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SNP analysis of stress-related genes reveals significant correlations with drug addiction in Jordan

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ABSTRACT

Objective: Drug addiction is a complex disorder caused by multiple factors, including environmental and genetic factors. Stress-related genes such as Galanin (GAL) and Oxytocin (OXT) have been linked to the reward pathways that contribute to the development and progression of substance addiction. This study aimed to explore the correlation between several polymorphisms of stress-related genes and drug addiction among Jordanian males.
Methods: The study included 500 participants, consisting of both healthy controls and drug-addicted Jordanian males. The genetic material and clinical data were collected, and 18 SNPs in four candidate genes were genotyped using the Sequenom MassARRAY® system. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 25.0 and the SNPStats website.
Results: The study identified a significant correlation between three SNPs of the GAL gene and drug addiction, specifically rs3136544, rs3136541, and rs694066. The study also found that different genotypes of these variants were significantly associated with drug addiction. Furthermore, different haplotypes of the GAL, GALR1, and OXTR polymorphisms were also significantly correlated with drug addiction. The study also identified a correlation between several drug addiction features and the studied variants, including the association of rs2717162 of Galanin receptor 1 (GALR1) with age at use onset and the association of rs3136541 of GAL with the type of substance and number of substances used.
Conclusion: Stress-related genes can play a significant role in the development and progression of addiction among the Jordanian population, and further investigations are necessary to understand the underlying mechanisms better and improve future treatment strategies.

1. Introduction

Addiction is a chronic neuropsychiatric disorder worldwide that is known as the failure to control drug consumption or cessation (Ducci and Goldman, 2008, Buisman-Pijlman et al., 2014, Torres-Berrio et al., 2018). It is presumed that alcohol, followed by cannabis, nicotine, cocaine, and amphetamine, are the most recurrently consumed substances globally, contributing to 0.4 % of annual deaths (Ducci and Goldman, 2008), (Torres-Berrio et al., 2018, Kim et al., 2015, Al-Eitan et al., 2012c, Al-Eitan et al., 2024). Addiction consequences conflict with a person's psychological and social life (Buisman-Pijlman et al., 2014, Levran et al., 2014b, McGregor and Bowen, 2012, Love et al., 2018). Addiction is affected by a complex interaction between environmental, genetic, and drug-induced factors (Levran et al., 2014b, Levran et al., 2014a, Levran et al., 2008). Stress is a key factor that may induce individuals to drug addiction and prolong the addiction cycle, including initiation, maintenance, and relapse (Kim et al., 2015, Levran et al., 2014b, Al-Eitan et al., 2012b). Stress is a behavioral and

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physiological response that lets organisms respond to the challenges of the environment perceived as threatening (Torres-Berrio et al., 2018, Levran et al., 2014b, Randesi et al., 2020). Several molecular genetic studies have proved the relationship between various polymorphisms in stress-related genes, such as the neuropeptide galanin (*GAL*) and Oxytocin *(OXT),* among other genes and affective disorders (Levran et al., 2014a, Belfer et al., 2006, Domschke and Reif, 2012).[1]

Previous studies conducted an association of heroin addiction involving several genes associated with stress response (Levran et al., 2008, Proudnikov et al., 2008, Levran et al., 2009). These investigations found a correlation between polymorphisms in the adrenocorticotropic hormone *ACTH* receptor gene (melanocortin 2 receptor MC2R) in Hispanics, the arginine vasopressin *AVP* receptor gene (AVPR1A) in African Americans, and the galanin gene (*GAL*) in European Americans.

A case-control hypothesis-driven association study investigated 112 SNPs in 26 genes related to stress response and heroin addiction in 1090 European subjects. Nineteen single nucleotide polymorphisms (SNPs) in 9 genes (AVP, corticotropin-releasing hormone receptor 1 (*CRHR1*), corticotropin-releasing hormone receptor 2 (*CRHR2*), FK506-binding protein 51 (*FKBP5*), nuclear receptor subfamily 3, group C, member 2 (mineralocorticoid receptor (*NR3C2*)), *AVPR1A*, *GAL*, galanin receptor 1 (*GLRA1)*, and neuropeptide Y receptor Y1 (*NPY1R*)) showed nominally significant association with heroin addiction (Levran et al., 2014a). Another study found that a different *AVPR1A* SNP was linked to general drug use disorders (Maher et al., 2011), and neuropeptide Y receptor Y2 (*NPY2R*) SNPs have been associated with alcohol and cocaine dependence (Wetherill et al., 2008).

Another study examined the association between 120 variants in 27 stress-related genes to determine the role of these variants in opioid dependence in Caucasian subjects from the Netherlands. They found that 12 SNPs in seven genes showed a nominally significant association with opioid dependence OD. Experiment-wise significant associations were found for three SNP pairs through an interaction effect: GALR1/GAL rs9807208/rs3136541, NPY1R/GAL rs4691910/rs1893679, NPY1R/GAL, and rs4691910/rs3136541. This corroborates evidence from prior reports on the association between stress-related variables and heroin dependence (Randesi et al., 2020).

