health: Turning gold into lead. Belastungen in der Kindheit und Gesundheit im Erwachsenenalter: die Verwandlung von Gold in Blei, Z. psychsom. Med. Psychother., 2002, 48(4), 359-369

[8] Latendresse S.J., Rose R.J., Viken R.J., Pulkkinen L., Kaprio J., Dick D.M., Parenting mechanisms in links between parents' and adolescents' alcohol use behaviors, Alcoholism Clin. Exp. Res., 2008,32,322–330

[9] Bandura A., Self-efficacy: toward a unifying theory of behavioral change, Psychol. Rev., 1977, 84(2), 191–215.

[10] Pompili M., Serafini G., Innamorati M., Dominici G., Ferracuti S., Kotzalidis G.D., et al., Suicidal behavior and alcohol abuse, Int. J. Environ. Res. Public Health, 2010, 7(4), 1392-1431

[11] Bandura A., Social Foundations of Thought and Action: A Social Cognitive Theory, NJ:Prentice-Hall, Englewood Cliffs, 1986

[12] Bandura A., Reese L., Adams N.E., Microanalysis of action and fear arousal as a function of differential levels of perceived self-efficacy, J. Pers. Soc. Psychol., 1982, 43, 5–21.

[13] Benight C., Bandura A., Social cognitive theory of posttraumatic recovery: The role of perceived self-efficacy, Behav. Res. Ther., 2004, 42(10), 1129-1148

[14] Hinnen C., Sanderman R., Sprangers M.A.G., Adult attachment as mediator between recollections of childhood and satisfaction with life, Clin. Psychol. Psychother., 2009, 16, 10–21

[15] Bruce L.C., Heimberg R.G., Goldin P.R., Gross J.J., Childhood maltreatment and response to cognitive behavioral therapy among individuals with social anxiety disorder, Depress. Anxiety, 2013, 30(7), 662-669

[16] Vilete L., Figueira I., Andreoli S.B., Ribeiro W., Quintana M.I., de Jesus Mari J., Resilience to trauma in the two largest cities of Brazil: a cross-sectional study, BMC Psychiatry, 2014, 14-257

[17] Suzuki H., Tomoda A., Roles of attachment and self-esteem: impact of early life stress on depressive symptoms among Japanese institutionalized children, BMC Psychiatry, 2015, 5, 15-18

[18] Sitharthan T., Kavanagh D.J., Role of self-efficacy in predicting outcomes from a programme for controlled drinking, 1991, Drug Alcohol Depend., 27, 87–94

[19] Rychtarik R., Prue D., Rapp S., King A., Self-efficacy, aftercare and relapse in a treatment program for alcoholics, 1992, J. Stud. Alcohol, 53, 435–440

[20] Jones B.T., McMahon J., Negative and positive alcohol expectancies as predictors of abstinence after discharge from a residential treatment program: a one-month and three-month follow-up study in men, J. Stud. Alcohol, 1994, 55, 543–548

[21] Greenfield S.F., Hufford M.R., Vagge L.M., Muenz L.R., Costello M.E., Weiss R.D., The relationship of self-efficacy expectancies to relapse among alcohol dependent men and women: a prospective study, J. Stud. Alcohol, 2000, 61, 345–351

[22] Trucco E.M., Connery H.S., Griffin M.L., Greenfield S.F., The relationship of self-esteem and self-efficacy to treatment outcomes of alcohol-dependent men and women, Am. J. Addict., 2007, 16(2), 85-92

[23] Brown S.M., Hariri A.R., Neuroimaging studies of serotonin gene polymorphisms: exploring the interplay of genes, brain, and behavior, Cogn. Affect. Behav. Neurosci., 2006, 6, 44–52

[24] Hanson J.L., Nacewicz B.M., Sutterer M.J., Cayo A.A., Schaefer S.M., Rudolph K.D., et al., Behavioral problems after early life stress: contributions of the hippocampus and amygdala, Biol. Psychiatry., 2015, 77(4), 314-323

[25] Sledjeski E.M., Speisman B., Dierker L.C., Does the number of lifetime traumas explain the relationship between PTSD and chronic medical conditions? Answers from the National Comorbidity Survey—Replication (NCS-R), J. Behav. Med., 2008, 31, 341–349

[26] Hruska B., Cullen P.K., Delahanty D.L., Pharmacological modulation of acute trauma memories to prevent PTSD: considerations from a developmental perspective, Neurobiol, Learn, Mem,, 2014, 112, 122-129

[27] Kluge C., Stoppel C., Szinyei C., Stork O., Pape H.C., Role of the somatostatin system in contextual fear memory and hippocampal synaptic plasticity, Learn. Mem., 2008, 15(4), 252-260

[28] Schwarzer R., Mueller I., Greenglass E., Assessment of perceived general self-efficacy on the internet: data collection in cyberspace, Anxiety Stress Coping, 1999, 12, 145-161

[29] Checkley S., The neuroendocrinology of depression and chronic stress, Br. Med. Bull., 1996, 52(3), 597–617

