Association of *PICK1* and *BDNF* Variations with Increased Risk of Methamphetamine Dependence Among Iranian population; A Case-Control Study

Amir Tajbakhsh
Shiraz University of Medical Sciences https://orcid.org/0000-0002-2311-6554
Maliheh Alimardani
Mashhad University of Medical Sciences
Mahla Asghari
Mashhad University of Medical Sciences
Soheila Abedini
Mashhad University of Medical Sciences
Sohrab Saghafi Khadem
Mashhad University of Medical Sciences
Abolfazl Nesaei Bajestani
Mashhad University of Medical Sciences
Forough Alipoor
Islamic Azad University of Torbat-e Jam
Maryam Alidoust
Mashhad University of Medical Sciences
Amir Savardashtaki
Shiraz University of Medical Sciences
Peyman Hashemian
Mashhad University of Medical Sciences
Alireza Pasdar (PasdarA@mums.ac.ir)
https://orcid.org/0000-0002-7864-9729

Research article

Keywords: Addiction, drug abuse, substance dependence, substance use disorder (SUD), dopamine pathway, glutamate pathway, variations, and polymorphisms

Posted Date: July 20th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-42133/v1

Abstract

Background: Genetic factors play an important role in susceptibility to methamphetamine. In this line, protein that interact with C-kinase-1 (PICK1) and brain-derived neurotrophic factor (*BDNF)* genes are linked to methamphetamine dependence (substance use disorder). Thus, in a case-control study, we investigated the association between polymorphisms of *PICK1* and *BDNF* genes and methamphetamine dependence in an Iranian population from 2015 to 2018.

Methods: Total of 235 cases and 204 controls were recruited. The *PICK1-rs713729*, *-rs2076369* and *BDNF-rs6265* genotypes were determined *via* ARMS-PCR assay. Statistical analysis was performed, using SPSS 20.0, PHASE 2.1.1 program as well as SNP Analyzer 2.0.

Results: In the present study, two polymorphisms including *PICK1-rs713729* (OR: 1.38 (CI: 1.08-1.52; $P\text{-}_{value}$: 0.004) in multiplicative and dominant models, and *PICK1-rs2076369* (OR: 1.31 (CI: 1.10-1.56; $P\text{-}_{value}$: 0.002) in multiplicative, dominant and co-dominant models were associated with the risk of methamphetamine abuse. Moreover, haplotype analysis methods showed a significant association between haplotype AG (OR: 2.50 (CI: 1.50-4.16; $P\text{-}_{value}$: 0.0002) in dominant, recessive and co-dominant models, and haplotype TT (OR: 0.67 (CI: 0.50-0.91; $P\text{-}_{value}$: 0.009) in dominant model and co-dominant model with the risk of methamphetamine abuse. None of the polymorphisms in this study had a high level of linkage disequilibrium.

Conclusion: Our findings indicate that the *PICK1* gene might be linked to methamphetamine dependence. Therefore, it can be stated that PICK1 might play a significant role in the pathophysiology of methamphetamine dependence in an Iranian population.

Background

Methamphetamine is the most extensively used illegal drug, which is a growing global problem [1–3]. Many changes occur in the human brain as a result of methamphetamine usage [4]. Methamphetamine leads to the release of synaptic dopamine, which might be a reason for the increased addiction [4]. In this line, methamphetamine can have neurotoxic effects on dopaminergic neurons and also contribute to changes in another neurotransmitter system, particularly glutamate [5, 6]. Glutamate-mediated excitotoxicity is the major mechanism by which methamphetamine damages the central nervous system (CNS) [7, 8]. Moreover, the primary target of methamphetamine is the dopamine transporters, which remove dopamine from the extracellular space at the synapse and controls dopamine signals [9]. The function and surface availability of the dopamine transporter are regulated *via* different cellular mechanisms [10, 11]. Additionally, it was stated that the density of dopamine transporter is low in the caudate/putamen of methamphetamine abusers, suggesting that the long-term use of methamphetamine results in damage to dopaminergic neurons [12, 13].

