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The rs4354668 polymorphism in the *SLC1A2* gene for the EAAT2 glutamate transporter is associated with an increased risk of harmful drug use – an exploratory study on a university student population

Bartosz Dawidowski[1], Barbara Olejniczak[2], Katarzyna Groblińska[1], Magdalena Knapińska[1], Urszula Kozicka[1], Michał Krasiński[1], Anna Kułak[1], Grzegorz Grelecki[1], Zuzanna Czaplińska[1], Oliwia Piotrowska[3], Klaudia Kościelecka[1], Piotr Podwalski[1*], Jerzy Samochowiec[1]

[1] Department of Psychiatry, Pomeranian Medical University
[2] Mossakowski Medical Research Institute, Polish Academy of Sciences NeuroOncology Laboratory
[3] Department of Pediatric Hematooncology and Gastroenterology, Pomeranian Medical University

Summary

Aim. Evidence suggests that decreased dopamine secretion in mesocorticolimbic pathways could predispose to increased susceptibility to substance addiction. It has been proposed to define such a phenomenon as the reward deficiency syndrome (RDS). Dopaminergic projections of the reward system receive glutaminergic projections from the cortex. Research indicates that a reduction in the stimulating glutamatergic transmission on the dopaminergic system could represent an alternative phenotype of RDS. A potential source of this type of abnormality is glutamate reuptake which depends on the function of excitatory amino acid transporter (EAAT) proteins. The most important of them is EAAT2, polymorphisms of which have been linked to several mental disorders. The aim of the presented research was to assess the association of the *rs4354668* gene variants for EAAT2 with the risk of substance use disorders.

Material and methods. We analyzed the genetic and psychometric data of 125 young adults ($n = 125$) for the effect of the rs4354668 polymorphism of the *SLC1A2* gene for EAAT2 on risky or harmful drug use (RHDU). After exploratory analysis we used logistic regression models to assess the probability of RHDU in individual groups.

Results. In the final model the T/T variant of rs4354668 was significantly associated with a lower probability of RHDU occurrence compared to the G/G variant (OR: 0.021; 95% CI: 0.001–0.275; $p = 0.009$). Other significant predictors of RHDU were smoking status and risky or harmful drinking of alcohol.

Conclusions. The results obtained may indicate a possible relationship of the risk of harmful drug use with variants of the rs4354668 polymorphism of the *SLC1A2* gene for EAAT2. Subjects with the T/T variant of this polymorphism appear to be less at risk of developing drug use disorders.

Key words: substance abuse, EAAT2, reward deficiency syndrome

Introduction

Substance addiction is a chronic mental disorder characterized by a persistent need to take psychoactive substances and loss of control over their use. Its etiopathogenesis involves complex interactions between genetic and environmental factors that cause abnormalities in the functioning of the brain circuits [1, 2]. Deficiencies in impulse control and learning are considered to be particularly important for the development of addictions (for example, addicts have increased reward dependence and reduced sensitivity to punishments) [3]. Addicted patients use drugs or engage in behavior related to psychoactive substances in a compulsive, maladaptive and sustained manner despite harmful health and life consequences [4]. Drug addiction is associated with an increased risk of mental disorders, cancer, infections, as well as cardiovascular, liver and lungs diseases [5]. It is also a significant risk factor for suicide [6]. Moreover, substance addiction is often associated with severe affective disorders and psychotic disorders [7, 8].

In 1996, Blum et al. [9] proposed a theoretical model to explain the neurophysiological basis of addiction and defined the so-called Reward Deficiency Syndrome (RDS) [9]. The alleged congenital dysfunction of the mesocorticolimbic pathways associated with decreased dopamine secretion would predispose to increased impulsivity, susceptibility to addiction, and to other disorders such as obesity [10]. The initial effect of enhancing the dependence on most psychoactive substances is related to the intensification of dopaminergic transmission in the nucleus accumbens, which is stimulated by the mesocorticolimbic pathways and is involved in the regulation of the reward system and motivational activities [2]. Such substance-induced enhancement of dopaminergic transmission may compensate for the functional dopamine deficiency associated with RDS and thus induce susceptible individuals to continue using the substance [10].

This model is consistent with the results of positron emission tomography (PET) studies, for example, in alcohol-dependent PET patients, a reduction in the availability of D2 and D3 receptors for dopamine in the putamen, ventral striatum and caudate nucleus was observed [11, 12]. Decreased dopaminergic transmission compared to healthy subjects also occurs in alcohol dependent patients in cortical regions including: dorsolateral prefrontal cortex, medial prefrontal cortex, orbitofrontal cortex, temporal cortex, and medial temporal lobe [13]. Similarly, in patients addicted to stimulant drugs such as amphetamines and cocaine, decreased dopamine burst and reduced availability of D2 and D3 receptors have been observed [14].

Glutamate, the main stimulating neurotransmitter of the central nervous system, plays a key role in the differentiation of neurons, responsible, among others, for the phenomenon of neuroplasticity related to learning and memory [15]. The nucleus accumbens and the striatum receive glutaminergic projections from the cortico-recumbent and cortico-striatal pathways, respectively, which enables higher cortical areas in the prefrontal cortex to control dopaminergic stimulation of the mesocorticolimbic pathways [16]. This control plays a role in regulation of drug-seeking behavior [2]. On the one hand, it may contribute to limiting the negative consequences of drug seeking, and on the other hand, to the potentialization of their impact on the dopaminergic system and behavior in response to circumstances and environmental influences that are associated in a learned manner with drug use [17, 18]. Reduction or blockage of glutamatergic transmission to the nucleus accumbens in an animal model induces increased substance seeking and exacerbates symptoms of addiction [2, 19]. Thus, a congenital reduction in the stimulatory effect of glutamatergic transmission on the dopaminergic system could represent an alternative phenotype of the reward deficiency syndrome to that associated with primary dopaminergic transmission deficiency [19].

Synaptic spillover of glutamate associated with its increased accumulation in the synapse may lead to excessive stimulation of non-synaptic glutamate receptors and, consequently, to an increase in oxidative stress exerting a cytotoxic effect on neurons and oligodendrocytes [20, 21]. The consequent disturbances in the structure of white matter have been associated with, among others, increased impulsivity and cognitive impairment in the course of bipolar disorder, and these features are also important in the etiopathogenesis of addiction [22-24]. As shown by meta-analyses, similar disorders of white matter integrity are also observed in patients addicted to cocaine and heroin [25, 26]. Moreover, genetic studies suggest the existence of polygenetic risk factors common to addiction and bipolar disorder [27]. For these reasons, a more detailed assessment of the role of glutamatergic disturbance in the etiopathogenesis of addiction, with particular emphasis on the mechanisms protecting against glutamate excitotoxicity, seems to be a promising research direction.

