Original article

Gene variants and serum levels of synaptic vesicle and presynaptic plasma membrane proteins in alcohol dependence and their relationship with impulsivity and temperament

Güliz Şenormancı[1]
https://orcid.org/0000-0001-8000-0075
Çetin Turan[1]
https://orcid.org/0000-0002-5259-6112
Sevim Karakaş Çelik[2]
https://orcid.org/0000-0003-0505-7850
Aycan Çelik[3]
https://orcid.org/0000-0003-0151-4081
Tuba Gökdoğan Edgünlü[4]
https://orcid.org/0000-0002-9300-9324
Ceren Bilgi[5]
https://orcid.org/0000-0002-0198-6401
Ayşe Semra Demir Akca[6]
https://orcid.org/0000-0002-2092-656X
Ömer Şenormancı[1]*
https://orcid.org/0000-0002-1407-4911

[1]Department of Psychiatry, University of Health Sciences Bursa Yuksek Ihtisas Training and Research Hospital, Bursa, Turkey
[2]Department of Medical Genetics, School of Medicine, Zonguldak Bulent Ecevit University, Zonguldak, Turkey
[3]Department of Molecular Biology and Genetics, Faculty of Sciences and Arts, Zonguldak Bülent Ecevit University, Zonguldak, Turkey
[4]Department of Medical Biology, School of Medicine, Muğla Sıtkı Koçman University, Muğla, Turkey
[5]Department of Molecular Biology and Genetics, Faculty of Science, Muğla Sıtkı Koçman University, Muğla, Turkey
[6]Department of Family Medicine, School of Medicine, Zonguldak Bülent Ecevit University, Zonguldak, Turkey

Received: 16-03-2020 – **Accepted:** 22-08-2020

DOI: 10.15761/0101-60830000000287

ABSTRACT

Background: Exocytosis-related gene variants have been suggested to be associated with externalizing behaviors.
Objective: This study aimed to examine VAMP2 26 bp Ins\Del, synaptotagmin XI (Syt11) rs3820594 and 33-bp promoter, Syntaxin 1A (Syn-1A) rs1569061 and SNAP-25 rs1051312 and rs3746544 polymorphisms, their serum levels and their relationship with impulsivity, temperament in individuals with alcohol dependence (AD) and healthy controls (HC).
Methods: The study included 107 individuals with AD and 104 HCs. Single-nucleotide polymorphisms (SNPs) were studied with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and serum levels with ELISA. Michigan Alcohol Screening Test (MAST), Barratt Impulsiveness Scale-11 (BIS-11) and Temperament Evaluation of Memphis, Pisa, Paris and San Diego Autoquestionnaire (TEMPS-A) were applied.
Results: Syn-1A rs1569061 C allele polymorphism was significantly higher in AD group. Syn-1A rs1569061 C allele was associated with 1.5 times increased risk of AD. All serum levels were significantly higher in the HC group. There was a relationship between Syn-1A rs1569061 polymorphism and BIS-11 motor impulsiveness in the AD group; Syt11 rs3820594 polymorphism and BIS-11 total, TEMPS-A depressive, hyperthymia in the HC group.
Discussion: In our study, gene variants and serum levels of synaptic vesicle and presynaptic plasma membrane proteins were related to AD, impulsivity and temperament.

Şenormancı G / Arch Clin Psychiatry. 2021;48(2):99-104.

Keywords: Alcohol dependence, VAMP2, Synaptotagmin XI, Syntaxin 1A, SNAP-25.