Several factors are involved in methamphetamine abuse. In this line, genetic factors play an important role in susceptibility to the use of methamphetamine [1–3]. The dopamine transporter, polymorphisms have been shown to be a risk factor for prognosis of prolonged-type methamphetamine psychosis, such as a single nucleotide polymorphism (SNP) [14, 15]. Moreover, in a systematic review, Bousman *et al.* showed that several genes are involved in methamphetamine-related disorders. In this line, three genes including catechol-O-methyltransferase (*COMT*), gamma-aminobutyric acid type A receptor subunit alpha1 (*GABRA1*) and dopamine receptor D4 (*DRD4*), nine genes including, brain-derived neurotrophic factor (*BDNF*), arrestin beta 2 (*ARRB2*), cytochrome P450 2D6 (*CYP2D6*), glutathione S-transferase mu 1 (*GSTM1*), glycine transporter-1 (*GLYT1*), glutathione S-transferase P1 (*GSTP1*), solute carrier family 22 member 3 (*SLC22A3*), prodynorphin (*PDYN*), and protein interacting with C kinase (*PICK1*), two genes including gamma-aminobutyric acid receptor subunit gamma-2 (*GABRG2*) and v-akt murine thymoma viral oncogene homolog 1 (*AKT1*) seems to be associated with methamphetamine abuse or dependence in Japanese and Han-Chinese populations [15]. It is indicated that the genetic epidemiology of methamphetamine abuse is very complex. There is an association between *PICK1* gene and drug addiction, such as methamphetamine abuse [16]. In line, the *PICK1* gene is mapped to chromosome *22q13.1* [17]. PICK1 interacts with dopamine transporter, which leads to the clustering of dopamine transporter on the cell surface and subsequently the improvement of dopamine transporter uptake activity [16, 18].

Furthermore, *BDNF-rs6265* is a functional SNP, which is accompanied with drug dependency [19]. *BDNF* gene is mapped to chromosome *11p*. It was found that *BDNF-rs6265* (Val66Met) SNP is linked to susceptibility to methamphetamine dependence in a Thai and Malaysian population [20–22]. In this respect, BDNF proteins are involved in the regulation of synaptic transmission as well as the process underlying substance use disorder (SUD) [23]. BDNF was shown to support the survival and protection of dopaminergic neurons following methamphetamine administration in mice [6]. The functional effects of SNPs, which are linked to SUD, are often unclear; hence, more investigations are required on different populations to define how these variants influence gene expression and function. In this case, worldwide participatory attempts are warranted to promote the accessibility of large population-based datasets/samples and to increase the ability of genetic associations [15]. On the other hand, a systematic review by Alam-MehrJerdi *et al.* indicated that the state of methamphetamine abuse requires further research on the epidemiology and health-related implications in Iran [24]. Hence, we attempt to investigate the association between *PICK1* and *BDNF* main SNPs and methamphetamine dependence (substance use disorder (SUD)) individuals in an Iranian population.

Methods

The aim, design and setting of the study

Data collection and analysis are important components of research. In this line, this case-control study involved 235 cases (with methamphetamine dependence (SUD) and 204 gender-matched controls (healthy individuals). In our study, SUD is recognized by the 11 criteria. The 11 criteria are divided into four categories of behavior, such as impaired control, social impairment, risky use and pharmacological indicators (tolerance and withdrawal) related to the substance use (Table 1) [25, 26].

Table 1
11 criteria for substance use disorders (SUD).

Num	Categories of behaviour	Criteria for Substance Use Disorders (SUD)
1	Impaired control	**Used larger amounts or longer**: Taking the drug in greater quantities or over prolonged periods of time.
2		**Repeated attempts to control use and/or quit**: Wanting to cut or avoid using the substance, but they haven't been successful.
3		**Much time spent using**: Spending a lot of time to get, using, or recover from substance using.
4		**Craving**: Cravings and encourages the substance to be used.
5	Social impairment	**Activities given up to use**: Not able to do what you can do at home, at work, or at school that you once liked because of substance use.
6		**Social or interpersonal problems related to use**: Continuing to use, even though it creates issues in your relationships or conflicts with others.
7		**Neglected major roles to use**: Giving up and refusing to perform significant social, occupational or recreational functions as a result of substance use.
8	Risky use	**Hazardous use**: Using substances again and again, including though you or others are in danger.
9		**Social or interpersonal problems related to use**: Continuing to use, even though you know that you have a physical or psychological condition which may have been triggered or exacerbated by the substance.
10	Pharmacological indicators	**Tolerance**: Need more substance to have the effect you like.
11		**Withdrawal**: Development of withdrawal symptoms and signs of withdrawal, which can be eased by taking more of the substance.

The *PICK1-rs713729*, *PICK1-rs2076369* and *BDNF-rs6265* genotypes were analyzed by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) assay. Subsequently, statistical analysis was performed, using SPSS 20.0 (IBM Inc., Chicago, IL, USA), PHASE 2.1.1 program as well as SNP Analyzer 2.0.

Table 1: 11 criteria for substance use disorders (SUD).

The Characteristics Of Participants

Study population and clinical data

This study was performed in accordance with the Declaration of Helsinki (1964) and its subsequent amendments. Moreover, approval was obtained from the local Ethics committee of Mashhad University of Medical Sciences (IR.MUMS.fm.REC.1394.421), 439 blood samples were collected from 204 controls and 235 cases from Mashhad, Iran from 2015 to 2018. After explaining the study objectives, a written informed consent was obtained from all participants. A questionnaire was used to collect demographic and other essential information from all participants (Table 2). The selection procedure included confirmed urine test (addiction test) and also the availability of complete patient's follow-up data. Moreover, healthy, individually matched on age were recruited from the Health Examination Centre, who were receiving routine medical examinations.