One of the main mechanisms of preventing glutamate excitotoxicity is its reuptake [28]. There are 5 types of excitatory amino acid transport (EAAT) proteins located in cells membranes: EAAT1, EAAT2, EAAT3, EAAT4 and EAAT5 [29]. EAAT2, which is encoded by the *SLC1A2* gene located on chromosome 11p13-12, is responsible for over 90% of glutamate reuptake and is expressed mainly in astrocytes, in about 10% in neurons, and also in other white matter cells such as oligodendrocytes [30-32]. Increased expression of *SLC1A2* in astrocytes has a neuroprotective effect under conditions of increased excitotoxicity, while heroin in an animal model lowers the expression of this gene and increases glutamate synaptic leakage, which mediates the time to re-initiate drug seeking [33-35]. Moreover, administration of ceftriaxone that increases *SLC1A2* expression in the nucleus accumbens reduces the seeking for cocaine and heroin in laboratory animals [35, 36]. This indicates the potential involvement of this transporter in the pathomechanism of addiction.

One of the *SLC1A2* polymorphisms that are the subject of research on mental disorders is *rs4354668* located 181 *bp* from the transcription site of the gene. This functional polymorphism is related to the substitution of T to G, which results in a 30% reduction in the activity of the *SLC1A2* promoter [32]. Previous studies have shown a beneficial effect of the T/T variant on cognitive dysfunction in schizophrenia, as well as on the frequency of relapses and white matter integrity disturbances in bipolar disorder [37-43]. In the case of schizophrenia, the protective effect of the T/T variant concerned executive functions and working memory, the impairment of which was confirmed meta-analytically in people abusing methamphetamine and cannabis, and in the case of executive functions also using ecstasy [44-48]. It has been also reported that white matter integrity disorders occur in people who abuse stimulants [49]. It is therefore possible that the beneficial effect of this *rs4354668* variant may be present in the case of substance abuse.

In summary, the higher expression of the *SLC1A2* gene encoding EAAT2, associated with the T/T variant *rs4354668*, may contribute to a more efficient glutamate reuptake, and thus its reduced excitotoxicity towards neurons whose activity is associated with dopaminergic stimulation within the reward system [16, 32, 33]. The other two variants (T/G and G/G) cause reduced EAAT2 expression, and thus less effective glutamate reuptake. Therefore, these variants can contribute to the loss of glutaminergic and dopaminergic stimulation and contribute to the occurrence of RDS predisposing to addiction [19, 20, 32]. Alternatively, the reduced glutamate concentrations persistent in the synaptic cleft in people with the T/T variant might be associated with less potentiating effects of psychoactive substances on the dopaminergic system and less contributing to the development of learned drug-seeking behavior than in people with T/G and G/G variants [17, 18, 32].

The aim of the presented study was to assess the relationship of *rs4354668* variants with the risk of psychoactive substance use disorders, as well as potential confounding factors affecting this relationship in a relatively homogeneous population of young adults studying at a medical university.

1. Material and methods

1.1. Study participants

The study was cross-sectional, exploratory and in two stages. In the first stage, the questionnaires were distributed among Polish-speaking students of the Pomeranian Medical University in Szczecin. The inclusion criteria for the study were: (1) consent for participation in the study and the processing of personal data, (2) age 18-30 years old, (3) complete and correct completion of the questionnaire of the first stage of the study, (4) providing correct contact information. The exclusion criteria from the study were: (1) failure to attend the second stage of the study to take blood samples for genetic testing, (2) diagnosed severe somatic diseases.

The upper age limit has been set to include people up to the upper quartile of the age of onset of disorders related to the use of cannabis, which is the most commonly used psychoactive substance other than alcohol in Poland [50, 51].

During the study, the following information was collected on the participants of the study: basic biometric data (age, sex, height, body weight, somatic diseases), demographic data (education level, basic information on the socioeconomic and health status of the family, number of siblings) lifestyle (smoking status, time spent on physical activity) and psychometric evaluations (Alcohol Use Disorder Identification Test – AUDIT, Drug Use Disorder Identification Test – DUDIT, Beck Depression Inventory – BDI).

Between March 1, 2021 and June 30, 2021, 427 (n = 427) questionnaires were distributed. Ultimately, 201 people (n = 201) returned correctly completed questionnaires and agreed to participate in the study. After the process of coding the questionnaires, a telephone contact was established in order to recruit for the second stage of the study, which involved taking a blood sample for genetic testing. The samples were collected from May 24 to July 9, 2021. Ultimately, 125 people (n = 125) took part in the second stage of the study, and their data were statistically analyzed.

All the described activities were performed in accordance with the Helsinki Declaration and approved by the local Bioethics Committee of the Pomeranian Medical University in Szczecin.

1.2. Psychometric tools

In the case of the study, it was decided to treat the use of alcohol and other psychoactive substances separately due to the existence of dedicated psychometric tools for alcohol use disorder and different legal status of alcohol in Poland. Completed AUDIT (Alcohol Use Disorder Identification Test), DUDIT (Drug Use Disorder Identification Test) and BDI-II (Beck Depression Inventory II) questionnaires were collected from all qualified participants. An AUDIT result equal to or higher than 6 for women, and for men – equal to or higher than 8 was assumed to suggest risky or harmful drinking of alcohol (RHDA) [52]. For DUDIT, a score of 2 or greater for women and 6 or greater for men, was considered to suggest risky or harmful drug use (RHDU) [53]. Risk of depression (RD) was considered present for participants who had a BDI-II score of 14 or more [54]. The respondents were provided with the maximum possible sense of security during the study. The participants completed the questionnaires on their own.

1.3. Genetic data

From each study participant (n = 125), 2.6 ml of peripheral blood was drawn. Genomic DNA was isolated using the Invisorb Spin Blood Mini Kit, Stratec Molecular. DNA samples were frozen until further analysis was carried out. Genotyping of DNA samples was performed using the real-time polymerase chain reaction (PCR) technique (Applied Biosystems StepOne) with TaqMan probes. Supplementary Table 1 shows a probe sequence and nomenclature that was used in the project (designed and synthesized by Applied Biosystems).

TaqMan Genotyping Master Mix was used in the study protocol. Each reaction contained 5 µl of master mix, 3.57 µl of nuclease-free water, 0.25 µl of twenty-fold diluted probe, and 1 µl of DNA, and 1 µl of nuclease-free water for the negative control. The PCR reaction profile was pre-denaturation at 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min.

1.4. Statistical Analysis

The statistical analysis was divided into the following stages: (1) comparison of the obtained values of continuous variables between the groups distinguished by stratification according to the *rs4354668* variant, (2) comparison of the obtained frequencies of categorical variables between the same groups, (3) comparison of the obtained values of continuous variables between the non-RHDU and RHDU groups, (4) comparison of the obtained frequencies of categorical variables between the same groups, (5) univariate logistic regression model with the occurrence status of RHDU as the dependent variable and the *rs4354668* variant as the independent variable, (6) multivariate logistic regression model with additional potential confounding factors that were significantly related to RHDU or *rs4354668* variants, (7) the fit of the multivariate logistic regression model with the highest goodness-of-fit to the data based on the Akaike information criterion (AIC). To compare the frequencies of the rs4354668 alleles in the research group with the frequencies expected in the case of the Hardy-Weinberg equilibrium, the X^2 test was used.