[30] Ambrus L., Lindqvist D., Träskman-Bendz L., Westrin Å., Hypothalamic-pituitary-adrenal axis hyperactivity is associated with decreased brain-derived neurotrophic factor in female suicide attempters, Nord. J. Psychiatry, 2016, 23, 1-7

[31] Lopez-Duran N.L., Kovacs M., George C.J., Hypothalamic-pituitary-adrenal axis dysregulation in depressed children and adolescents: a meta-analysis, Psychoneuroendocrinology, 2009, 34(9), 1272-1283

[32] Patel Y.C., Somatostatin and its receptor family, Front. Neuroendocrinol., 1999, 20, 157-198

[33] Yeung M., Engin E., Treit D., Anxiolytic-like effects of somatostatin isoforms SST 14 and SST 28 in two animal models (Rattus norvegicus) after intra-amygdalar and intra-septal microinfusions, Psychopharmacology, 2011, 216(4), 557-567

[34] Yeung M., Treit D., The anxiolytic effects of somatostatin following intra-septal and intra-amygdalar microinfusions are reversed by the selective sst2 antagonist PRL2903, Pharmacol. Biochem. Behav., 2012, 101(1), 88-92

[35] Stefanelli T., Bertollini C., Lüscher C., Muller D., Mendez P., Hippocampal Somatostatin Interneurons Control the Size of Neuronal Memory Ensembles, Neuron, 2016, 89(5), 1074-1085

[36] Rohrer L., Raulf F., Bruns C., Buettner R., Hofstaedter F., Schule R., Cloning and characterization of a fourth human somatostatin receptor, Proc. Nat. Acad. Sci., 1993, 90, 4196-4200

[37] Meyer M.A., Highly expressed genes within hippocampal sector CA1: implications for the physiology of memory, Neurol. Int., 2014, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4077213/

[38] Gastambide F., Viollet C., Lepousez G., Epelbaum J., Guillou J.L., Hippocampal SSTR4 somatostatin receptors control the selection of memory strategies, Psychopharmacology, 2009, 202: 153-163

[39] Gastambide F., Lepousez G., Viollet C., Loudes C., Epelbaum J., Guillou J.L., Cooperation between hippocampal somatostatin receptor subtypes 4 and 2: functional relevance in interactive memory systems, Hippocampus, 2010, 20(6), 745-57

[40] Lin L.C., Sibille E., Transcriptome changes induced by chronic psychosocial/environmental or neuroendocrine stressors reveal a selective cellular vulnerability of cortical somatostatin (SST) neurons, compared with pyramidal (PYR) neurons, Mol. Psychiatry, 2015, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4494784/

[41] Lin L.C., Sibille E., Reduced brain somatostatin in mood disorders: a common pathophysiological substrate and drug target?, Front. Pharmacol., 2013, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3766825/

[42] Sandoval K.E., Farr S.A., Banks W.A., Niehoff M.L., Morley J.E., Crider A.M., et al., Chronic peripheral administration of somatostatin receptor subtype-4 agonist NNC 26-9100 enhances learning and memory in SAMP8 mice, Eur. J. Pharmacol., 2011, 654(1), 53-59

[43] Sandoval K.E., Farr S.A., Banks W.A., Crider A.M., Morley J.E., Witt K.A., Somatostatin receptor subtype-4 agonist NNC 26-9100 mitigates the effect of soluble Aβ(42) oligomers via a metalloproteinase-dependent mechanism, Brain Res., 2013, 1520:145-156

[44] Serafini G., Pompili M., Hansen K.F., Obrietan K., Dwivedi Y., Shomron N., et al., The involvement of microRNAs in major depression, suicidal behavior, and related disorders: a focus on miR-185 and miR-491-3p, Cell Mol. Neurobiol., 2014, 34(1):17-30

[45] World Health Organization, The ICD-10 Classification of Mental and Behavioural Disorders: Clinical Description and Diagnostic Guidelines, WHO, Geneva, 1992

[46] Daeppen J.B., Yersin B., Landry U., Pécoud A., Decrey H., Reliability and validity of the Alcohol Use Disorders Identification Test (AUDIT) imbedded within a general health risk screening questionnaire: results of a survey in 332 primary care patients, Alcohol Clin. Exp. Res., 2000, 24(5), 659-65

[47] Felitti V.J., Anda R.F., Nordenberg D., Williamson D.F., Spitz A.M., Edwards V., et al., Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults: the Adverse Childhood Experiences (ACE) Study, Am. J. Prev. Med., 1998, 14, 245–258

[48] [Juczyński Z. [Instruments for measurement in health promotion and health psychology], 2nd ed., Pracownia Testów Psychologicznych, Warsaw, 2012 [in Polish]

[49] Kaplan M.S., Huguet N., McFarland B.H., Caetano R., Conner K.R., Giesbrecht N., et al., Use of alcohol before suicide in the United States, Ann. Epidemiol., 2014, 24(8), 588-592