Table 2
Demographic and clinical characteristics of controls and methamphetamine dependence (SUD).

Variable		Controls	Methamphetamine dependence (SUD)	P-*value*
Gender	Female	38.9%	35.7%	P < 0.001
	Male	68.1%	64.3%	
Age		31.96 (8.44 ± 0.58)	38.91 (9.11 ± 0.68)	P = 0.423
Marriage status	Married	56.7%	43.4%	P < 0.001
	Separated	-	11.6%	
	Widow	-	4.2%	
	Divorced	1.1%	20.6%	
	Single	42.1%	20.1%	

Table 2: Demographic and clinical characteristics of controls and methamphetamine dependence (SUD).

Description Of Materials

Blood collection and DNA extraction

For DNA extraction, approximately 10 millilitres (ml) of peripheral blood was obtained from each participant and immediately subdivided into tubes containing sterile ethylene diamine tetra acetic acid (EDTA) [27]. DNA extraction from whole blood was extracted using salting-out technique. Then, the extracted DNA was quantified by the ratio of absorbance at 260 nanometres (nm) and 280 nm ($A_{260/280}$) *via* BioTek™ Epoch™ Microplate Spectrophotometer (Winooski, VT, USA,) as well as *via* gel electrophoresis and finally stored at -20 °C until used.

Target Single Nucleotide Polymorphisms (snps) Determinations (marker Selection)

In this study, the SNPs were selected using available SNPs data bases and published articles. Such articles were examined intron and exon SNPs, which might alter the affinity of *PICK1-rs713729*, *PICK1-rs2076369* and *BDNF-rs6265* to methamphetamine dependence (SUD) (Table 3). Moreover, potential functional SNPs were included in order to meet the following criteria: minor

allele frequency (MAF) > 0.05 (5%), heterozygosity > 0.15 (15%) and also validated SNPs in articles and databases. Furthermore, in order to inhibit redundancy in SNPs genotyping, SNPs that are not located in strong linkage disequilibrium (LD) were chosen.

Table 3
The investigated polymorphisms characteristics in this study.

Rs number	Gene	Protein	Position	Exon/ Intron	Variant length	Allele	Function	Haplotype distance (bp)
rs713729	*PICK1*	Non-coding	22:38059462	Intron 3	1	T > A	Intron variant	8183
rs2076369	*PICK1*	Non-coding	22:38067645	Intron 4	1	T > G	Intron variant	
rs6265	*BDNF*	NP_001137277.1:p.Val66Met	11:27658369	Exon 4	1	C > T	Missense	-

Table 3: The investigated polymorphisms characteristics in this study.

Genotyping

To determine the genotype frequency of *PICK1-rs713729*, *PICK1-rs2076369* and *BDNF-rs6265* an ARMS-PCR method was used. Specific primers for PCR amplification were designed *via* web tools, such as Primer1 and also WASP (web-based allele-specific primer designing tool) [28].

PCR amplifications for *PICK1-rs713729, PICK1-rs2076369* and *BDNF-rs6265* were conducted in a 10–15 microliter (μl) volume per reaction, containing 3 μl Taq 2x master mix (Ampliqon, Germany), 10 μM of each primer and 100 nanogram (ng) DNA. Moreover, the specific primers used to detect *PICK1-rs713729, PICK1-rs2076369* and *BDNF-rs6265* SNPs are listed in Table 4. For *PICK1-rs2076369*, we also used Betaine (Ampliqon, Germany) as an enhancer in PCR.

Table 4
Primer sequences used for genotyping in ARMS-PCR.

SNPs	Primers	Sequences	Primer Length	PCR products
rs6265	FO	CTACAGTTCCACCAGGTGAGAAGAGTG	27	400
	RO	ATGGACATGTTTGCAGCATCTAGGTA	26	
	FI©	TGGTCCTCATCCAACAGCTCTTCTATaAC	29	253
	RI(t)	TTGGCTGACACTTTCGAACcCA	22	201
rs713729	FO	CTTTCTAGCGGAATCCCGACTGTG	24	407
	RO	CAGTGAAAAAGCAAACCAGGACACTG	26	
	FI(a)	CTTCTCATTCTTGAGGTCTGACCCACA	27	196
	RI(t)	AGGTGGTCAGAAAGCCCCTCAGA	23	265
rs2076369	FO	CATGTTGCCCAAGCTGGTCTCAAACTC	27	299
	RO	CTGGACACCCGTAACTGCTCTGACC	35	
	FI(g)	AGGAGTCTCAGTCCAGAACAGTCTTGACG	29	191
	RI(t)	CTCCACACCCTGAGCCCCTTCTCA	24	165

FOP: Forward outer primer; **FIP**: Forward inner primer; **RIP**: Reverse inner primer; **ROP**: Reverse outer primer.