For continuous variables, medians, mean and standard deviation in individual groups were calculated as descriptive statistics. For categorical variables, the frequencies and the percentage share of the variable levels in individual groups were calculated.

In stages 1 and 3, the normality of the distribution in the groups was determined using the visual assessment of the histograms and the Shapiro-Wilk test. Additionally, the homogeneity of variance in the groups was assessed with the use of Levene's test.

In stage 1, in the case of meeting the assumption of homogeneity of variance and normality of distribution in groups, one-way analysis of variance (ANOVA) was used to assess differences between the groups. Otherwise, the Kruskal-Wallis test was used. In stage 3, if the assumptions were met, the Student's t-test was used, and if they were not met, the Mann-Whitney test. In stages 2 and 4, the X^2 test was used in the case of the frequency of occurrence of individual categorical variables in groups equal or greater than 5, while in the case of lower frequencies, Fisher's exact test was used. Due to the exploratory nature of the analysis, the *post-hoc* analysis was not performed in stages 1-4.

In step 6 of the analysis, in order to avoid the correlation between continuous variables and the corresponding categorical variables (e.g., total AUDIT score and AUDIT score above the cut-off point), only categorical variables were introduced into the model, which for the validated scales should be assumed to have higher predictive power.

All stages of the statistical analysis were performed with the use of R studio version 4.1.2. In stage 7, the glmulti package was used to find the model with the highest goodness-of-fit to the data. Candidate variables included all those that obtained a statistically significant relationship with RHDU or with the *rs4354668* variants. An exhaustive screening algorithm was used to check all possible combinations of variables included in the starter set without any interactions between them. AIC was used as a criterion for assessing goodness-of-fit and models with AIC not greater than 2 than the AIC of the model with the best goodnessof-fit to the data were presented in results. The model with the lowest number of predictors was then chosen as the model best fit for the data.

In the case of multivariate models, the multicollinearity was assessed using the generalized variance inflation factor (GVIF). For all logistic regression models, the odds ratios of individual predictors were also calculated, as well as the accuracy and precision of the prediction.

Due to the exploratory nature of the study and the lack of data on the prevalence of RHDU in the studied population, the statistical power of the analyses was assessed *post-hoc*. In order to take into account the influence of the other predictors included in the model on the obtained statistical power, the pseudoR^2 value was calculated using the Nagelkerke method. The G*Power program in version 3.1.9.7 was used for the calculations.

For all tests, $\alpha = 0.05$ was assumed. The obtained values of $p > 0.05$ and $p < 0.1$ were considered as the statistical trend.

2. Results

The frequencies of alleles in the studied population did not differ from those predicted on the basis of the Hardy-Weinberg equilibrium ($nG = 99$; $nT = 151$; $p = 0.579$).

The assessment of the normality of the distribution of variables in the groups distinguished according to the *rs4354668* variant is presented in Supplementary Table 2. The only variable for which the distribution did not differ from normal in all groups was age (G/G: $W = 0.962$, $p = 0.550$; T/G: $W = 0.970$, $p = 0.174$; T/T: $W = 0.205$, $p = 0.967$). In the case of this variable, the variance was additionally homoscedastic (F (2;122) = 0.942; $p = 0.393$). Therefore, for its further analysis, ANOVA was used, and in the case of other variables, the Kruskal-Wallis test was used. Table 1 presents a summary of descriptive statistics and a statistical analysis of the relationships of continuous variables with individual *rs4354668* genotypes. Initial data exploration showed no statistically significant relationship of any continuous variable with the *rs4354668* genotypes. However, a statistical trend was shown for the DUDIT score (X^2: 5.679; $p = 0.058$).

Table 1. **Comparison of medians or means of continuous variables included in the study by groups distinguished on the basis of *rs4354668* variants**

Variable	G/G *n* = 21 Median (Mean ± SD)	T/G *n* = 57 Median (Mean ± SD)	T/T *n* = 47 Median (Mean ± SD)	*F/X*	*p*
Age	23.833 (23.722 ± 1.976)	23.833 (23.452 ± 2.385)	23.167 (23.576 ± 2.128)	0.121[a]	0.886
BMI	21.604 (22.199 ± 3.944)	21.671 (22.006 ± 2.969)	21.968 (22.811 ± 3.751)	0.975[b]	0.614
Cigarettes/day	< 0.001 (1.333 ± 3.773)	< 0.001 (0.877 ± 3.301)	< 0.001 (1.638 ± 4.12)	1.600[b]	0.449
Hours of exercise/week	3.000 (2.905 ± 1.814)	3.000 (3.772 ± 3.063)	3.000 (3.553 ± 3.074)	0.503[b]	0.778
Number of siblings	1.000 (1.286 ± 0.784)	1.000 (1.193 ± 1.076)	1.000 (1.213 ± 0.858)	1.117[b]	0.572
AUDIT	5.000 (5.381 ± 3.748)	3.000 (4.632 ± 3.917)	5.000 (5.191 ± 3.728)	1.389[b]	0.499
DUDIT	< 0.001 (0.952 ± 1.802)	< 0.001 (0.684 ± 1.814)	< 0.001 (0.426 ± 2.234)	5.679[b]	*0.058*
BDI	9.000 (10.714 ± 7.798)	9.000 (10.526 ± 8.279)	6.000 (8.638 ± 7.903)	2.297[b]	0.317

The number in front of the parentheses represents the median, and numbers in the parentheses represent the mean ± standard deviation. BMI – body mass index; AUDIT – Alcohol Use Disorders Identification Test; DUDIT – Drug Use Disorders Identification Test; BDI – Beck Depression Inventory; *n* – group size; SD – standard deviation; *F* – Fisher statistics; *p* – *p* value, X^2 – Chi square; [a] ANOVA – analysis of variance was used; [b] – the Kruskal-Wallis test was used. The *p* values corresponding to the statistical trend are in *italics*.

Table 2 presents the frequency of occurrence of the levels of the main categorical variables and their percentage share in the groups as well as the statistical analysis of the relationships with individual *rs4354668* variants. Supplementary Table 3 presents similar information on the additional categorical variables included in the study. Initial data exploration showed a statistically significant relationship between the *rs4354668* variants and RHDU ($p = 0.030$).

Table 2. **Comparison of frequencies of the main categorical variables and their percentage share in particular groups distinguished on the basis of *rs4354668* variants**

Variable	G/G *n* = 21	%G/G	T/G *n* = 57	%T/G	T/T *n* = 47	% T/T	X^2	*p*
Female	16	76.19	38	66.67	32	68.09	0.666	0.717
Male	5	23.81	19	33.33	15	31.91	0.666	-
Diagnosed mental disorders	4	19.05	12	21.05	7	14.89	-	0.712
Smoking	3	14.29	6	10.53	9	19.15	-	0.490
RHDA	8	38.10	15	26.32	17	36.17	1.581	0.454
RHDU	4	19.05	8	14.04	1	2.13	-	**0.030**
RD	7	33.33	17	29.82	12	25.53	0.484	0.785

n = group size; X^2 – Chi square; *p* – *p*-value; RHDA – risky or harmful drinking of alcohol; RHDU – risky or harmful drug use; RD – risk of depression. The *p* values corresponding to statistical significance are in **bold**.