Brain-derived neurotrophic factor (BDNF) levels have been associated with several aspects of cocaine addiction (Corominas-Roso et al., 2013). Therefore, BDNF levels have been proposed as a biomarker of cocaine use-related outcomes (von Diemen et al., 2016). These outcomes and even susceptibility to drug addiction are similarly predicted by the functioning of the hypothalamic–pituitary–adrenal (HPA) axis (Sinha, 2013). Interestingly, existing literature links the activation of the HPA axis with altered BDNF expression in the brain, suggesting that variants in stress-related genes may modulate peripheral BDNF and drug abuse risk (Suri and Vaidya, 2013).

The neuropeptide galanin (*GAL*) is broadly expressed in the Central Nervous System (CNS), including limbic areas that control emotionality and contribute to drug addiction and reward (Belfer et al., 2006, Jackson et al., 2011, Unschuld et al., 2010, Hawes et al., 2008, Fagerberg et al., 2014). Human galanin (*GAL*), which is located on chromosome 11q13.3, is composed of six exons and 30 amino-acid neuropeptides (Levran et al., 2008, Belfer et al., 2006, Unschuld et al., 2010, Juhasz et al., 2014, Holmes and Picciotto, 2006).

It has been demonstrated that *GAL* regulates numerous activities such as feeding, intestinal secretion, learning, memory, mood behavior, neurological diseases, and nociception, as well as the neuroendocrine control of systems like the hypothalamic–pituitary–adrenal axis (Butzkueven and Gundlach, 2010, Ogren et al., 2010, Shen and Gundlach, 2010, Autio et al., 2020, Zhu et al., 2022).

G protein-coupled receptors (GPCRs) bind to Galanin and modify the intracellular signaling pathways that participate in the addiction (Hawes et al., 2008, Gold et al., 2012). Galanin applies its action via three G-protein-coupled receptors: Galanin receptor 1 (*GALR1)*, Galanin receptor 2 (*GALR2)*, and Galanin receptor 3 (*GALR3)* that are expressed in the brain areas that are involved in drug dependence, including the locus coeruleus (LC), nucleus accumbens (NAc), and ventral midbrain (VMB) (Jackson et al., 2011, Unschuld et al., 2010, Juhasz et al., 2014, Šípková et al., 2017).

Galanin modifies the mesolimbic dopamine system through dopaminergic transmission inhibition that is necessary for the rewarding properties of drug abuse (McGregor and Bowen, 2012, Jackson et al., 2011, King et al., 2020). Galanin has been implicated in alcohol addiction (Genders et al., 2020), and in population susceptibility to alcoholism (Belfer et al., 2006).

The central nervous system contains a variety of neuromodulators that can alter processes involved in arginine vasopressin (*AVP*) and oxytocin (*OXT*) synthesis and release (Ciosek and Drobnik, 2013). Two members of the galanin neuropeptide family: “parental” galanin (*GAL*) and its cousin galanin-like peptide (*GALP*), are potential modulators (Wodowska and Ciosek, 2014). Justyna Wodowska et al. found that Galanin (*GAL*) and galanin-like peptide *GALP* modulate the release of *AVP* and *OXT* at every level of the hypothalamo-neurohypophysial system. Gal acts in the rat central nervous system as an inhibitory neuromodulator for *AVP* and *OXT* release via galanin receptors (Wodowska and Ciosek, 2014).

Oxytocin (*OXT*) is a small neuropeptide hormone that is produced in CNS and various peripheral tissues and organs, including the uterine epithelium, vascular endothelium, heart, and genital organs (Buisman-Pijlman et al., 2014, Love et al., 2018). Oxytocin is released due to different situations, such as close physical contact in a safe environment, conditioned fear, and novel environments (Buisman-Pijlman et al., 2014, N AL-Eitan et al., 2012, Al-Eitan et al., 2014, Al-Eitan et al., 2021). *OXT* inhibits stress-induced activity in the HPA axis and performs a vital role in response to stress (Neumann, 2008). *OXT* regulates many social behaviors, including anxiety reduction, inflammatory and immune responses, pain reduction, and information and memory processing (Buisman-Pijlman et al., 2014, Love et al., 2018, King et al., 2020).

OXT and its receptor (*OXTR*) are known to be associated mainly with stress reactivity and empathy and play an important role in numerous neuropsychiatric disorders, including alcohol and drug addiction (Buisman-Pijlman et al., 2014).

The *OXTR*, which is a member of the rhodopsin-type 1 G protein-coupled receptor (GPCR) (Buisman-Pijlman et al., 2014, Levran et al., 2008) is activated by the binding of *OXT* to its outer membrane domain, then phospholipase C, the G-protein alpha subunit, and protein kinase C are activated, finally leading to several downstream cellular responses that regulate the social and emotional behaviors and rewards (Buisman-Pijlman et al., 2014, King et al., 2020, Enck and Klosterhalfen, 2009). We are not aware of reports on the association between polymorphisms in stress-related genes, especially oxytocin and galanin, and drug addiction in the Jordanian population. Therefore, this study aimed to investigate the association, if any, between four stress-related genes (*GAL* and *OXT* and their receptors) polymorphisms and addiction in the Jordanian Arab population.