The ARMS-PCRs condition for each primer is as follows, Table 5. In general, initial denaturation at temperature 94 °C for five minutes, then 35 cycles including denaturation at 94 °C for 25 seconds, annealing at alternative °C for 25 seconds (based on each primer), an elongation at 72 °C for 30 seconds followed by 72 °C for seven minutes as the final elongation step (Table 5).

Table 5
The ARMS-PCRs condition for targeted SNPs was as follows.

		First Denaturation		**35 cycles**						**Last extension**	
SNPs	Primers	Tm	Min	Denaturation		Annealing		Extension		Tm	Min
				Tm	Min	Tm°C	Min	Tm	Min		
rs6265	FO	94 °C	7 min	94 °C	30 s	61.5	25 s	72 °C	45 s	72 °C	7 min
	RO										
	FI©										
	RI(t)										
rs713729	FO					61	30 s		30 s		5 min
	RO										
	FI(a)										
	RI(t)										
rs2076369	FO					64	25 s		30 s		5 min
	RI(t)										
	FI(g)					67	30 s				
	RO										

FOP: Forward outer primer; **FIP**: Forward inner primer; **RIP**: Reverse inner primer; **ROP**: Reverse outer primer; **TM**: Temperature; **Min**: Minute; **S**: Seconds; **°C**: Centigrade;

The DNA fragments of PCR products *via* the absence or presence of bands unique for mutant or wild primers were detected, using electrophoresis in 2.5-3% agarose gel by ultraviolet (UV) trans illuminator (Gel Doc; U:Genius).

Table 4: Primer sequences used for genotyping in ARMS-PCR.

Table 5: The ARMS-PCRs condition for targeted SNPs was as follows.

Statistical analysis

A Hardy–Weinberg equilibrium (HWE) method was used to evaluate the differences in data for statistical significance. HWE assumption was investigated by the Pearson χ^2 distribution with 1 degree of freedom. Allele and genotype frequencies were calculated, and the differences between groups were evaluated by Chi-squared tests. Then, the association between methamphetamine, risk factors and alleles/genotypes was evaluated by binary logistic regression, estimating Odds ratios (ORs) and also 95% confidence intervals (CIs). Three logistic regression models were used to analyse the SNPs, using different genetic models (additive, dominant, and recessive). For the analysis of SNP-SNP interactions, an adjusted logistic regression model was used to estimate the multiplicative interaction effect of the SNPs, located on the same haplotype. $P\text{-}_{value} = 0 < 0.05$ was considered to be statistically significant. SPSS 20.0 (Inc., Chicago, IL, USA), PHASE program as well as SNP Analyser 2 software were used for further statistical analysis [29].

Haplotype Analysis

Haplotypes were generated and assembled from the genotyped data by PHASE program, to reconstruct haplotypes, and SNP Analyzer 2 software [29, 30]. In the present study, P-$_{values}$ of less than 0.05 were considered to be statistically significant. Moreover, Bonferroni correction was also used to account for multiple testing; thus, a two-tailed P-$_{value}$ < 0.016 (=0.05/3 SNPs) was considered to be statistically significant in the present study.

Results

Identification of single nucleotide polymorphisms (SNPs) and association studies

There were no significant differences between *BDNF-rs6265* and the risk of methamphetamine dependence (SUD). On the contrary, a significant difference was observed between two SNPs; *PICK1-rs713729* (OR: 1.38 (CI: 1.08–1.52; P-$_{value}$: 0.004) in multiplicative and dominant models, and *PICK1-rs2076369* (OR: 1.31 (CI: 1.10–1.56; P-$_{value}$: 0.002) in multiplicative, dominant and co-dominant models were shown to be associated with the risk of methamphetamine dependence (SUD) (Table 6). Moreover, haplotype analysis showed that specific haplotypes related to these SNPs were accompanied by methamphetamine dependence (SUD) individuals. In this line, analysis of haplotypes in our population between *PICK1-rs713729* and *PICK1-rs2076369* showed that haplotype AG (OR: 2.50 (CI: 1.50–4.16; P-$_{value}$: 0.0002) in dominant, recessive and co-dominant models and haplotype TT (OR: 0.67 (CI: 0.50–0.91; P-$_{value}$: 0.009) in dominant model and co-dominant model have presented a significant association with the risk of methamphetamine dependence SUD) (Table 7).

Table 6
Case-Control study- Association study.