The assessment of the normality of the distribution of variables in the non-RHDU and RHDU groups is presented in Supplementary Table 4. The only variable for which the distribution did not differ from the normal in both groups was age. In the case of this variable, the variance was additionally homoscedastic (F (1;123) = 1; p = 0.393). Therefore, for its further analysis, the *t*-test was used, and in the case of other variables, the Mann-Whitney test was used. Table 3 presents a summary of descriptive statistics and statistical analysis of the relationship of continuous variables with RHDU. Initial data exploration showed a statistically significant relationship between the RHDU and cigarettes/day (U = 348; p <0.001), the AUDIT score (U = 370.5; p = 0.004) and the BDI score (U = 0.017; p = 0.017).

Table 3. **Comparison of medians or means of continuous variables included in the study by groups distinguished on the basis of occurrence of risky or harmful drug use**

Variable	non-RHDU *n* = 112 Median (Mean ± SD)	RHDU *n* = 13 Median (Mean ± SD)	*t/U*	*p*
Age	23.417 (23.501 ± 2.256)	24.000 (23.917 ± 1.806)	– 0.764[a]	0.455
BMI	21.787 (22.428 ± 3.439)	21.358 (21.597 ± 3.516)	832.500[b]	0.400
Cigarettes/day	< 0.001 (0.795 ± 3.044)	2.000 (5.077 ± 6.116)	348.000[b]	**<0.001**
Hours of exercise/week	3.000 (3.656 ± 2.915)	2.000 (2.577 ± 2.597)	905.000[b]	0.150

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Number of siblings	1.000 (1.214 ± 0.895)	1.000 (1.231 ± 1.363)	807.500[b]	0.473
AUDIT	4.000 (4.643 ± 3.666)	7.000 (7.769 ± 3.94)	370.500[b]	**0.004**
BDI	7.000 (9.259 ± 7.804)	12.000 (14.923 ± 8.684)	431.500[b]	**0.017**

The number in front of the parentheses represents the median, and numbers in parentheses represent the mean ± standard deviation. RHDA – risky or harmful drinking of alcohol; RHDU – risky or harmful drug use; RD – risk of depression; BMI – body mass index; AUDIT – Alcohol Use Disorders Identification Test; DUDIT – Drug Use Disorders Identification Test; BDI – Beck Depression Inventory; *n* – group size; SD – standard deviation; *t* – *t* statistic; U – *U* statistic; *p* – *p*-value, [a] – *t*-test was used; [b] – Mann-Whitney test was used. The *p* values corresponding to the statistical significance are in **bold**.

Table 4 presents frequency of occurrence of levels of the main categorical variables and their percentage share in groups as well as the statistical analysis of the relationships with RHDU. Similar information for additional variables included in the study is presented in Supplementary Table 5. Initial data analysis showed a statistically significant relationship between RHDU and the diagnosis of mental disorders (OR = 6.837; 95% CI: 1.726 – 28.265; $p = 0.002$), smoking status (OR = 15.630; 95% CI: 3.729 – 73.743; $p < 0.001$), RHDA (OR = 5.783; 95% CI: 1.483 – 27.616; $p = 0.004$), and the statistical trend for prevalence of hypothyroidism (OR = 3.339; 95% CI: 0.658 – 14.293; $p = 0.078$).

Table 4. **Comparison of frequencies of the main categorical variables and their percentage share in particular groups distinguished on the basis of occurrence of risky or harmful drug use**

Variable	non-RHDU *n* = 112	% non-RHDU	RHDU *n* = 13	% RHDU	OR	95% CI	*p*
Female	75	66.96	11	84.62	0.371	0.038 – 1.833	0.342
Male	37	33.04	2	15.38	-	-	-
Diagnosed mental disorders	16	14.29	7	53.85	6.837	1.726 – 28.265	**0.002**
Hypothyroidism	13	11.61	4	30.77	3.339	0.658 – 14.293	*0.078*
Smoking	10	8.93	8	61.54	15.63	3.729 – 73.743	**< 0.001**
RHDA	31	27.68	9	69.23	5.783	1.483 – 27.616	**0.004**
RD	30	26.79	6	46,15	2.325	0.594 – 8.825	0.194

n = group size; OR – odds ratio; 95% CI – 95% confidence interval; *p* – *p*-value; RHDA – risky or harmful drinking of alcohol; RHDU – risky or harmful drug use; RD – risk of depression. The *p* values corresponding to statistical significance are in **bold** and those corresponding to statistical trend are in *italics*.

In the univariate logistic regression model, the T/T *rs4354668* variant was statistically significantly associated with a lower probability of RHDU occurrence compared to the G/G variant (OR: 0.092; 95% CI: 0.005-0.678; $z = -2.065$; $p = 0.039$), while the participants with the T/G variant did not differ from those with the G/G variant (OR: 0.694; 95% CI: 0.192-2.865; $z = -0.542$; $p = 0.588$). To put it differently, participants with the T/T variant had an average 90.8% lower chance of developing RHDU than participants with the G/G variant. Table 5 presents the model statistics in more detail. Figure 1 shows a plot of the odds ratios for this model.

Table 5. **Summary of statistics of the univariate logistic regression model with the G/G variant as the indicator level**

Predictor	*β*	SD	*z*	*p*	OR	OR 95% CI
Variant G/G	-1.447	0.556	-2.604	**0.009**	–	–
Variant T/G	-0.366	0.674	-0.542	0.588	0.694	0.192 – 2.865
Variant T/T	-2.382	1.154	-2.065	**0.039**	0.092	0.005 – 0.678

β – estimated *β* coefficient; SD – standard deviation; *z* – Wald *z* statistics; *p* – *p*-value; OR – odds ratio; OR 95% CI – odds ratio confidence interval. The *p* values corresponding to statistical significance are in **bold**.