[1] FKBP5: FK506-binding protein 51; GAL: Galanin; GALP:galanin-like peptide;GALR1:Galanin receptor 1;GALR2:Galanin receptor 2;GALR3:Galanin receptor 3; GPCRs: G protein-coupled receptors;GWAS: Genome-wide association study;HPA; hypothalamic–pituitary–adrenal; HWE: Hardy-Weinberg Equilibrium;IRP: Institutional Review Board; LC: locus coeruleus; MC2R:melanocortin 2 receptor;NAc: nucleus accumbens; NCRA: National Centre for Rehabilitation of Addicts;NPY1R:neuropeptide Y receptor Y1, PY2R; neuropeptide Y receptor Y2;R3C2: nuclear receptor subfamily 3, group C, member 2;OD: opioid dependence;OXT: Oxytocin; SNP: single nucleotide polymorphism; SNPs: single nucleotide polymorphism; SPSS: Statistical Package for the Social Sciences; SUD; substance use disorders;; SNPs: single nucleotide polymorphism; VMB: ventral midbrain;

2. Materials and methods

2.1. Study subjects

This study has been approved by the Institutional Review Board (IRB) of Jordan University of Science and Technology (43/114/ 2018). This study was also approved by the Ministry of Health (MOH/ REC/ 180057), the Public Security Directorate (C/2/46/21546), and King Abdullah University Hospital (43/114/2018). This study consisted of one thousand Jordanian Arab participants; 500 Jordanian-addicted males were recruited from the National Centre for Rehabilitation of Addicts (NCRA) of the Ministry of Health and the Drug Rehabilitation Centre of the Jordanian Public Security Directorate (DRC-PSD), and 500 healthy controls were selected based on the criteria of being healthy individuals with no history of substance use or psychiatric disorders. The average age (Mean ± SD) was 28.4 ± 6.88 and 30.1 ± 6.81 for patients and controls, respectively. Demographic data of the participants are shown in Table 1.

The addicted individuals were selected depending on the substance use criteria according to the Manual of Mental Disorders (DSM-IV) criteria (APA, 2013) (Online, 2013). Inclusion criteria were that participants had to be residents of Jordan, be 18 years of age or older, provide written informed consent, and meet DSM-IV criteria for a substance abuse problem (Online, 2013). Exclusion criteria were the presence of severe psychiatric disorders (unless a substance use disorder was the underlying cause), neurological diseases (e.g., epilepsy, multiple sclerosis, Parkinson's disease), pregnancy, dementia, severe somatic conditions like cardiovascular disorders, and current participation in another clinical study.

In Jordan, about 134,947 adult males exhibit substance use disorder, constituting a prevalence rate of 2.5 % among the total population of 9,531,712 (https://www.who.int/publications/m/item/jordan—who-special-initiative-for-mental-health). The sample size required was computed using the OpenEpi program, version 3.01, with a 95 % confidence interval. Given a substance use disorder prevalence of 2.5 % in Jordan, a precision of 3 %, and a design effect of 1, the estimated sample size required was 105 participants. The case sample in our study comprised 500 individuals, which exceeded the specified sample size.

2.2. DNA extraction, genotyping, and SNP selection

We used the Sequenom MassARRAY iPLEX Gold system to customize the SNP genotyping of the selected SNPs. Genomic DNA was extracted from the whole blood of each participant using the standard kit procedure (the Gentra ® Puregene ® Blood Kit, Qiagen, Germany). The extracted DNA's quantity and quality were measured using the NanoDrop ND-1000 (Bio Drop, UK) and gel electrophoresis. The DNA samples were sequenced in the Australian Genome Research Facility (AGRF; Melbourne Node, Melbourne, Australia) using the Sequenom MassARRAY® system (iPLEX GOLD) (Sequenom, San Diego, CA, USA) in this study. Eighteen polymorphisms were investigated in this study: rs2740210, rs3761248, rs4813625, rs877172, rs237887, rs237902, rs4686301, rs7632287, rs2513304, rs3136544, rs1546309, rs3136541, rs694066, rs2717162, rs5374, rs5376, rs1942578 and rs9807208, as shown in Table 2.

Table 1
Demographic data of the participants.

	Cases n (%)	Controls n (%)
Employment		
Yes	341 (70.3 %)	397 (79.4 %)
No	144 (29.7)	103 (20.6 %)
Marital status		
Single	346 (71 %)	205 (41 %)
Divorced	14 (2.9 %)	2 (0.4 %)
Widowed	0 (0 %)	1 (0.2 %)
Married	127 (26.1)	292 (58 %)
Smoking		
Yes	426 (87.8 %)	331 (66.2 %)
No	59 (12.2 %)	169 (33.8 %)
Age (Mean ± SD)	28.4 ± 6.88	30.1 ± 6.81
Age at the first use (Mean ± SD)	23.9 ± 6.56	–
Cases with family history of addiction	29 (5.8 %)	–

2.3. Statistical and haplotype analyses

Genetic associations and haplotype analysis analyses were performed in this study. In addition, the genotypic and allelic distribution and the minor allele frequencies for both groups, as well as the Hardy-Weinberg Equilibrium (HWE) equation, were conducted using SNPStats web (HTTPs: //www. snpstats.net/start.htm) (2006 Institute Català d'Oncologia). $P<0.05$ was considered significant. The P-value of HWE is > 0.05 to be considered normally distributed. The Chi-square test and Bonferroni multivariate analysis were performed using the Statistical Package for the Social Sciences (SPSS), version 25.0 (SPSS, Inc., Chicago, IL).