SNP number	Gene	Position	Genetic models	OR	95%CI	P$_{-value}$	Bonferroni correction
							P$_{-value}$
rs713729	*PICK1*	*22: 38059462*	Multiplicative	2.12	1.34–3.36	0.001	0.003
			Dominant	2.08	1.21–3.57	0.007	0.020
			Recessive	2.51	0.97–6.52	0.05	0.149
			Co-dominant	2.71	1.04–7.06	0.02	0.061
rs2076369	*PICK1*	*22: 38067645*	Multiplicative	0.68	0.05–0.91	0.009	0.028
			Dominant	0.51	0.34–0.76	0.0008	0.003
			Recessive	0.92	0.51–1.67	0.806	-
			Co-dominant	0.64	0.34–1.21	0.003	0.008
rs6265	*BDNF*	*11: 27658369*	Multiplicative	1.09	0.74–1.61	0.65	-
			Dominant	1.05	0.68–1.63	0.81	-
			Recessive	1.75	0.43–7.13	0.64	-
			Co-dominant	1.76	0.43–7.18	0.89	-

PICK1: Protein interacting with C-kinase-1; BDNF: Brain-derived neurotrophic factor.

Table 7
Case-Control study- Haplotype analysis.

Genetic models	ID	P_{value}	OR	Lower CI	Higher CI
Multiplicative	H3	**0.0002**	2.5	1.502	4.161
	H2	**0.009**	0.678	0.505	0.91
	H1	0.742	1.047	0.794	1.382
	H4	0.745	0.84	0.292	2.416
Dominant	H2	**0.001**	0.532	0.358	0.79
	H3	**0.007**	2.123	1.217	3.704
	H1	0.735	0.924	0.582	1.466
	H4	0.762	0.838	0.266	2.643
Recessive	H3	**0.003**	11.569	1.499	89.293
	H1	0.436	1.175	0.782	1.766
	H4	0.555	0.842	0.052	13.549
	H2	0.651	0.867	0.466	1.611
Co-dominant	H3	**0.004**	12.331	1.595	95.316
	H2	**0.006**	0.627	0.326	1.206
	H1	0.571	1.044	0.620	1.757
	H4	0.840	0.838	0.052	13.492

H1: TG; **H2**: TT; **H3**: AT; **H4**: AG.

Table 6: Case-Control study- Association study.

Table 7: Case-Control study- Haplotype analysis

Distribution Of Single Nucleotide Polymorphisms (snps)

Table 6 shows the frequency of the genotypes and the allele frequencies of all the tested SNPs. In this study, two haplotypes, AG and TT were significantly different between the normal and methamphetamine dependence (SUD) individuals. The prevalence of TT haplotype in the case group (27%) was lower than that of the normal individuals (34%), and the frequency of the AG haplotype in the methamphetamine dependence (SUD) individuals was higher (12%) than that (3%) of the normal individuals (Table 8). We also examined whether the three SNPs were in LD. In this line, there was no strong LD between these SNPs (Table 9).

Table 8
Haplotype frequencies in population.

Control samples			
No	Haplotype_ID	Sequence	Frequency
1	H1	TG	0.57898
2	H2	TT	0.34348
3	H3	AT	0.04155
4	H4	AG	0.03599
Case samples			
No	Haplotype_ID	Sequence	Frequency
1	H1	TG	0.57417
2	H2	TT	0.27492
3	H3	AG	0.12853
4	H4	AT	0.02237
Case & control samples			
No	Haplotype_ID	Sequence	Frequency
1	H1	TG	0.57309
2	H2	TT	0.30956
3	H3	AG	0.08951
4	H4	AT	0.02785

Table 9
LD map and LD block.

Control samples							
No	Marker1	Marker2	Distance	|D'|	r2	LOD	P_value
1	0	1	8183	0.26	0.00915	1.629	0.06
2	0	2	10401093	0.43	0.00271	1.055	0.31
3	1	2	10409276	0.023	0.00014	-0.019	0.82
Case samples							
No	Marker1	Marker2	Distance	|D'|	r2	LOD	P_value
1	0	1	8183	0.49	0.01879	6.88	0.003
2	0	2	10401093	0.07	0.00017	0.062	0.786
3	1	2	10409276	0.075	0.00244	0.719	0.29
Case & control samples							
No	Marker1	Marker2	Distance	|D'|	r2	LOD	P_value
1	0	1	8183	0.28	0.00540	3.379	0.03
2	0	2	10401093	0.17	0.00074	0.540	0.43
3	1	2	10409276	0.07	0.00184	0.287	0.21
Marker1: rs713729; **Marker2**: rs2076369; **Marker3**: rs6265; **LOD**: Logarithm of odds;							

Table 8: Haplotype frequencies in population.

Table 9: LD map and LD block.