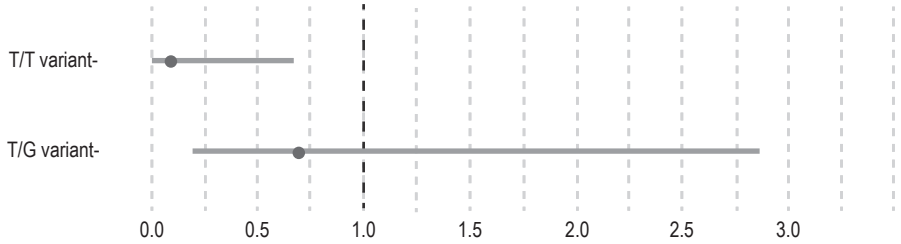


Figure 1. **Odds ratio plot for logistic regression model with variants of rs4354668 as predictor**

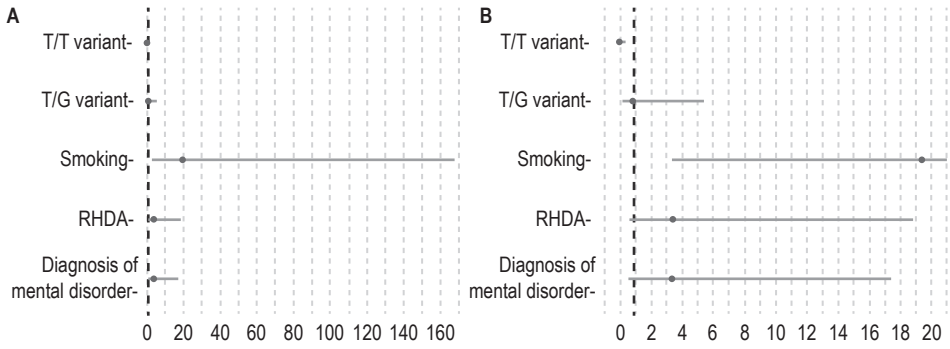
In the multivariate logistic regression model taking into account potential confounders, for which a statistically significant relationship with the *rs4354668* variants or RHDU was demonstrated at earlier stages of the analysis, the statistically significant effect of the T/T variant on the incidence of RHDU was maintained. The statistical significance of this predictor increased and the associated odds ratio decreased (OR: 0.037; 95% CI: 0.001-0.433; $z = -2.336$; $p = 0.019$). The second highly significant predictor was smoking status (OR: 19.301; 95% CI: 3.336–168.063; $z = 3.064$; $p = 0.002$), while the diagnosis of any mental disorder (OR: 3.392; 95% CI: 0.585-17.425; $z = 1.458$; $p = 0.145$), RHDA (OR: 3.391; 95% CI: 0.633-18.804; $z = 1.458$; $p = 0.145$) and T/G variant (OR: 0.785; 95% CI: 0.134–5.461; $z = -0.265$; $p = 0.791$) did not reach statistical significance. The model was also statistically significantly

better fitted to the data compared to the univariate model (respectively: AIC = 59.821 and AIC = 82.368). However, accounting for the small size of the research sample, as well as the number of predictors included, overfitting of the model and incorrect estimation of its parameters cannot be ruled out. These problems were addressed in the next stage of the analysis. Table 6 presents the detailed statistics of the model, while Supplementary Table 6 presents the GVIF values of each predictor. The GVIF values did not indicate the occurrence of multicollinearity. Figure 2 shows a plot of the odds ratios for this model.

Table 6. **Summary of statistics for the multivariate logistic regression model including all potentially significant confounders with the G/G variant as the indicator level**

Predictor	*β*	SD	*z*	*p*	OR	OR 95% CI
Intercept	-3.107	0.939	-3.309	**0.001**	–	–
Variant T/G	-0.242	0.915	-0.265	0.791	0.785	0.134 – 5.461
Variant T/T	-3.295	1.410	-2.336	**0.019**	0.037	0.001 – 0.433
Diagnosed mental disorders	1.222	0.838	1.458	0.145	3.392	0.585 – 17.425
Smoking	2.960	0.966	3.064	**0.002**	19.301	3.336 – 168.063
RHDA	1.221	0.838	1.458	0.145	3.391	0.633 – 18.804

β – estimated *β* coefficient; SD – standard deviation; *z* – Wald *z* statistics; *p* – *p*-value; OR – odds ratio; OR 95% CI – odds ratio confidence interval; RHDA – risky or harmful drinking of alcohol. The *p* values corresponding to statistical significance are in **bold**.



A) Full scope. B) Cropped to more accurately show predictors other than smoking.

Figure 2. **Odds ratio plot for logistic regression model with variants of rs4354668 and potential confounding factors as predictors**

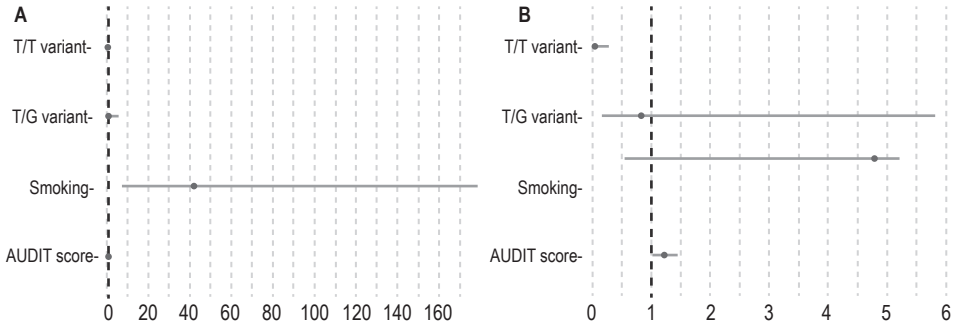
The algorithm for selecting the best-fit model with the use of glmulti selected 8 models that did not differ in the AIC value from the best-fit model by more than 2. Supplementary Table 7 provides a summary of the predictors included in these models

and their AIC values. Each of the models contained the *rs4354668* variant as a predictor, which might indicate that it was statistically significant regardless of the confounding factors included. Only one of the models obtained contained 3 predictors and therefore it was considered the best fit for the data. The statistics of this model are summarized in Table 7. Statistically significant predictors were the T/T variant (OR: 0.021; 95% CI: 0.001-0.275; $z = -2.618$; $p = 0.009$), smoking status (OR: 42.446; 95% CI: 7.919-359.472; $z = 3.982$; $p < 0.001$) and RHDA (OR: 1.200; 95% CI: 1.009-1.435; $z = 2.117$; $p = 0.034$). An insignificant predictor was the T/G variant (OR: 0.823; 95% CI: 0.137-5.819; $z = -0.211$; $p = 0.833$). Thus, it can be assumed that, when controlling for confounding factors, participants in the study with the T/T rs435466 variant had an average 97.90% lower risk of RHDU than participants with the G/G variant. A summary of the GVIF values for the model is presented in the Supplementary Table 8. Figure 3 shows a plot of the odds ratios for this model.

Table 7. **Summary of statistics for the multivariate logistic regression model that, after adjusting for some potential confounders, was the best fit for the data**

Predictor	*β*	SD	*z*	*p*	OR	OR 95% CI
Intercept	-3.505	1.027	-3.412	**0.001**	–	–
Variant T/G	-0.195	0.924	-0.211	0.833	0.823	0.137 – 5.819
Variant T/T	-3.858	1.473	-2.618	**0.009**	0.021	0.001 – 0.275
Smoking	3.748	0.941	3.982	**< 0.001**	42.446	7.919 – 359.472
AUDIT score	0.182	0.086	2.117	**0.034**	1.200	1.009 – 1.435

In the case of genotypes, the G/G variant was used as a reference point for calculating the odds ratios, while in the case of the AUDIT scores, the AUDIT score = 1 was used. *β* – the estimated *β* coefficient; SD – standard deviation; *z* – Wald *z* statistics; *p* – *p*-value; OR – odds ratio; OR 95% CI – odds ratio confidence interval; AUDIT –



A) Full scope. B) Cropped to more accurately show predictors other than smoking.