3. Results

This study was conducted with five hundred participants from each group (the healthy controls and SUD patients). The genetic association between the studied polymorphisms and drug addiction revealed that three SNPs in the *GAL* gene (rs3136541, rs694066, and rs3136544) were significantly associated with drug addiction with P-values (0.0039, 0.0042, and 0.015), respectively (Table 3). Furthermore, none of the other fourteen SNPs were in correlation with drug addiction among Jordanians ($P>0.05$) (Supplementary Table 1).

The genetic model analysis (see Table 3)revealed that the codominant model of the rs3136541 SNPs (OR = 0.57, P = 0.003) and the recessive model (OR = 1.62, P = 0.002) were significantly associated with drug addiction. Regarding the rs3136541 polymorphism, 30 % of controls had the (TT) genotype compared to 21 % in addicted patients. So, according to the genotype distribution between cases and controls, we conclude that the (TT) genotype and the (T) allele could be protective factors against drug addiction. For the SNP rs694066, the different models indicated as genetic risk factors for drug addiction include the dominant model (OR = 0.62, P = 0.0010), the codominant model (OR = 1.60, P = 0.0044), and the Overdominant model (OR = 0.64, P = 0.0024) for drug addiction. However, the (A/G) genotype in controls was lower (20.0 %) than it (29 %) in the addicted patients. Therefore, the (G/A) genotype could be considered a risk factor that increases the risk of addiction among Jordanians. As illustrated, the rs3136544

Table 2
The characteristics of the studied polymorphisms within the candidate genes.

Gene	Rs #	Chr. Position	SNP	functional consequence
GALR1	rs9807208	18:77262299	G>A	intron variant
GALR1	rs2717162	18:77256371	T>C	intron variant
GALR1	rs5374	18:77250689	T>C	coding sequence variant
GALR1	rs5376	18:77268853	G>A	missense variant
GALR1	rs1942578	18:77481057	T>C	NA
GAL	rs1546309	11:68687214	C>T	intron variant
GAL	rs3136541	11:68690475	C>T	upstream transcript variant
GAL	rs694066	11:68685517	G>A	intron variant
GAL	rs3136544	11:68692195	A>G	NA
GAL	rs2513304	11:68693677	T>G	NA
OXT	rs2740210	20:3072609	C>A	downstream transcript variant
OXT	rs3761248	20:3069747	T>C	intron variant
OXT	rs4813625	20:3069074	G>C	intron variant
OXT	rs877172	20:3069244	T>G	intron variant
OXTR	rs237887	3:8755356	G>A	intron variant
OXTR	rs237902	3:8767498	G>A	NA
OXTR	rs4686301	3:8756900	C>T	intron variant
OXTR	rs7632287	3:8749759	G>A	downstream transcript variant

NA: Not Applicable.

Table 3
The genotype association and genetic model analysis of the significant associated SNPs and SUD.