Introduction

Specific soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes consist of Syntaxin 1A (Syn-1A), vesicle-associated membrane protein 2 (VAMP2) and synaptosomal associated and protein 25 kDa (SNAP-25) [1]. The SNARE complex plays an important role in neurotransmission. It takes part in the regulation of the release of neurotransmitters in the presynaptic area, through exocytosis [2]. VAMP forms the part of the SNARE complex in the vesicle, whereas syntaxin and

Address for correspondence: Ömer Şenormancı, Psychiatrist, University of Health Sciences Bursa Yuksek Ihtisas Training and Research Hospital, Mimar Sinan Mah. Emniyet Cad. 16310, Yıldırım/Bursa, Turkey; Tel: 0902242955000; E-mail: senorman_7@hotmail.com

SNAP-25 are plasma membrane proteins. The complex constituted by syntaxin and SNAP-25 is like a receptor for VAMP. The SNARE complex is the result of a quartet complex. One of these four complex bonds consists of syntaxin, the second one is VAMP and the latter two are SNAP-25 [3]. Despite the fact that alcohol has a wide variety of synaptic effects, it is not clear which proteins and processes are affected at the presynaptic terminal and how these arrangements occur [4].

Current studies have suggested that exocytosis-related gene variants are associated with externalizing behavioral problems such as dependence [5,6]. Impulsivity is one of the main features of recurrent severe externalizing behavioral problems such as alcohol/ substance dependence, risky sexual behaviors and sudden outbursts [7]. It has been claimed that impulsivity is hereditary in approximately 45% [8]. People with AD are characterized by high level of impulsivity, which is associated with the hedonic effects of alcohol [9].

Temperament is defined as emotional responses and behavioral patterns, which develop through childhood. Given that it has a permanent nature and hereditary basis, it can also be conceptualized as the biological base of personality. Reactions against the reward cues concerning the development and maintenance of dependence are related to temperament [10,11]. AD has been suggested to be associated with various temperament types, particularly cyclothymic temperament [12]. Impulsivity and externalizing behaviors are some characteristic features of cyclothymic, hyperthymic and irritable temperament [10]. SNARE protein polymorphisms have also been shown to be associated with impulsivity [13] and temperament [14]. Thus, there may be relations between AD, SNARE proteins, impulsivity, and temperament. In our study, it was aimed to compare polymorphisms and serum levels of 2 synaptic vesicle proteins (VAMP2 26 bp ins\del and synaptotagmin XI [Syt11] rs3820594 and 33 bp in the promoter) and 2 presynaptic plasma membrane proteins (Syn-1A rs1569061 and SNAP-25 rs1051312 and rs3746544) among people with AD and HC. Moreover, the relationships between polymorphisms and serum levels of SNARE proteins, related to externalizing behaviors in both groups, and impulsivity and temperament were searched.

Methods

Participant characteristics and evaluation

The study consisted of 107 inpatients, diagnosed with AD depending on the Diagnostic and Statistical Manual of Mental Disorders fifth edition (DSM 5) criteria for dependence at the Research, Treatment and Training Center for Alcohol and Substance Dependence, University of Health Sciences Bursa Yuksek Ihtisas Training and Research Hospital and 104 healthy subjects with no history of any substance dependence. Individuals with AD diagnoses of at least one year duration were included in the study. The control group was recruited from the hospital staff and their relatives with no history of psychiatric illness or substance use. Having intellectual disability or a serious medical disease (thyroid disorder, diabetes, etc.) were applied as exclusion criteria. HC whose Michigan Alcohol Screening Test (MAST) score ≤5 were included in the study. All participants were Turkish. After all participants were informed and written consent was obtained, SNARE polymorphisms, blood serum levels and blood samples were collected. MAST, Barratt Impulsiveness Scale-11 (BIS-11) and Temperament Evaluation of Memphis, Pisa, Paris and San Diego Autoquestionnaire (TEMPS-A) were applied to the participants and their relationship with SNARE polymorphisms and blood serum levels were inquired. The scales were administered at least 30 days after the last alcohol use in order for the results not to be affected by withdrawal symptoms, especially depressive symptoms. The amount of alcohol consumed was calculated in terms of a standard drink in this study. Accordingly, rakı, whiskey, gin, brandy and vodka were accepted to contain almost equal amounts of alcohol and 70 cl of high alcoholic beverages were calculated as 30 units. 0.33 L beer, 0.15 L wine were recorded as 1 unit after self-reporting. Ethical approval was granted by the Clinical Research Ethics Committee of Bülent Ecevit University Research Hospital (No. 33479383-2018/01). The study was carried out with the financial support of the Scientific Research Projects Unit of the University of Health Sciences (Project No: 2018/029).