Discussion

The SUD is a persistent relapsing disorder with harmful consequences [31]. In this respect, the brain reward system such as mesocortical dopamine system is the common feature of this disorder [32, 33]. Furthermore, abnormal dopamine and glutamate systems are associated with the pathophysiology of SUD and dependency. However, the development of drug dependency is affected *via* factors, such as pharmacological effects on mental status, environmental and individual factors, such as genetics. In this respect, genetic factors are suggested to have a greater effect on drug dependency. It was shown that drugs abuse and addiction cause dopamine and opioid peptides to be released into the ventral striatum, which causes “high” sensation in abusers [33, 34].

The use of methamphetamine has become a serious health concern in Iranian methadone dependent patients [24, 35]. In Iran, it is used to improve sexual performance and promote physical energy due to its stimulating effects [36]. It is shown that methamphetamine abuse has surged from 3.9% amongst both genders in 2007 to 60.3% for men in 2014 and 89.5% for women in 2015–2016. Remarkably, the frequency of methamphetamine dependence amongst female methadone dependent patients in the Iranian population was higher. This dependency has been linked to multiple health issues in the social and health contexts of both genders, particularly women [35].

The key findings of the present study were to discover any association between *PICK1-rs713729* and *PICK1-rs2076369* in the *PICK1* gene promoter and methamphetamine dependence individuals. Consistent with our study, Matsuzawa *et al.* showed that *PICK1-rs713729* and *PICK1-rs2076369* were significantly associated with methamphetamine abuse in a Japanese population. Additionally, they revealed that *PICK1-rs713729* was linked to those with spontaneous relapse of psychosis [16]. Moreover, *PICK1*

gene was reported as the beginning of methamphetamine addiction, worse prognosis as well as spontaneous relapse [37]. *PICK1* is involved in the targeting and localization of synaptic membranes proteins and also in the surface of dopamine transporter clustering [18, 38].

Based on previous studies, it was reported that gene variations, which are associated with glutamatergic systems leads to differences between individuals in the risk of SUD and/or dependency. It is indicated that glutamate-related genes are involved in the risk of SUD and/or dependency. The glutamate receptor genes were suggested to interact with BDNF by BDNF- tropomyosin kinase B (TrKB) transduction signaling cascade. BDNF is a neurotrophic factor, which influences the expansion, maintenance and survival of dopaminergic neurons in CNS [39]. In the present study, no association was found between *BDNF-rs6265* and the risk of methamphetamine dependence (SUD) in Iranian population. This finding is consistent with the result of methamphetamine dependent male Caucasian individuals, which showed no association in 193 non-psychotic males (117 methamphetamine-dependent cases and 76 controls) [40]. However, Cheng *et al.* showed that there was an association between methamphetamine-dependency and *BDNF* gene in 103 methamphetamine abusers and 122 normal controls. They noted that the lower 66Met carriers were linked to substance abuse [21]. Further, Sim *et al.* described an increase in a Chinese subgroup of Malaysian methamphetamine-dependent subjects (n = 24), which was not found among other Malaysian ethnic groups [22]. The various results for this SNP might be due to different sample sizes and also different ethnicities as well as genetic diversity.

Conclusion

Collectively, the variation in the *PICK1* gene was associated with methamphetamine dependence (SUD), which is significant in the discovery of biological mechanisms, making a bridge between pathways and methamphetamine dependence (SUD). Our findings suggest that the *PICK1* gene might be involved in susceptibility to SUD and therefore PICK1 might play a role in its pathophysiology in the Iranian population. These findings can be helpful in rehabilitation programs and psycho-education for those who have substance dependency. In this case, potential genetic predictors can be used for individual susceptible to SUD. All in all, understanding genetic variations might help to understand the biological mechanisms of progression, suppression and accuracy of methamphetamine. In addition, our findings provide the basis for future genetic research as well as functional studies on the use of methamphetamine and other neurological disorders in Iranian population.

Declarations

Ethics approval and consent to participate

The study was approved by the Mashhad University f Science Ethics Committee (ethical approval code: IR.MUMS.fm. REC.1394.421).

In this context, written informed consent has been obtained from all individuals.

Consent to publish

All individuals whose data are described have signed a written informed consent form.

Availability of data and materials

Data sharing is not applicable to this article because no datasets were generated or analyzed during this study.

Competing interests

All authors have read and declare that they have no competing interest in this article.

Funding

This study was financially supported by the Mashhad University of Medical Sciences (Grant Number: 931681).