Gene	Polymorphism Ref. SNP # / Group (n)	%					*P* value	Model	Genotype	OR (95 % CI)	*P* value
	rs2513304	G/G	G/T	T/T	G	T		Codominant	G/G, G/T, T/T	0.76 (0.58–1.00)	0.062
										0.69 (0.48–1.01)	
	controls	46.0	40.0	14.0	66.0	34.0	0.06	Dominant	G/G, G/T+T/T	0.74 (0.58–0.96)	0.021
	patients	38.0	45.0	17.0	61.0	39.0		Recessive	G/G+G/T, T/T	0.80 (0.56–1.13)	0.2
								Overdominant	G/G+T/T, G/T	0.84 (0.65–1.08)	0.17
	rs3136544	A/A	A/G	G/G	A	G	0.015	Codominant	A/A, A/G, G/G	1.13 (0.84–1.53)	0.015
										1.62 (1.15–2.29)	
	controls	26.0	45.0	29.0	49.0	51.0		Dominant	A/A, A/G+G/G	1.29 (0.98–1.70)	0.073
	patients	31.0	47.0	21.0	55.0	45.0		Recessive	A/A+A/G, G/G	0.64 (1.13–2.01)	0.005
								Overdominant	A/A+G/G, A/G	0.90 (0.70–1.16)	0.43
	rs3136541	C/C	C/T	T/T	C	T	0.0039	Codominant	T/T, C/T, C/C	1.13 (0.84–1.52)	0.003
										1.74 (1.23–2.45)	
GAL	controls	27.0	43.0	30.0	49.0	51.0		Dominant	T/T, C/T+C/C	1.32 (1.00–1.73)	0.05
	patients	33.0	47.0	21.0	56.0	44.0		Recessive	T/T+C/T, C/C	1.62 (1.21–2.17)	0.002
								Overdominant	T/T+C/C, C/T	0.88 (0.68–1.13)	0.30
	rs694066	A/A	A/G	G/G	A	G	0.0042	Codominant	G/G, G/A, A/A	0.62 (0.46–0.84)	0.004
										0.61 (0.29–1.31)	
	controls	2.0	20.0	77.0	13.0	87.0		Dominant	G/G, G/A+A/A	0.62 (0.47–0.83)	0.001
	patients	3.0	29.0	68.0	18.0	82.0		Recessive	G/G+G/A, A/A	0.69 (0.33–1.47)	0.33
								Overdominant	G/G+A/A, G/A	0.64 (0.47–0.85)	0.002
	rs1546309	C/C	C/T	T/T	C	T	0.125	Codominant	T/T, C/T, C/C	0.83 (0.63–1.08)	0.12
										0.68 (0.46–1.02)	
	controls	11.0	42.0	48.0	32.0	68.0		Dominant	T/T, C/T+C/C	0.79 (0.62–1.02)	0.068
	patients	14.0	44.0	42.0	36.0	64.0		Recessive	T/T+C/T, C/C	0.75 (0.51–1.10)	0.14
								Overdominant	T/T+C/C, C/T	0.90 (0.70–1.15)	0.39
	rs2717162	C/C	C/T	T/T	C	T	0.644	Codominant	T/T, C/T, C/C	0.93 (0.71–1.21)	0.64
										0.82 (0.54–1.26)	
	controls	10.0	44.0	46.0	32.0	68.0		Dominant	T/T, C/T+C/C	0.91 (0.71–1.17)	0.45
	patients	12.0	45.0	43.0	34.0	66.0		Recessive	T/T+C/T, C/C	0.86 (0.57–1.27)	0.44
								Overdominant	T/T+C/C, C/T	0.97 (0.75–1.24)	0.79
	rs5374	C/C	C/T	T/T	C	T	0.910	Codominant	C/C, C/T, T/T	0.97 (0.74–1.27)	0.91
										0.92 (0.62–1.36)	
	controls	41.0	47.0	13.0	64.0	36.0		Dominant	C/C,C/T+T/T	0.96 (0.74–1.24)	0.75
	patients	40.0	47.0	14	63.0	37.0		Recessive	C/C+C/T, T/T	0.93 (0.64–1.35)	0.71
								Overdominant	C/C+T/T, C/T	0.99 (0.77–1.27)	0.95
	rs5376	A/A	A/G	G/G	A	G	0.597	Codominant	G/G, G/A, A/A	1.06 (0.56–2.01)	0.49
										NA (0.00-NA)	
GALR1	controls	96.0	4.0	0.0	98.0	2.0		Dominant	G/G, G/A+A/A	1.11 (0.59–2.09)	0.75
	patients	96.0	4.0	0.0	98.0	2.0		Recessive	G/G+G/A, A/A	NA (0.00-NA)	0.24
								Overdominant	G/G+A/A, G/A	1.05 (0.56–2.00)	0.87
	rs9807208	A/A	A/G	G/G	A	G	0.219	Codominant	G/G, G/A, A/A	0.79 (0.60–1.03)	0.22
										0.91 (0.61–1.37)	
	controls	46.0	42.0	13.0	67.0	33.0		Dominant	G/G, G/A+A/A	0.82 (0.63–1.05)	0.11
	patients	41.0	47.0	12.0	64.0	36.0		Recessive	G/G+G/A, A/A	1.03 (0.71–1.51)	0.87
								Overdominant	G/G+A/A, G/A	0.81 (0.63–1.04)	0.091
	rs1942578	C/C	T/C	T/T	C	T	0.746	Codominant	T/T, T/C, C/C	1.11 (0.83–1.48)	0.75
										1.11 (0.77–1.59)	
	controls	20.0	50.0	30.0	45.0	55.0		Dominant	T/T, T/C+C/C	1.11 (0.85–1.46)	0.44
	patients	19.0	49.0	32.0	44.0	56.0		Recessive	T/T+T/C, C/C	1.04 (0.76–1.42)	0.81
								Overdominant	T/T+C/C, T/C	1.07 (0.83–1.37)	0.61
	rs2740210	A/A	A/C	C/C	A	C	0.213	Codominant	C/C, A/C, A/A	1.23 (0.94–1.60)	0.21
										1.35 (0.83–2.18)	
	controls	9.0	38.0	53.0	28	72		Dominant	C/C, C/A+A/A	1.25 (0.97–1.61)	0.085
	patients	7	35.0	58	24	76		Recessive	C/C+C/A, A/A	1.24 (0.78–1.99)	0.36
								Overdominant	C/C+A/A, C/A	1.18 (0.91–1.53)	0.2
	rs3761248	C/C	C/T	T/T	C	T	0.568	Codominant	T/T, C/T, C/C	0.87 (0.66–1.13)	0.57
										0.92 (0.54–1.55)	
	controls	6.0	21.0	62.0	22.0	78.0		Dominant	T/T, C/T+C/C	0.87 (0.68–1.13)	0.3
	patients	6.0	35.0	59.0	24.0	76.0		Recessive	T/T+C/T, C/C	0.97 (0.58–1.62)	0.89
OXT								Overdominant	T/T+C/C, C/T	0.87 (0.67–1.14)	0.31
	rs4813625	C/C	C/G	G/G	C	G	0.627	Codominant	C/C, C/G, G/G	0.92 (0.70–1.23)	0.63
										1.08 (0.76–1.55)	
	controls	20.0	47.0	33.0	44.0	56.0		Dominant	C/C,C/G+G/G	0.97 (0.74–1.26)	0.8
	patients	18.0	49.0	32.0	43.0	57.0		Recessive	C/C+C/G, G/G	1.14 (0.83–1.56)	0.43
								Overdominant	C/C+G/G, C/G	0.90 (0.70–1.15)	0.39
	rs877172	G/G	G/T	T/T	G	T	0.279	Codominant	T/T, G/T, G/G	0.79 (0.59–1.06)	0.28
										0.83 (0.59–1.17)	
	controls	23.0	43.0	34.0	44.0	56.0		Dominant	T/T, C/T+C/C	0.81 (0.62–1.05)	0.12
	patients	24.0	47.0	30.0	47.0	53.0		Recessive	T/T+C/T, C/C	0.95 (0.70–1.27)	0.72
								Overdominant	T/T+C/C, C/T	0.86 (0.67–1.11)	0.24