Assessment tools

Michigan Alcoholism Screening Test (MAST)

MAST, developed by Gibbs (1983), is an assessment tool including 25 self-report questions that examine whether a person has alcohol use problems [21]. Turkish validity and reliability study of the test was accomplished by Coşkunol et al. (1995) [22].

Barratt Impulsiveness Scale-11 (BIS-11)

BIS-11 was developed by Patton and Barrat (1995). It is a self-report form that evaluates impulsivity with regard to its three main components [23]. Turkish validity and reliability study was conducted by Güleç et al. (2008) [24].

Temperament Evaluation of Memphis, Pisa, Paris and San Diego Autoquestionnaire (TEMPS-A)

It consists of 5 sub-scales named cyclothymic, depressive, irritable, hyperthymic and anxious, it was developed by Akiskal et al. (2005) [25]. Turkish validity and reliability study of 5 sub-scales was done by Vahip et al. (2005) [26].

Genotyping

Genomic DNA was extracted from peripheral blood leucocytes (200 µl of total blood) by using Macherey-Napel (MN) Nucleospin blood® DNA extraction kit (Cat no. 740.951.250) according to manufacturer instructions. A polymerase chain reaction (PCR)-based restriction fragment-length polymorphism (RFLP) method was used for Syn-1A rs1569061, SNAP-25 rs1051312 and rs3746544, Syt11 rs3820594 polymorphism and VAMP2 26 bp ins/del polymorphism and Syt11 33-bp polymorphism were genotyped using polymerase chain reaction. In a total reaction volume of 25 µL, 2.5 µL 10×buffer, 20 pM each primer, 2.5 µL genomic DNA, 2.5 µL dNTP mixture were combined. The primer sequences and annealing temperature for each SNP were shown in Table 1. The PCR products (15 µL) were used for digestion with the appropriate restriction enzyme (Table 2), and digests were analyzed by electrophoresis in a 3% agarose gel.

Serum SNARE Measurement

Serum VAMP2, Syt11, Syn-1A and SNAP-25 levels were measured by Sandwich Enzyme Linked Immunosorbent Assay (ELISA). YL-Biont's commercial Human PDYN ELISA kit (Ca, USA, Cat. No: YLA3974HU) was used for measurements. The PDYN concentrations of the studied samples were calculated from the absorbance measurement at 450 nm wavelength and the plotted standard curve graph after the incubations made before the study according to the kit, procedure used. The PDYN concentrations of the samples were calculated from the absorbance measurement at 450 nm wavelength and the plotted standard curve chart after the incubations made before the study.

Statistical analyses

Statistical analysis was performed with SPSS 18.0 package (SPSS Inc., Chicago, IL). The normality of the numerical variables was examined with Shapiro-Wilk test. For group comparisons, one-way analysis of variance analysis (ANOVA) was used for continuous

Table 1. Primer sequences and binding temperatures for PCR

SNP ID	Name of gene	Primer sequences (5'-3')	Binding Temperatures (°C)
26bp in\del polimorfizmi	VAMP2 (Synaptobrevin)	F:ACAAAGTGCGCCTTATACGC R:GGGATTTTCCTTGACGACACTC	57
33 bp repeat in the promotor and rs3820594	Synaptotagmin XI	F:TCTACCTATGCTTCTTACCC R:TGTCGTAATCAGAGGCTGTTGCT	62
rs1569061	Syntaxin 1A	F: CAATGCTGCTGCTGAACTC R: CGCTGACATTTATGTGACC	57
rs3746544 rs1051312	SNAP-25	F:TTCTCCTCCAAATGCTGTCG R:CCACCGAGGAGAGAAAATG	58