Figure 3. **Odds ratio plot for logistic regression best-fit model with variants of rs4354668 and potential confounding factors as predictors**

Alcohol Use Disorders Identification Test. The *p* values corresponding to statistical significance are in **bold**.

The obtained statistical power was calculated for the last presented model. Considering that the value of pseudoR^2 = 0.393 for the remaining predictors, the obtained statistical power was very low (1-β = 0.062). Accounting for the odds ratio obtained in the study, the frequency of RHDU in groups distinguished on the basis of the *rs4354668* variants and the impact of other predictors, the minimum sample size for obtaining a statistical power of 0.8 was n = 433.

3. Discussion

To our knowledge, the presented study is the first study of the potential impact of variants of the *rs4354668* polymorphism of the *SLC1A2* gene on risky or harmful drug use (RHDU). The presented results may indicate the potential protective effect of the T/T variant against the occurrence of such disorders. Both the exploratory analysis and the univariate logistic regression model showed a relationship between the T/T variant and a lower incidence of RHDU. Moreover, the individual *rs4354668* variants were not statistically significantly associated with any of the potential confounding factors taken into account, including the diagnosis of mental disorders, the risk of depression and the risk of alcohol use disorder. Also controlling for the variables associated with RHDU itself not only did not reduce the statistical significance of the relationship between the T/T variant and the less frequent occurrence of RHDU but even significantly improved it. This relationship is therefore independent of the diagnosis of mental disorders, RHDA, smoking status, AUDIT and BDI scores, and the number of cigarettes smoked per day. Based on the model with the best fit for the obtained data, we estimated that individuals with the T/T variant of the *rs4354668* polymorphism of the *SLC1A2* gene for EAAT2 had an average 97.90% lower probability of the occurrence of RHDU.

Our results are consistent with the research conducted so far on the role of *rs4354668* in other mental disorders. Patients with schizophrenia with the T/T *rs4354668* variant showed lower cognitive deficits associated with the prefrontal cortex, as well as a more significant improvement due to treatment [39, 40, 42, 55]. Similarly, in the case of bipolar disorder, patients with the T/T variant treated with lithium salts had a lower frequency of relapses of the disease [37]. Moreover, subjects diagnosed with bipolar disorder who have the T/T variant are less sensitive to the adverse impact of stress experienced in early life on the integrity of white matter [41]. Also, in a recent study on the Polish population of patients diagnosed with a depressive disorder, it was shown that the G/G *rs4354668* variant, associated with lower EAAT2 expression, is more frequent in the group of patients than in the control group, which indicates that *SLC1A2* polymorphisms may contribute to increased risk of this disorder [56]. Although in the studied population we did not detect a statistically significant relationship between the *rs4354668* polymorphism and the BDI scores or the diagnosis of mental disorders, we

did not control the results in terms of risk states of psychosis, hypomania and early stress, which in themselves may be associated with a higher risk of addiction [57-59]. It cannot therefore be ruled out that this polymorphism did not contribute directly to the occurrence of RHDU, but is connected to a phenotype susceptible to schizophrenia, bipolar disorder or the effects of early-life stress. Alternatively, our results may point to a partially common neurobiological basis of these disorders and of RHDU.

Other statistically significant predictors of RHDU in the studied population were the AUDIT score and smoking status. Previous research suggests that earlier alcohol consumption may increase the likelihood of using other psychoactive substances in the future [60-62]. In the case of laboratory animals, it has also been shown that both alcohol and nicotine can increase the expression of dopamine receptors (but not glutamate receptors) and affect the subsequent sensitivity to the addictive effects of amphetamine [63]. Smoking tobacco itself is also associated with more frequent abuse of psychoactive substances and a tendency to relapse of their abuse [64]. Smokers not only abuse psychoactive substances more often, but also suffer from depressive disorders more frequently [65, 66]. In turn, the comorbidity of nicotinism and bipolar disorder is not only common, but also these disorders share genetic underpinnings [67]. Such results are in line with our findings showing that smoking and the AUDIT score are better predictors of RHDU than RD alone or the BDI score. Moreover, the positive effect of controlling for smoking status on the goodness-of-fit of the logistic regression model may be related to the fact that the promoting influence of nicotine on the use of other psychoactive substances is mainly mediated by the dopaminergic system, and not by the glutamatergic system [63]. Thus, participants who were influenced by co-morbid alcohol abuse, depressive symptoms or smoking may be partially separate from those for whom the effect of specific *rs4354668* variants and their associated RDS was more significant. This hypothesis seems to be confirmed by the lack of a statistically significant relationship between *rs4354668* variants and factors other than RHDU, obtained in our analysis.

Reward dependence is a personality trait defined by Cloninger associated with low noradrenergic and dopaminergic activity, which manifests itself as “resistance to extinction of previously rewarded behavior” [68]. Although the results of studies on the role of reward dependence in addiction are inconclusive, some of them suggest that its low level may be associated with opioid abuse [69-71]. Moreover, it has been suggested that high reward dependence may have a protective effect against addiction in opioids users as well as against relapse of alcohol dependence [72, 73]. The T/T *rs4354668* variant seems to affect reward dependence and people with this genotype achieve lower reward dependence values [74]. Based on this, people with the T/T variant should be more prone to substance abuse, which is not in line with our results. This inconsistency may be due to: 1) different relationships between reward dependence and substance abuse, which we did not control in our study, or 2) the lack of a significant relationship between reward dependence and RHDU in the study population. It can also be hypothesized that due to the recruitment of the research group among medical

students, people with the T/T variant who became addicted were not able to start or continue their studies, and therefore the research group included participants with the T/T variant belonging mostly to the non-RHDU group. Additional research is required to further evaluate possible relationships between substance abuse, *rs4354668* variants, and personality traits.

Glutamate synaptic spillover is one of the important mechanisms of several psychiatric disorders, including schizophrenia, depression, addiction and neurodegenerative diseases [21, 29]. Increased spillover may result in non-specific activation of glutamate receptors, which are crucial for neuroplasticity, and thus for memory and learning processes [2, 75]. The long-term effect of glutamate spillover on neuroplasticity in the nucleus accumbens contributes to the risk of addiction relapse and the persistence of its symptoms [2]. Decreased activity of the main glutamate transporter, EAAT2, may intensify this process, while increased activity may have a potentially protective effect [76]. In addition, increased glutamate spillover contributes to the intensification of oxidative stress, which causes apoptosis of nervous tissue cells. In the case of neurodegenerative diseases, an analogous effect of EAAT2 on this mechanism has been proven [21].