(*continued on next page*)

Table 3 (*continued*)

Gene	Polymorphism Ref. SNP # / Group (n)	%					*P* value	Model	Genotype	OR (95 % CI)	*P* value
	rs237887	A/A	A/G	G/G	A	G	0.273	Codominant	G/G, G/A, A/A	1.06 (0.78–1.43)	0.27
										0.82 (0.57–1.17)	
	controls	26.0	54.0	21.0	53.0	47.0		Dominant	G/G, G/A+A/A	0.98 (0.73–1.30)	0.87
	patients	25.0	50.0	25.0	50.0	50.0		Recessive	G/G+G/A, A/A	0.79 (0.58–1.06)	0.12
								Overdominant	G/G+A/A, G/A	1.16 (0.90–1.49)	0.24
	rs237902	A/A	A/G	G/G	A	G	0.073	Codominant	G/G, G/A, A/A	1.19 (0.91–1.56)	0.072
										1.58 (1.05–2.37)	
	controls	15.0	44.0	41.0	37.0	63.0		Dominant	G/G, G/A+A/A	1.27 (0.98–1.64)	0.066
	patients	11.0	42.0	47.0	32.0	68.0		Recessive	G/G+G/A, A/A	1.45 (0.99–2.13)	0.056
OXTR								Overdominant	G/G+A/A, G/A	1.08 (0.83–1.39)	0.057
	rs4686301	C/C	C/T	T/T	C	T	0.085	Codominant	C/C, C/T, T/T	0.94 (0.72–1.22)	0.083
										0.59 (0.37–0.94)	
	controls	50.0	43.0	7.0	72.0	28.0		Dominant	C/C,C/T+T/T	0.87 (0.68–1.11)	0.26
	patients	47.0	43.0	11.0	68.0	32.0		Recessive	C/C+C/T, T/T	0.61 (0.38–0.96)	0.03
								Overdominant	C/C+T/T, C/T	1.01 (0.79–1.30)	0.92
	rs7632287	A/A	A/G	G/G	A	G	0.395	Codominant	G/G, G/A, A/A	0.97 (0.74–1.28)	0.39
										1.43 (0.83–2.47)	
	controls	7.0	31.0	62.0	22.0	78.0		Dominant	G/G, G/A+A/A	1.03 (0.80–1.34)	0.81
	patients	5.0	32.0	63.0	21.0	79.0		Recessive	G/G+G/A, A/A	1.44 (0.84–2.47)	0.18
								Overdominant	G/G+A/A, G/A	0.94 (0.72–1.23)	0.67

*P-value less than 0.05 is considered significant.

polymorphism was found to have a relationship with drug addiction in this study; the codominant model (OR = 1.13, $P = 0.015$) and the recessive model (OR = 0.64, $P = 0.0056$) were significantly associated with drug addiction.

The distribution of (G/G) genotype among controls was 29.0 % compared to 21.0 % in the people with an addiction. This significant difference between the controls and patients may pose the (G/G) genotype as a protective factor for drug addiction that may decrease the risk of the disorder's development and progression. Moreover, the rs2513304 SNP showed an association with drug addiction regarding the dominant genetic model (OR = 0.74, $P = 0.021$), and the (G/G) genotype could be considered as a risk factor according to the results of the genotype frequency results as shown in Table 3. For the SNP rs4686301, the polymorphism was found to be associated with drug addiction under the recessive model (OR = 0.61, $P = 0.03$). The remaining studied polymorphisms showed no significant association, as shown in (Table 3).

The association analysis between the clinical data of the addicted individuals and the studied SNPs revealed that four SNPs were significant; rs2717162 was associated with the age at the use onset ($P = 0.02$), and rs3136541 was associated with the types of substances abused and multi substance use ($P = 0.03$ and $P = 0.04$, respectively). Additionally, rs5376 and rs694066 were associated with types of substances abused ($P = 0.02$ and P = 6e-9, respectively), as shown in Supplementary Table 1.