SNP=Single nucleotide polymorphism

Table 2. Enzymes specific to polymorphism and restriction conditions

SNP ID	Restrictive Enzyme	Restriction conditions
26bp in\del polymorphism	-	-
33 bp repeat in the promotor and rs3820594	HphI (NEB-Time saver)	15 min incubation at 37°C
rs1569061	TaiI (Thermo Scientific)	3 h incubation at 65 °C
rs3746544 T>G	MnlI (Thermo Scientific)	Overnight incubation at 37 °C
rs1051312 T>C	DdeI (Thermo Scientific)	Overnight incubation at 37 °C

SNP=Single nucleotide polymorphism

variables that meet the assumption of parametric test, Mann Whitney U test or Kruskal Wallis test was used for continuous variables that do not meet the assumption. Serum SNARE level and scale scores were evaluated using Spearman correlation analysis. $\chi 2$ (chi-square) test was carried out to compare the genotype frequency of polymorphisms between AD group and HC group. The relationship between polymorphisms and AD was modelled through binary logistic regression analysis. OR value and 95% confidence interval were calculated to compare the risk of dependence among genotypes. Numerical variables were shown as mean ± standard deviation (Mean ± SD) or median (Min-Max), and categorical variables were indicated with the number of observations and percentage (n-%) notation. A value of $\rho<0.05$ was considered statistically significant.

Results

There was a significant difference between AD group and control group in terms of age ($p<0.001$). SNARE genotype and allele frequencies of alcohol dependence and control groups were shown in Table 3

The relationship between SNARE polymorphisms and levels and BIS-11, TEMPS-A in the AD group

As BIS-11 motor impulsiveness scores of TT [14.5 (9-17), n=27], TC [15 (10-26), n=51] and CC [17 (12-24), n=29] genotypes of syn-1A rs1569061 polymorphism were compared using Kruskal Wallis test, a statistically significant difference was obtained (p=0.021). As a result of Mann-Whitney U test and Bonferroni correction, performed to confirm the significance level across groups, BIS-11 motor impulsiveness score of CC genotype was significantly higher than TT genotype ($p=0.005$, $\alpha^*=\alpha/3=0.05/3=0.016$).

There was a weak-moderate negative correlation between SNAP 25 and MAST (r=-0.28, p=0.033).

The relationship between SNARE polymorphisms and levels and BIS-11, TEMPS-A in the control group

When TT (54.6±7.2, n=59), TC (56.7±8.2, n=41) and CC (64.7±12.4, n=4) genotypes of Syt11 rs3820594 polymorphism were compared through ANOVA in terms of BIS-11 total scores, significant difference existed (p=0.048). The results of post hoc Tukey test conducted to determine the significance indicated that the scores of those with CC genotype were significantly higher than those with TT genotype (p=0.046).

As TEMPS-A depressive scores of Syt11 rs3820594 polymorphism TT [3.3 (1-8), n=59], TC [4.8 (1-12), n=41] and CC [9.5 (5-14), n=4] genotypes were contrasted with Kruskal Wallis test, a statistically significant difference was obtained (p=0.006). The TEMPS-A depressive score of CC genotype was significantly higher than TT genotype ($p=0.002$, $\alpha^*=\alpha/3=0.05/3=0.016$), in consequence of Mann-Whitney U test and Bonferroni correction performed to detect the significance level.