Authors' contributions

AT: Conception and design, sample and data collection, extraction of genomic DNA, genome genotyping, data analysis and writing the manuscript; **MA**: Conception and design, sample data collection, extraction of genomic DNA, genome genotyping, and data analysis; **MA**: Sample and data collection, extraction of genomic DNA, and genome genotyping; **SA**: Sample and data collection, extraction of genomic DNA, and genome genotyping; **SSKh**: Sample and data collection, and data analysis; **ANB**: Sample and data collection; **FA**: Data collection; **MA**: Data collection; **AS**: Data analysis and critical review; **PH**: Conception and design, supervision of the project, approval of the manuscript, overall responsibility; **AP**: Conception and design, supervision of the project, approval of the manuscript, overall responsibility. All authors have read and approved the manuscript.

Acknowledgements
Authors wish to thank Mashhad University of Medical Sciences.

References

1. Kendler KS, Karkowski LM, Neale MC, Prescott CA. Illicit psychoactive substance use, heavy use, abuse, and dependence in a US population-based sample of male twins. Arch Gen Psychiatry. 2000;57(3):261–9.
2. Uhl GR, Liu QR, Naiman D. Substance abuse vulnerability loci: converging genome scanning data. Trends Genet. 2002;18(8):420–5.
3. Goldman D, Oroszi G, Ducci F. The genetics of addictions: uncovering the genes. Nat Rev Genet. 2005;6(7):521–32.
4. Volkow N, Li TK. The neuroscience of addiction. Nat Neurosci. 2005;8(11):1429–30.
5. Kerdsan W, Thanoi S, Nudmamud-Thanoi S. Changes in the neuronal glutamate transporter EAAT3 in rat brain after exposure to methamphetamine. Basic Clin Pharmacol Toxicol. 2012;111(4):275–8.
6. Joyce JN, Renish L, Osredkar T, Walro JM, Kucera J, Dluzen DE. Methamphetamine-induced loss of striatal dopamine innervation in BDNF heterozygote mice does not further reduce D3 receptor concentrations. Synapse. 2004;52(1):11–9.
7. Mark KA, Soghomonian JJ, Yamamoto BK. High-dose methamphetamine acutely activates the striatonigral pathway to increase striatal glutamate and mediate long-term dopamine toxicity. J Neurosci. 2004;24(50):11449–56.
8. Yamamoto BK, Bankson MG. Amphetamine neurotoxicity: cause and consequence of oxidative stress. Crit Rev Neurobiol. 2005;17(2):87–117.
9. Fumagalli F, Gainetdinov RR, Valenzano KJ, Caron MG. Role of dopamine transporter in methamphetamine-induced neurotoxicity: evidence from mice lacking the transporter. J Neurosci. 1998;18(13):4861–9.
10. Blakely RD, Bauman AL. Biogenic amine transporters: regulation in flux. Curr Opin Neurobiol. 2000;10(3):328–36.
11. Robinson MB. Regulated trafficking of neurotransmitter transporters: common notes but different melodies. J Neurochem. 2002;80(1):1–11.
12. Volkow ND, Chang L, Wang GJ, Fowler JS, Leonido-Yee M, Franceschi D, Sedler MJ, Gatley SJ, Hitzemann R, Ding YS, et al. Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. Am J Psychiatry. 2001;158(3):377–82.
13. Sekine Y, Iyo M, Ouchi Y, Matsunaga T, Tsukada H, Okada H, Yoshikawa E, Futatsubashi M, Takei N, Mori N. Methamphetamine-related psychiatric symptoms and reduced brain dopamine transporters studied with PET. Am J Psychiatry. 2001;158(8):1206–14.
14. Ujike H, Harano M, Inada T, Yamada M, Komiyama T, Sekine Y, Sora I, Iyo M, Katsu T, Nomura A, et al. Nine- or fewer repeat alleles in VNTR polymorphism of the dopamine transporter gene is a strong risk factor for prolonged methamphetamine psychosis. The pharmacogenomics journal. 2003;3(4):242–7.
15. Bousman CA, Glatt SJ, Everall IP, Tsuang MT. Genetic association studies of methamphetamine use disorders: A systematic review and synthesis. Am J Med Genet B Neuropsychiatr Genet. 2009;150B(8):1025–49.