Supplementary Tables 2 and 5 show that the haplotype analysis of the *GAL, GALR1,* and *OXTR* gene SNPs revealed associations of specific SNP arrangements with drug addiction. It could increase the risk of susceptibility to addiction.

These arrangements were CCAAT of the GAL gene (OR = 0.63, P = 0.001) and GACT of the GALR1 gene (OR = 0.32, P = 0.045). The last significant haplotypes were (AACG) and (AACA) of the *OXYR* gene (OR = 1.59, P = 0.014, and OR = 3.67, P = 0.011, respectively). The haplotype analysis for the remaining polymorphisms showed no significant association with drug addiction among Jordanians, as illustrated in Supplementary Tables 2, 3, 4, and 5.

The calculation of D′ values, a standardized measure of linkage disequilibrium (LD), and their corresponding P-values were conducted for all SNP pairs within our sample. Supplementary Tables 6, 7, 8, and 9 show that most SNPs exhibited strong and statistically significant linkage disequilibrium.

In our efforts to uncover the correlation between haplotypes and clinical and demographic conditions, we observed a correlation between the specific haplotypes and these conditions, as shown in Supplementary Table 10.

4. Discussion

Drug Addiction is a complex chronic disorder that is caused by a combination of genetic, environmental, and drug-induced factors (Buisman-Pijlman et al., 2014, Randesi et al., 2020). Stress is a severe risk factor affecting both the development and the relapse of addiction behaviour and disorders (Levran et al., 2014a, Randesi et al., 2020). Therefore, it is essential to determine the genetic vulnerability to drug addiction by investigating genetic variants of genes biologically involved in drug metabolism and transportation (Hawes et al., 2008, Baracz et al., 2020, Al-Eitan et al., 2020). The neuropeptide galanin is a well-defined stress-related gene that has been reported as a protective factor against the progression of drug abuse (Al-Eitan et al., 2012b, Hawes et al., 2008). This study aimed to identify the association between stress-related genes variants and drug addiction in Jordan. We analyzed 18 variants in four stress-related genes in 500 Jordanians addressed as addicted males in addition to 500 healthy controls; the *GAL* gene (rs2513304, rs3136544, rs1546309, rs3136541, and rs694066), the *GALR1* gene (rs2717162, rs5374, rs5376, rs1942578, and rs9807208), the *OXT* gene (rs2740210, rs3761248, rs877172 and rs4813625), and the *OXTR* gene (rs877172, rs237887, rs237902, and rs4686301).

The genetic association between the studied polymorphisms of the *GAL* and *GALR1* genes and drug addiction revealed that three SNPs of the *GAL* gene; rs3136541 $P = 0.0039$), rs694066 ($P = 0.0042$) and rs3136544 ($P = 0.015$) are highly associated. On the other hand, the remaining polymorphisms showed no significant association with drug addiction ($P>0.05$). The association analysis between the clinical data of the addicted individuals and the studied SNPs revealed an association with five SNPs; rs2717162 was associated with the age at substance use onset ($P = 0.02$). rs3136541 was associated with the types of substances abused and the number of used substances ($P = 0.03$ and $P = 0.04$, respectively). rs5376, rs694066, and rs3136544 were associated with the types of substances ($P = 0.02$, $P =$ 6e-9, and $P = 0.03$, respectively) (Supplementary Table 1).

The *GALR1* and *GAL* genes are highly expressed in the regions of the human brain that are critical for drug and cue-induced craving and implicated in emotional behavior (Hawes et al., 2008, Gold et al., 2012, Online, 2013). Functional changes in the *GALR1* gene could lead to a

depletion in dopamine release, resulting in reduced drug reward (Lori et al., 2011, Richardson et al., 2014). Many studies indicated that variations in the *GALR1* and *GAL* genes affect the probability of developing a tobacco addiction, the risk of relapse, and the efficacy of smoking cessation aids (Richardson et al., 2014).

Our study revealed that *GAL* SNP rs3136541 had a significant association with drug addiction, and the genotype (TT) /the (T) allele could be assigned as a protective factor against drug addiction among Jordanians. Our results are in accordance with studies in African–American and European populations (Randesi et al., 2020). A plausible correlation in this current study was detected between rs694066 and drug addiction ($P = 0.0042$), and our findings suggest that the GG genotype/ G allele may be considered a factor in increasing the risk of addiction. However, the variant rs694066 was implicated with weight loss from alcoholism in Finnish and Native American males (Levran et al., 2008, Belfer et al., 2006, Holmes and Picciotto, 2006). The substantial findings suggest that galanin may act as a protective or increased risk factor for drug addiction depending on the variant's distribution within different populations (Jackson et al., 2011, Unschuld et al., 2010, Lori et al., 2011).