TEMPS-A hyperthymia scores of TT [11.8 (4-18), n = 59], TC [12.2 (4-18), n=41] and CC [6 (2-10), n=4] genotypes of Syt11 rs3820594 polymorphism were compared using Kruskal Wallis test; a statistically significant difference was recorded ($p = 0.036$). As a result of Mann-Whitney U test and Bonferroni correction, performed to determine the significance, the TEMPS-A hyperthymia score of TT genotype was significantly higher than that of CC genotype ($p=0.009$, $\alpha^*=\alpha/3=0.05/3=0.016$). The TEMPS-A hyperthymia score of TC genotype was significantly higher than CC genotype ($p=0.009$, $\alpha^*= \alpha/3=0.05/3=0.016$).

There was a weak-moderate positive correlation between VAMP2 and BIS-11 attentional impulsiveness (r=0.34, p=0.003). There was a weak-moderate positive correlation between VAMP2 and BIS-11 total (r=0.29, p=0.012).

There was a weak positive correlation between Syn-1A and BIS-11 attentional impulsiveness (r=0.24, p=0.040).

Evaluation of serum SNARE levels of alcohol dependence and control groups was shown in Table 5.

Discussion

In the study of people with AD, patients with schizophrenia and HCs, the synapsin II variants related to SNARE proteins were provided to be associated with people with AD compared to patients

Table 3. Clinical features of individuals with alcohol dependence (n=107)

	Med	min-max
The onset age of alcohol	19	11-46
Amount of alcohol use (daily)	15	7.5-86
Years of alcohol use	22	1-45
Number of Hospitalizations in the Alcohol and Substance Dependence Treatment Center in the past	0	0-16

Table 4. SNARE genotype and allele frequencies of alcohol dependence (n=107) and control (n=104) groups

SNP (SNARE)	Genotype/allele	Alcohol (n=107)	Control (n=104)	OR (95% CI)	p
	ins/ins	82 (76.6%)	72 (69.2%)	Reference	
VAMP2 ins/del	ins/del	24 (22.4%)	30 (28.8%)	0.642 (0.332-1.242)	0.446
	del/del	1 (0.9%)	2 (1.9%)	0.434 (0.037-5.155)	
	1/2 repeat	1 (0.9%)	0 (0.0%)	*	
Synaptotagmin XI 33 bp repeat	2/2 repeat	87 (81.3%)	82 (78.8%)	Reference	0.629
	2/3 repeat	17 (15.9%)	20 (19.2%)	0.801 (0.393-1.635)	
	3/3 repeat	2 (1.9%)	2 (1.9%)	0.943 (0.130-6.847)	
	TT	45 (42.1%)	59 (56.7%)	Reference	
Synaptotagmin XI rs3820594	TC	54 (50.5%)	41 (39.4%)	1.460 (0.790-2.700)	0.748
	CC	8 (7.5%)	4 (3.8%)	3.449 (0.812-14.638)	
	TT	59 (55.1%)	57 (54.8%)	Reference	
SNAP-25 rs1051312	TC	40 (37.4%)	43 (41.3%)	0.820 (0.438-1.533)	
	CC	8 (7.5%)	4 (3.8%)	1.911 (0.506-7.215)	0.482
	TT	46 (43.0%)	43 (41.3%)	Reference	
SNAP-25 rs3746544	TG	52 (48.6%)	45 (43.3%)	1.094 (0.582-2.055)	
	GG	9 (8.4%)	16 (15.4%)	0.443 (0.157-1.250)	0.279
	TT	27 (25.2%)	24 (23.1%)	Reference	
Syntaxin 1A rs1569061	TC	51 (47.7%)	55 (52.9%)	0.780 (0.388-1.565)	0.082
	CC	29 (27.1%)	25 (24.0%)	1.106 (0.493-2.483)	
VAMP2 ins/del	ins	188 (87.9%)	174 (83.7%)	Reference	0.265
	del	26 (12.1%)	34 (16.3%)	0.708 (0.408-1.228)	
	1 repeat	1 (0.5%)	0	*	
Synaptotagmin XI 33 bp repeat	2 repeat	191 (9.8%)	184 (88.5%)	Reference	0.434
	3 repeat	21 (86.6%)	24 (11.5%)	0.839 (0.451-1.556)	
Synaptotagmin XI rs3820594	T	144 (67.3%)	159 (76.4%)	Reference	0.040
	C	70 (32.7%)	49 (23.6%)	1.577 (1.027-2.423)	
SNAP-25 rs1051312	T	158 (73.8%)	157 (75.5%)	Reference	0.738
	C	56 (26.2%)	51 (24.5%)	1.091 (0.703-1.692)	
SNAP-25 rs3746544	T	144 (67.3%)	131 (63.0%)	Reference	0.360
	G	70 (32.7%)	77(37.0%)	0.827 (0.554-1.235)	
Syntaxin 1A rs1569061	T	105 (49.1%)	103(49.5%)	Reference	1.000
	C	109 (50.9%)	105(50.5%)	1.018 (0.695-1.492)	