[50] Schrijvers D.L., Bollen J., Sabbe B.G., The gender paradox in suicidal behavior and its impact on the suicidal process, J. Affect. Disord., 2012, 138(1-2), 19-26

[51] Bertolote J.M., Fleischmann A., Suicidal behavior prevention: WHO perspectives on research, Am. J. Med. Genet. C. Semin. Med. Genet., 2005, 133, 8-12

[52] Sepúlveda N., Paulino C.D., Carneiro J., Penha-Gonçalves C., Allelic penetrance approach as a tool to model two-locus interaction in complex binary traits, Heredity, 2007, 99(2), 173-1784

[53] Ogino S., Wilson R.B., Genotype and haplotype distributions of MTHFR677C>T and 1298A>C single nucleotide polymorphisms: a meta-analysis, J. Hum. Genet., 2003, 48, 1–7

[54] Petronis A., Epigenetics as a unifying principle in the aetiology of complex traits and diseases, Nature, 2010, 465, 721–727

[55] Serafini G., Pompili M., Innamorati M., Giordano G., Montebovi F., Sher L., et al., The role of microRNAs in synaptic plasticity, major affective disorders and suicidal behavior, Neurosci. Res., 2012, 73(3), 179-190

[56] Cortés-Mendoza J., Díaz de León-Guerrero S., Pedraza-Alva G., Pérez-Martínez L., Shaping synaptic plasticity: the role of activity-mediated epigenetic regulation on gene transcription, Int. J. Dev. Neurosci., 2013, 31, 359–369

[57] Egan M.F., Kojima M., Callicott J.H., Goldberg T.E., Kolachana B.S., Bertolino A., et al., The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function, Cell, 2003, 112, 257-269

[58] Roth T.L., Lubin F.D., Funk A.J., Sweatt J.D., Lasting epigenetic influence of early-life adversity on the BDNF gene, Biol. Psychiatry, 2009, 65(9), 760–769

[59] Herman A.I., Philbeck J.W., Vasilopoulos N.L., Depetrillo P.B., Serotonin transporter promoter polymorphism and differences in alcohol consumption behavior in a college student population, Alcohol, 2003, 38, 446-449

[60] Munafo M.R., Lingford-Hughes A.R., Johnstone E.C., Walton R.T., Association between the serotonin transporter gene and alcohol consumption in social drinkers, Am. J. Med. Genet. Neuropsychiat. Genet., 2005, 135B: 10-14

[61] Covault J., Tennen H., Armeli S., Conner T.S., Herman A.I., Cillessen A.H.N., et al., Interactive Effects of the Serotonin Transporter 5-HTTLPR Polymorphism and Stressful Life Events on College Student Drinking and Drug Use, Biol. Psychiatry, 2007, 61, 609-616

[62] van Jzendoorn M.H., Belsky J., Bakermans-Kranenburg M.J., Serotonin transporter genotype 5HTTLPR as a marker of differential susceptibility? A meta-analysis of child and adolescent gene-by-environment studies, Transl. Psychiatry, 2012, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3432188/

[63] van Jzendoorn M.H., Caspers K., Bakermans-Kranenburg M.J., Beach S.R.H., Philibert R., Methylation Matters: Interaction Between Methylation Density and Serotonin Transporter Genotype Predicts Unresolved Loss or Trauma, Biol. Psychiatry, 2010, 68, 405–407

[64] Pakstis A.J., Speed W.C., Kidd J.R., Kidd K.K., Candidate SNPs for a universal individual identification panel, Hum. Genet., 2007, 121(3-4), 305-317

[65] Kim J.C., Kim S.Y., Cho D.H., Roh S.A., Choi E.Y., Jo Y.K., et al., Genome-wide identification of chemosensitive single nucleotide polymorphism markers in colorectal cancers, Cancer Sci, 2010, 101(4), 1007–1013

[66] Kidd K.K., Kidd J.R., Speed W.C., Fang R., Furtado M.R., Hyland F.C., et al., Expanding data and resources for forensic use of SNPs in individual identification, Forensic Sci. Int. Genet., 2012, 6(5), 646-652

[67] Bellis M.A., Hughes K., Leckenby N., Hardcastle K.A., Perkins C., Lowey H., Measuring mortality and the burden of adult disease associated with adverse childhood experiences in England: a national survey, J. Public Health (Oxf), 2015, 37(3), 445-454

[68] Kilpatrick D.G., Saunders B.E., Smith D.W., Youth Victimization: Prevalence and Implications, U.S. Department of Justice, Office of Justice Program, National Institute of Justice, 2003, http://www.ncjrs.gov/pdffiles1/nij/194972.pdf

[69] Feng F., Li S., Chen H., Impacts of Stress, Self-Efficacy, and Optimism on Suicide Ideation among Rehabilitation Patients with Acute Pesticide Poisoning, PLoS One, 2015, http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0118011

[70] Kobayashi Y., Fujita K., Kaneko Y., Motohashi Y., Self-Efficacy as a Suicidal Ideation Predictor: A Population Cohort Study in Rural Japan, Open J. Prev. Med., 2015, 5, 61-71