The association analysis in our study between the *GALR1* polymorphisms studied and the substance use disorders (SUD). SUD was non-significant. In contrast to other studies, a nominal association between *GALR1* SNPs rs5376 and drug addiction was estimated in the African population. At the same time, rs2717162 was claimed to be correlated with drug addiction in African–Americans (Levran et al., 2014b, Randesi et al., 2020). Different studies found that the (G) allele of rs948854 has a gender-specific association with anxiety severity (female carriers of the G-allele were more anxious) (Randesi et al., 2020). Certain studies showed an association between *GALR1* SNP rs2717162, which is associated with craving for tobacco in European and African-American smokers (Levran et al., 2014b, Gold et al., 2012), and rs3136541 with alcoholism in European ancestry that could increase alcohol consumption (Levran et al., 2014b, Jackson et al., 2011, Richardson et al., 2014). Various studies have suggested that the *GALR1* SNP rs9807208 reduces responses to the use of several drugs of abuse, such as cocaine, morphine, amphetamines, and opioids (Richardson et al., 2014, Al-Eitan et al., 2012a). Otherwise, several studies have reported no association between rs9807208 and drug addiction, which is consistent with this study in Jordan (Hawes et al., 2008, Richardson et al., 2014).

Numerous studies have revealed that *OXT* gene expression in the brain is altered due to exposure to alcohol and drugs like nicotine, cocaine, methamphetamine, and morphine (King et al., 2020). A variant in the *OXT* gene (rs4813625) was found in association with adolescent alcohol drinking, which is mediated by an interaction with the dopamine system and is sex dependent (Buisman-Pijlman et al., 2014). Furthermore, two polymorphisms (rs4564970 and rs1488467) of the *OXTR* gene appeared to moderate the aggressive behaviour of alcohol in adult men (Buisman-Pijlman et al., 2014, Baracz et al., 2020). In contrast to our study, which showed a non-significant association between the studied polymorphisms of oxytocin and oxytocin receptor genes and drug addiction in Jordanian males. As appeared from the haplotype analysis for the studied polymorphisms of the *GAL* and *OXT* genes and their receptors, there was no association with drug addiction except for four arrangements that could increase the risk of addiction in the Jordanian population; (CCAAT) of the GAL gene (OR = 0.63, P = 0.001), (GACT) of the GALR1 gene (OR = 0.32, P = 0.045), and (AACG) and (AACA) of the OXYR gene (OR = 1.59, P = 0.014, and OR = 3.67, P = 0.011, respectively).

The relationship between SNPs and addiction might be non-linear. Traditional correlation measures capture linear relationships and might miss complex, non-linear interactions (Moore and Williams, 2002). Interactions with other genetic, environmental, or lifestyle factors could influence the effect of SNPs on addiction. These interactions might mask a direct correlation but still result in significant associations when analyzed through more complex statistical models that account for these interactions (Moore and Williams, 2002, Phillips, 2008). In addition, significant associations might be present in specific population subgroups, such as individuals with certain genetic backgrounds or environmental exposures. These subgroup-specific effects could lead to significant overall associations without a clear correlation in the general population (Marchini et al., 2004; Thomas and Witte, 2002). Confounding factors might also play a role. There could be other variables that influence both the SNPs and addiction, creating an indirect pathway that leads to significant associations (Thomas and Witte, 2002, Smith and Ebrahim, 2003). Advanced statistical techniques can adjust for these confounders to reveal true associations. Addiction is a complex trait influenced by multiple genetic and environmental factors. The genetic architecture of addiction might involve many small-effect SNPs contributing to the risk in an intricate manner that is not captured by simple correlation measures (Manolio et al., 2009, Kreek et al., 2005).

5. Conclusion and future directions

Drug addiction is a multifactorial disorder worldwide. Stress-related genes such as GAL play a crucial role in the development and risk of addiction, thus improving diagnosis and treatment. Our study investigated the association between several SNPs in four stress genes (GAL, OXY, and their receptors). Our analysis revealed that several SNPs in stress-related genes, especially in the GAL gene, were strongly associated with drug addiction among Jordanians. This may contribute to an increased or decreased risk of developing the disorder. To the best of our knowledge, no studies have identified a direct link between the GAL gene and OXT in terms of stress and drug addiction. Additional research is needed to investigate this area. The present study had other limitations that should be acknowledged. First is the omission to assess psychiatric comorbidity in patients with substance use disorders (SUD). We were unable to collect data on dual diagnoses in addicted patients. Future studies should address this area to provide a more comprehensive understanding of SUD. Integrating genetic findings into the pharmacological treatment of SUD and dual diagnosis holds significant promise. Identifying genetic variations in stress-related genes associated with addiction may have clinical implications. It determines therapy targets and supports treatment alternatives proposed by animal studies. It also offers the potential to improve therapeutic interventions by identifying patients at specific risk for stress-related relapse and patients who may benefit from particular interventions depending on their genotype, particularly early in the addiction cycle(Al-Eitan and Haddad, 2014; Al-Eitan and Tarkhan, 2016; AL-Eitan, 2020).

Genome-wide association study (GWAS) is a successful candidate gene study approach. However, a critical question is whether GWAS can be effectively used in diverse populations to understand the associations between genotype and substance use disorder more comprehensively. Therefore, further association and functional studies, a much larger sample size, including patients from different ethnic groups, and broader SNP coverage are needed to confirm these findings further and determine the role of GAL, OXY, and their receptors as well as the other genes variations indicated, as contributing factors to drug addictions.

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CRediT authorship contribution statement

Laith AL-Eitan: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hana Abu Kharmah:** Writing – review & editing, Writing – original draft, Investigation,

Formal analysis. **Mansour Alghamdi:** Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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