Chi square test, p<0.05, * Probability ratio could not be calculated.
SNARE=N-ethylmaleimide-sensitive factor attachment protein receptor complexes, SNP=Single nucleotide polymorphism, OR (95% CI)=Odds ratio 95% confidence interval

Table 5. Evaluation of serum SNARE levels of alcohol dependence (n=107) and control (n=104) groups

	Alcohol (n=107)	Control (n=104)	
VAMP 2	21.5 (9.3-239.1) ng/L	35.5 (7.0-587.0) ng/L	p<0.001
Syt11	2.9 (1.2-240.5) ng/L	35.0 (2.5-453.7) ng/L	p<0.001
SNAP-25	75.2 (44.6-502.9) pg/ml	78.4 (60.0-829.8) pg/	p=0.017
Syn-1A	15.8 (9.2-380.3) ng/L	28.6 (18.5-390.4) ng/L	p<0.001

Mann Whitney U test, p<0.05
SNARE=N-ethylmaleimide-sensitive factor attachment protein receptor complexes,

with schizophrenia and HCs[27]. In our study, Syt11 rs3820594 C allele polymorphism was significantly higher in AD group than HC group, in accordance with literature findings. Regression analysis revealed that carrying the Syt11 rs3820594 C allele augments the AD risk 1.5 times.

Even though the BIS-11 and SNAP-25 rs1051312 polymorphism were found to be associated in the community-based study conducted on Hungarian participants, in our study, no correlation was detected between impulsivity and SNAP-25 rs1051312 polymorphism in both AD and HC groups [13]. In a study performed using Temperament and Character Inventory (TCI) on participants who have committed crimes and antisocial personality traits, SNAP-25 rs1051312 and rs3746544 polymorphisms were found to be related to TCI reward dependence and TCI novelty seeking [28]. Having stated that the relationships between the TC genotype of SNAP-25 rs1051312 polymorphism and TCI novelty seeking, TCI harm avoidance and TCI cooperativeness scores, used in the study, have been determined in fibromyalgia patients [14]. Impulsivity and temperament are hereditary traits and are associated with various neurotransmitter systems [8,10]. SNARE proteins have important roles in exocytosis. Alterations of SNARE proteins lead to changes in many neurotransmitter systems related to impulsivity and temperament [28]. Although the TEMPS-A and TCI scales used in our study were divergent scales, they showed a high correlation as compared with regard to temperament characteristics they assess. In our study, the BIS-11 motor impulsiveness score of the Syn-1A rs1569061 polymorphism CC genotype was significantly higher in the AD group than in the TT group. In the literature, SNAP-25, one of the genes encoding SNARE protein, was found to be correlated with impulsivity and temperament in other psychiatric conditions, but no correlation was obtained between SNAP-25 polymorphism and impulsivity and temperament in the present study. According to the results of our study, it can be stated that the effects of having Syn-1A rs1569061 polymorphism CC genotype on neurotransmitter system for people with AD is related to increased BIS-11 motor impulsiveness scores.