The results obtained by us seem to fit the assumed model, in which increased glutamate reuptake in people with the T/T rs4354668 variant contributes to a weaker association of psychoactive substances with learned behaviors and environmental stimuli or to more effective protection against glutamate excitotoxicity leading to changes in neuroplasticity which result in insufficient stimulation of the dopaminergic system associated with the reward deficiency syndrome [17, 18, 32, 33, 77]. Such individuals may have less need to compensate for dopamine deficiency in the mesocorticolimbic pathways and thus be less likely to develop RHDU.

The presented study has certain limitations. The study group, consisting almost entirely of medical students, may not be representative of the general adult population. Moreover, there was a time gap between the first stage, in which psychometric, sociodemographic and lifestyle information was collected, and the second stage, in which genetic material was acquired. This could affect the reliability of the data obtained from the AUDIT, DUDIT and BDI scales. The collected questionnaires were also not anonymous, which, taking into account the legal status of almost all psychoactive substances in Poland, except alcohol, could have influenced the honesty of the answers given by the participants. For a similar reason, we did not collect information on the types of substances used by the participants and the presence of specific psychiatric diagnoses, which prevented a more detailed analysis of their effects on RHDU. What is particularly important, all the psychometric methods used were based on self-assessment and the participants were not examined by psychiatric specialists, which may also contribute to the lower quality of the data analyzed in the study. We also did not control the results for the presence of other symptoms of mental illness, such as anxiety, high risk of psychosis or hypomania. Due to the very limited number of current, validated in Polish personality assessment tools, we were also unable to

correct the obtained results in terms of such personality traits as impulsivity, novelty seeking, harm avoidance or reward dependence.

The most significant limitation of the presented study is the sample size. Due to the lack of data on the prevalence of RHDU in the studied population and the exploratory nature of the study, we were unable to estimate the required sample size *a priori*. The statistical power obtained was ultimately very small ($1\text{-}\beta = 0.062$); however, we were able to estimate the sample size for future studies with similar objectives and study populations at $n = 433$. Although the size of the research sample allowed to obtain statistically significant results, the multivariate type of the analysis performed could make it impossible to detect weaker relationships. The analysis also did not take into account potential interactions between the variables included in the analysis.

4. Conclusions

The results obtained in the presented study may indicate a possible relationship of the risk of abuse of psychoactive substances other than alcohol with variants of the *rs4354668* polymorphism of the *SLC1A2*. People with the T/T variant of this polymorphism appear to be less at risk of developing drug use disorders. This may indirectly confirm the suspected role of EAAT2 in the etiopathogenesis of addictions and the role of the glutamatergic system in shaping the phenotype of reward deficiency syndrome. Future, prospective studies based on thorough analysis are necessary to confirm the cause-and-effect nature of the observed relationships.

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Address: Piotr Podwalski
Department of Psychiatry, Pomeranian Medical University
71-460 Szczecin, Broniewskiego Street 26
e-mail: piotr.podwalski@pum.edu.pl

ANNEX: SUPPLEMENTARY TABLES

SUPPLEMENTARY TABLE 1: **Probes used in the study**

Gene	VIC/AM	Sequence	NCBI SNP nomenclature	ID
SLC1A2	T/G	GCGCGTGTGCGGGTGTGTGCGCGCC[T/G] GGGGAGGCGGTGGAGGCCGCTGCGC	rs4354668	C__27142767_20

SUPPLEMENTARY TABLE 2: **Assessment of the normality of the distributions of the values of continuous variables in groups distinguished by variant of rs4354668**

Variable	G/G *p* (*W*) *n* = 21	T/G *p* (*W*) *n* = 57	T/T *p* (*W*) *n* = 47
Age	0.550 (0.962)	0.174 (0.970)	0.205 (0.967)
BMI	0.089 (0.921)	0.170 (0.970)	< 0.001 (0.819)
Cigarettes/day	< 0.001 (0.417)	< 0.001 (0.298)	< 0.001 (0.462)
Hours of exercise/week	0.214 (0.940)	< 0.001 (0.849)	< 0.001 (0.870)
Number of siblings	0.001 (0.800)	< 0.001 (0.794)	< 0.001 (0.733)
AUDIT	0.171 (0.935)	< 0.001 (0.833)	0.005 (0.926)
DUDIT	< 0.001 (0.600)	< 0.001 (0.439)	< 0.001 (0.193)
BDI	0.058 (0.911)	0.001 (0.923)	< 0.001 (0.883)

n = group size; *W* – *W* statistic; *p* – *p*-value; BMI – body mass index; AUDIT – Alcohol Use Disorders Identification Test score; DUDIT – Drug Use Disorder Identification Test score; BDI – Beck Depression Inventory score.

SUPPLEMENTARY TABLE 3: **Comparison of the frequencies of the additional categorical variables and their percentage share in groups distinguished on the basis of rs4354668 variants**

Variable	GG *n* = 21	% GG	TG *n* = 57	% TG	TT *n* = 47	% TT	X^2	*p*
Cohabitation with the family	9	42.86	21	36.84	17	36.17	0.302	0.860
Complete family	19	90.48	46	80.70	41	87.23	-	0.565
Both parents alive	21	100	53	92.98	46	97.87	-	0.394
Parent residing abroad	0	0.00	8	14.04	6	12.77	-	0.219
Parent working abroad	2	9.52	10	17.54	7	14.89	-	0.715
Unemployed parent	2	9.52	12	21.05	6	12.77	-	0.394
No siblings	2	9.52	13	22.81	6	12.77	-	0.300
Hypothyroidism	3	14.29	9	15.79	5	10.64	-	0.780

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Irritable bowel syndrome	2	9.52	4	7.02	1	2.13	-	0.282
Asthma	1	4.76	3	5.26	2	4.26	-	1.000
Acne	3	14.29	10	17.54	9	19.15	-	0.952
Obese relative	6	28.57	12	21.05	6	12.77	-	0.259
Pre-diabetes in a relative	3	14.29	2	3.51	4	8.51	-	0.234
Type 2 diabetes in a relative	2	9.52	5	8.77	2	4.26	-	0.650
Hypercholesterolemia in a relative	8	38.10	14	24.56	11	23.40	1.795	0.408
Hypertriglyceridemia in a relative	2	9.52	6	10.53	4	8.51	-	1.000
Depression in a relative	2	9.52	9	15.79	13	27.66	-	0.180
Alcoholism in a relative	2	9.52	10	17.54	7	14.89	-	0.715
Any mental disorder in a relative	3	14.29	17	29.82	17	36.17	3.338	0.188
Secondary education	20	95.24	52	91.23	41	87,23	-	0.667
Higher education	1	4.76	5	8.77	6	12,77	-	-
Mother's primary education	1	4.76	1	1.75	0	0	-	0.903
Mother's secondary education	4	19.05	12	21.05	9	19.15	-	-
Incomplete higher education of the mother	1	4.76	5	8.77	4	8.51	-	-
Higher education of the mother	15	71.43	39	68.42	34	72.34	-	-
Father's primary education	1	4.76	1	1.75	1	2.13	-	0.884
Father's secondary education	3	14.29	9	15.79	10	21.28	-	-
Incomplete higher education of the father	5	23.81	11	19.30	7	14.89	-	-
Father's higher education	12	57.14	36	63.16	29	61.7	-	-

n = group size; X^2 – Chi square; *p* = *p*-value; RHDA – risky or harmful drinking of alcohol; RHDU – risky or harmful drug use; RD – risk of depression