16. Matsuzawa D, Hashimoto K, Miyatake R, Shirayama Y, Shimizu E, Maeda K, Suzuki Y, Mashimo Y, Sekine Y, Inada T, et al. Identification of functional polymorphisms in the promoter region of the human PICK1 gene and their association with methamphetamine psychosis. Am J Psychiatry. 2007;164(7):1105–14.
17. Stober G, Meyer J, Nanda I, Wienker TF, Saar K, Knapp M, Jatzke S, Schmid M, Lesch KP, Beckmann H. Linkage and family-based association study of schizophrenia and the synapsin III locus that maps to chromosome 22q13. Am J Med Genet. 2000;96(3):392–7.
18. Deken SL, Beckman ML, Quick MW. PICKing on transporters. Trends Neurosci. 2001;24(11):623–5.
19. Haerian BS. BDNF rs6265 polymorphism and drug addiction: a systematic review and meta-analysis. Pharmacogenomics. 2013;14(16):2055–65.
20. Iamjan SA, Thanoi S, Watiktinkorn P, Nudmamud-Thanoi S, Reynolds GP. BDNF (Val66Met) genetic polymorphism is associated with vulnerability for methamphetamine dependence. Pharmacogenomics. 2015;16(14):1541–5.
21. Cheng CY, Hong CJ, Yu YW, Chen TJ, Wu HC, Tsai SJ. Brain-derived neurotrophic factor (Val66Met) genetic polymorphism is associated with substance abuse in males. Brain Res Mol Brain Res. 2005;140(1–2):86–90.
22. Sim MS, Mohamed Z, Hatim A, Rajagopal VL, Habil MH. Association of brain-derived neurotrophic factor (Val66Met) genetic polymorphism with methamphetamine dependence in a Malaysian population. Brain Res. 2010;1357:91–6.
23. Bolanos CA, Nestler EJ. Neurotrophic mechanisms in drug addiction. Neuromolecular Med. 2004;5(1):69–83.
24. Alam-mehrjerdi Z, Mokri A, Dolan K. Methamphetamine use and treatment in Iran: A systematic review from the most populated Persian Gulf country. Asian J Psychiatr. 2015;16:17–25.
25. MCLELLAN AT. Substance Misuse and Substance use Disorders: Why do they Matter in Healthcare? Trans Am Clin Climatol Assoc. 2017;128:112.
26. Hasin DS, O'Brien CP, Auriacombe M, Borges G, Bucholz K, Budney A, Compton WM, Crowley T, Ling W, Petry NM. DSM-5 criteria for substance use disorders: recommendations and rationale. Am J Psychiatry. 2013;170(8):834–51.
27. Mardan-Nik M, Saffar Soflaei S, Biabangard-Zak A, Asghari M, Saljoughian S, Tajbakhsh A, Meshkat Z, Ferns GA, Pasdar A, Ghayour-Mobarhan M. A method for improving the efficiency of DNA extraction from clotted blood samples. Journal of clinical laboratory analysis. 2019;33(6):e22892.
28. Liu J, Huang S, Sun M, Liu S, Liu Y, Wang W, Zhang X, Wang H, Hua W. An improved allele-specific PCR primer design method for SNP marker analysis and its application. Plant Methods. 2012;8(1):34.
29. Yoo J, Lee Y, Kim Y, Rha SY, Kim Y. SNPAnalyzer 2.0: A web-based integrated workbench for linkage disequilibrium analysis and association analysis. BMC Bioinformatics. 2008;9(1):290.
30. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet. 2001;68(4):978–89.
31. Association AP: **Diagnostic and statistical manual of mental disorders** (**DSM-5®**): American Psychiatric Pub; 2013.
32. Koob GF, Bloom FE. Cellular and molecular mechanisms of drug dependence. Science. 1988;242(4879):715–23.
33. Volkow ND, Fowler JS, Wang GJ. The addicted human brain: insights from imaging studies. J Clin Invest. 2003;111(10):1444–51.
34. Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Jayne M, Ma Y, Pradhan K, Wong C. Profound decreases in dopamine release in striatum in detoxified alcoholics: possible orbitofrontal involvement. J Neurosci. 2007;27(46):12700–6.
35. Alammehrjerdi Z, Ezard N, Dolan K. Methamphetamine dependence in methadone treatment services in Iran: the first literature review of a new health concern. Asian J Psychiatr. 2018;31:49–55.
36. Shariatirad S, Maarefvand M, Ekhtiari H. Methamphetamine use and methadone maintenance treatment: an emerging problem in the drug addiction treatment network in Iran. Int J Drug Policy. 2013;24(6):e115–6.
37. Ujike H. [Potential genetic predictors for individual vulnerability to substance dependence]. Nihon shinkei seishin yakurigaku zasshi = Japanese journal of psychopharmacology. 2008;28(1):11–7.
38. Torres GE, Yao WD, Mohn AR, Quan H, Kim KM, Levey AI, Staudinger J, Caron MG. Functional interaction between monoamine plasma membrane transporters and the synaptic PDZ domain-containing protein PICK1. Neuron.

2001;30(1):121–34.

39. Li X, Wolf ME. Brain-derived neurotrophic factor rapidly increases AMPA receptor surface expression in rat nucleus accumbens. Eur J Neurosci. 2011;34(2):190–8.
40. Bousman CA, Glatt SJ, Cherner M, Atkinson JH, Grant I, Tsuang MT, Everall IP, Group H. Preliminary evidence of ethnic divergence in associations of putative genetic variants for methamphetamine dependence. Psychiatry research. 2010;178(2):295–8.