When the relationship between SNARE polymorphisms and levels of HC group and BIS-11, TEMPS-A were examined, individuals with Syt11 rs3820594 polymorphism CC genotype had higher BIS-11 total and TEMPS-A depressive scores than those with Syt11 rs3820594 polymorphism TT genotype. In terms of TEMPS-A hyperthermia scores, Syt11 rs3820594 polymorphism TT genotype and Syt11 rs3820594 polymorphism TC genotype were significantly higher than CC genotype. There was a positive correlation between BIS-11 attentional impulsiveness and VAMP2 and Syn-1A. In conclusion, it can be stated that Syt11 rs3820594 polymorphism is associated with impulsivity and temperament in HCs, except that it increases the risk of dependence in the AD group.

There are also studies evaluating SNARE protein levels on dependent people. A postmortem investigation of nucleus accumbens in people with chronic heroin abuse confirmed the downregulation of SNARE proteins. This down-regulation of presynaptic protein gene transcripts has been thought to be a compensatory mechanism developed due to chronic use of the substance [29]. There is only one study inquiring of SNARE protein levels in people with AD. The autopsy study in which dorsolateral prefrontal cortex and anterior cingulate cortex analysed in patients with schizophrenia and bipolar disorder and participants with no psychiatric illness, pointed out a low SNAP-25b BA24 levels in people with AD [30]. In our study, serum levels of VAMP2, Syt11, Syn-1A and SNAP-25 were significantly higher in the control group than AD group. There was a negative correlation between SNAP-25 and MAST scores in people with AD. Our study is the first one to indicate the low serum levels of SNARE proteins in people with AD. Unlike preclinical studies in the literature, alcohol use generated alleviation in SNARE proteins in individuals. The synaptic effectiveness of alcohol may have a different pattern in humans distinct from animals.

Our study has some limitations. The number of participants was small. Participants consisted of males, so gender-specific differences in SNARE polymorphisms, impulsivity, and temperament characteristics could not be evaluated. Another limitation was that the medical history and alcohol use history of the participants were taken as a self-report. Our study was conducted on Turkish people with AD. It would be useful to carry out studies involving both gender and other ethnic groups with a higher number of participants.

Disclosure

No conflicts of interest are declared by the authors.

Financial disclosure

The study was carried out with the financial support of the Scientific Research Projects Unit of the University of Health Sciences (Project No: 2018/029).

References

1. Marz KE, Hanson PI. Sealed with a twist: Complexin and the synaptic SNARE complex. Trends Neurosci 2002;25(8):381-3.
2. Montecucco C, Schiavo G, Pantano S. SNARE complexes and neuroexocytosis: How many, how close? Trends Biochem Sci 2005;30(7):367-2.
3. An SJ, Almers W. Tracking SNARE complex formation in live endocrine cells. Science 2004;306(5968):1042-6.
4. Barclay JW, Graham ME, Edwards MR, Johnson JR, Morgan A, Burgoyne RD. Presynaptic targets for acute ethanol sensitivity. Biochem Soc Trans 2010;38(Pt 1):172-6.
5. Cupertino RB, Schuch JB, Bandeira CE, da Silva BS, Rovaris DL, Kappel DB, et al. Replicated association of Synaptotagmin (SYT1) with ADHD and its broader influence in externalizing behaviors. Eur Neuropsychopharmacol 2017;27(3):239-47.
6. da Silva BS, Cupertino RB, Schuch JB, Kappel DB, Sanvicente-Vieira B, Bandeira CE, et al. The association between SYT1-rs2251214 and cocaine use disorder further supports its role in psychiatry. Prog Neuropsychopharmacol Biol Psychiatry 2019;94:109642.
7. Links PS, Kolla NJ, Guimond T, McMain S. Pro-spective risk factors for suicide attempts in a treated sample of patients with borderline personality disorder. Can J Psychiatry 2013;58(2):99-106.
8. Congdon E, Canli T. A neurogenetic approach to impulsivity. J Pers 2008;76(6):1447-84.
9. Westman JG, Bujarski S, Ray LA. Impulsivity moderates subjective responses to alcohol in alcohol-dependent individuals. Alcohol Alcohol 2017;52(2):249-55.
10. Rihmer Z, Akiskal KK, Rihmer A, Akiskal HS. Current research on affective temperaments. Curr Opin Psychiatry 2010;23(1):12-8.
11. Clarke TK, Krause K, Li T, Schumann G. An association of prodynorphin polymorphisms and opioid dependence in females in a Chinese population. Addict Biol 2009;14(3):366-70.
12. Yehya Y, Haddad C, Obeid S, Tahan F, Nabout R, Hallit S, et al. Affective temperaments in Lebanese people with substance use disorder. Perspect Psychiatr Care 2019;55(3):478-85.
13. Németh N, Kovács-Nagy R, Székely A, Sasvári-Székely M, Rónai Z. Association of impulsivity and polymorphic microRNA-641 target sites in the SNAP-25 gene. PLoS One 2013;8(12):e84207.
14. Dogru A, Balkarli A, Tepeli E, Aydin E, Cobankara V. Association of synaptosomal-associated protein 25 (SNAP-25) gene polymorphism with temperament and character traits in women with fibromyalgia syndrome. Arch Clin Psychiatry 2018;45(4):88-93.
15. Gibbs LE. Validity and reliability of the Michigan alcoholism screening test: A review. Drug Alcohol Depend 1983;12(3):279-85.