SUPPLEMENTARY TABLE 4: **Assessment of the normality of the distributions of the values of continuous variables in groups distinguished by occurrence of risky or harmful drug use**

Variable	**non-RHDU *p* (*W*) *n* = 112**	**RHDU *p* (*W*) *n* = 13**
Age	0.094 (0.980)	0.337 (0.930)
BMI	< 0.001 (0.896)	0.558 (0.947)
Cigarettes/day	< 0.001 (0.290)	0.011 (0.817)
Hours of exercise/week	< 0.001 (0.859)	0.072 (0.880)

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Number of siblings	< 0.001 (0.803)	< 0.001 (0.688)
AUDIT	< 0.001 (0.877)	0.941 (0.974)
BDI	< 0.001 (0.901)	0.690 (0.956)

RHDU – risky or harmful drug use; *n* = group size; *W* – *W* statistic; *p* – *p*-value; BMI – body mass index; AUDIT – Alcohol Use Disorders Identification Test score; DUDIT – Drug Use Disorder Identification Test score; BDI – Beck Depression Inventory score.

SUPPLEMENTARY TABLE 5: **Comparison of the frequencies of the additional categorical variables and their percentage share in groups distinguished on the basis of occurrence of risky or harmful drug use**

Variable	**non-RHDU *n* = 112**	**%**	**RHDU *n* = 13**	**%**	**OR**	**95% CI**	***p***
Cohabitation with the family	45	40.18	2	15.38	0.273	0.028 – 1.340	0.129
Complete family	96	85.71	10	76.92	0.559	0.124 – 3.496	0.416
Both parents alive	108	96.43	12	92.31	0.448	0.040 – 23.691	0.428
Parent residing abroad	12	10.71	2	15.38	1.509	0.146 – 8.291	0.639
Parent working abroad	18	16.07	1	7.69	0.437	0.010 – 3.322	0.690
Unemployed parent	19	16.96	1	7.69	0.410	0.009 – 3.099	0.691
No siblings	18	16.07	3	23.08	1.560	0.252 – 6.912	0.457
Irritable bowel syndrome	6	5.36	1	7.69	1.467	0.030 – 13.823	0.546
Asthma	5	4.46	1	7.69	1.773	0.035 – 17.915	0.490
Acne	21	18.75	1	7.69	0.363	0.008 – 2.721	0.462
Obese relative	20	17.86	4	30.77	2.031	0.415 – 8.223	0.273
Pre-diabetes in a relative	7	6.25	2	15.38	2.697	0.245 – 16.794	0.236
Type 2 diabetes in a relative	7	6.25	2	15.38	2.697	0.245 – 16.794	0.236
Hypercholesterolemia in a relative	29	25.89	4	30.77	1.270	0.265 – 4.99	0.743
Hypertriglyceridemia in a relative	11	9.82	1	7.69	0.767	0.016 – 6.214	1.000
Depression in a relative	21	18.75	3	23.08	1.297	0.211 – 5.657	0.714
Alcoholism in a relative	16	14.29	3	23.08	1.790	0.286 – 8.041	0.416
Any mental disorder in a relative	32	28.57	5	38.46	1.557	0.372 – 5.885	0.525
Secondary education	102	91.07	11	84.62	-	-	0.362

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Higher education	10	8.93	2	15.38	-	-	-
Mother's primary education	2	1.79	0	0	-	-	0.826
Mother's secondary education	22	19.64	3	23.08	-	-	-
Incomplete higher education of the mother	10	8.93	0	0	-	-	-
Higher education of the mother	78	69.64	10	76.92	-	-	-
Father's primary education	3	2.68	0	0	-	-	1.000
Father's secondary education	20	17.86	2	15.38	-	-	-
Incomplete higher education of the father	21	18.75	2	15.38	-	-	-
Father's higher education	68	60.71	9	69.23	-	-	-

n = group size; OR – odds ratio; 95% CI – 95% confidence interval; *p* – *p*-value; RHDA – risky or harmful drinking of alcohol; RHDU – risky or harmful drug use; RD – risk of depression.

SUPPLEMENTARY TABLE 6: **Summary of the values of the generalized variance inflation coefficient (GVIF)**

Predictor	**GVIF**	**df**	**GVIF** $^{(1 / (2 * df))}$
Variant of *rs4354668*	1.299	2	1.068
Diagnosed mental disorders	1.065	1	1.032
Smoking	1.338	1	1.157
RHDA	1.129	1	1.062

$GVIF^{(1/(2*df))}$ values below 2 indicate that the model does not have predictor multicollinearity. RHDA – risky or harmful drinking of alcohol; df – degrees of freedom.

SUPPLEMENTARY TABLE 7: **AIC values of models selected by the *glmulti* algorithm that did not differ from the model with the highest AIC value by more than 2**

Predictors	**AIC**
Variant of *rs4354668* + diagnosed mental disorders + smoking + AUDIT score	57.461
Variant of *rs4354668* + smoking + AUDIT score	**57.557**
Variant of *rs4354668* + smoking + AUDIT score + BDI score	58.007
Variant of *rs4354668* + diagnosed mental disorders + smoking + AUDIT score + BDI score	58.703
Variant of *rs4354668* + smoking + AUDIT score + cigarettes/day	59.314

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Variant of *rs4354668* + diagnosed mental disorders + smoking + AUDIT score + cigarettes/day	59.348
Variant of *rs4354668* + diagnosed mental disorders + RHDA + smoking + AUDIT score	59.389
Variant of *rs4354668* + RHDA + smoking + AUDIT score + BDI score	59.398

AIC – Akaike information criterion; AUDIT – Alcohol Use Disorders Identification Test; BDI – Beck Depression Scale; RHDA – risky or harmful drinking of alcohol. The model considered to be the best fit for the data is **bolded**.

SUPPLEMENTARY TABLE 8: **Summary of generalized variance inflation coefficient (GVIF) values for the best-fit model**

Predictor	**GVIF**	**df**	**GVIF** $^{(1 / (2 * df))}$
Variant of *rs4354668*	1.320	2	1.072
Smoking	1.307	1	1.143
AUDIT score	1.021	1	1.011

GVIF $^{(1 / (2 * df))}$ values below 2 indicate that the model does not have predictor multicollinearity. df – degrees of freedom; AUDIT – Alcohol Use Disorders Identification Test.