16. Coşkunol H, Bağdiken İ, Sorias S, Saygılı R. Michigan alkolizm tarama testinin (MATT) geçerliliği. Ege Tıp Dergisi 1995;34(1-2):15-8.
17. Patton JH, Stanford MS, Barratt ES. Factor structure of the Barratt impulsiveness scale. J Clin Psychol 1995;51(6):768-74.
18. Güleç H, Tamam L, Güleç MY, Turhan M, Karakuş G, Zengin M, et al. Barrat dürtüsellik ölçeği-11 (BIS-11)'nin Türkçe uyarlamasının psikometrik özellikleri. Klinik Psikofarmakoloji Bul. 2008;18(4):251-8.
19. Akiskal HS, Akiskal KK, Haykal RF, Manning JS, Connor PD. TEMPS-A: progress towards validation of a self-rated clinical version of the Temperament Evaluation of the Memphis, Pisa, Paris, and San Diego Autoquestionnaire. J Affect Disord 2005;85(1-2):3-16.
20. Vahip S, Kesebir S, Alkan M, Yazıcı O, Akiskal KK, Akiskal HS. Affective temperaments in clinically-well subjects in Turkey: initial psychometric data on the TEMPS-A. J Affect Disord 2005;85(1-2):113-25.
21. Grebb JA, Greengard P. An analysis of synapsin II, a neuronal phosphoprotein, in postmortem brain tissue from alcoholic and neuropsychiatrically ill adults and medically ill children and young adults. Arch Gen Psychiatry 1990;47(12):1149-56.
22. Basoglu C, Oner O, Ates A, Algul A, Bez Y, Cetin M, et al. Synaptosomal-associated protein 25 gene polymorphisms and antisocial personality disorder: association with temperament and psychopathy. Can J Psychiatry 2011;56(6):341-7.
23. Albertson DN, Schmidt CJ, Kapatos G, Bannon MJ. Distinctive profiles of gene expression in the human nucleus accumbens associated with cocaine and heroin abuse. Neuropsychopharmacology. 2006;31(10):2304-12.
24. Thompson PM, Cruz DA, Fucich EA, Olukotun DY, Takahashi M, Itakura M. SNAP-25a/b Isoform levels in human brain dorsolateral prefrontal cortex and anterior cingulate cortex. Mol Neuropsychiatry. 2015;1(4):220-34.