Association of \textit{PICK1} and \textit{BDNF} variations with increased risk of methamphetamine dependence among Iranian population: a case–control study

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Abstract

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

\textbf{Methods:} Total of 235 cases and 204 controls were recruited in a period between 2015 to 2018. The \textit{PICK1-rs713729}, \textit{-rs2076369} and \textit{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.

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

\textbf{Conclusion:} Our findings indicate that the \textit{PICK1} gene polymorphism might affect the risk of methamphetamine dependency in our population.

\textbf{Keywords:} Addiction, Drug abuse, Substance dependence, Substance use disorder (SUD), Dopamine pathway, Glutamate pathway, Variations, And polymorphisms

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\section*{Background}

Methamphetamine is the most extensively abused illegal drug and this is a growing global problem [1–3]. Many changes occur in the human brain as a result of methamphetamine abuse [4]. Methamphetamine leads to the release of synaptic dopamine, which might be a reason for the increased addiction [4]. In this context,
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. It has been shown that 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 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) seem 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 and drug addiction, such as methamphetamine abuse [16]. In this respect, the PICK1 gene is mapped to chromosome 22q13.1 [17]. Interestingly, 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 affects 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 feasibility of genetic association studies [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]. Therefore, we aimed to investigate the association between PICK1 and BDNF main SNPs and methamphetamine dependence (substance use disorder (SUD)) in a cohort of Iranian individuals.

Methods
The aim, design and setting of the study
This case–control study involved 235 cases (with methamphetamine dependence (SUD)) and 204 gender-matched controls (healthy individuals). In our study, SUD was 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].

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.

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 participants, individually matched on age, were recruited from the Health Examination Centre who were receiving routine medical examinations.

Description of materials

**Blood collection and DNA extraction**

In order to extract the DNA, approximately 10 millilitres (ml) of peripheral blood was obtained from all participants 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 nm (nm) and 280 nm (A260/280) 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 databases and published articles. Such articles 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.

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### Table 1 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 |

*In the present study, substance use disorders (SUD) is recognized by these 11 criteria

### 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 (SD)                  | 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%                            |           |

*Significant P-value < 0.05. SD: Standard deviation
To determine the genotype frequency of \( \text{PICK1-rs713729} \), \( \text{PICK1-rs2076369} \) and \( \text{BDNF-rs6265} \), 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 \( \text{PICK1-rs713729} \), \( \text{PICK1-rs2076369} \) and \( \text{BDNF-rs6265} \) were conducted in a 10–15 \( \mu l \) (\( \mu l \)) volume per reaction, containing 3 \( \mu l \) Taq \( 2 \times \) master mix (Ampliqon, Germany), 10 \( \mu M \) of each primer and 100 nanogram (ng) DNA. Moreover, the specific primers used to detect \( \text{PICK1-rs713729} \), \( \text{PICK1-rs2076369} \) and \( \text{BDNF-rs6265} \) SNPs are listed in Table 4. For \( \text{PICK1-rs2076369} \), we also used betaine (Ampliqon, Germany) as an enhancer in PCR.

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 s, annealing at alternative °C for 25 s (based on each primer), an elongation at 72 °C for 30 s followed by 72 °C for seven minutes as the final elongation step (Table 5). Absence or presence of mutant or normal PCR products were detected via gel electrophoresis in 3% agarose gel by ultraviolet (UV) trans illuminator (Gel Doc; U:Genius).

### Table 3 Characteristics of the investigated polymorphisms in this study

| Rs number | Gene | Protein | Position | Exon/Intron | Variant length | Allele | Function | Haplotype distance (bp) |
|-----------|------|---------|----------|-------------|----------------|--------|----------|------------------------|
| rs713729  | PICK1| Non-coding | 22:38,059,462 | Intron 3 | 1 | T > A | Intron variant | 8183 |
| rs2076369 | PICK1| Non-coding | 22:38,067,645 | Intron 4 | 1 | T > G | Intron variant | |
| rs6265    | BDNF | NP_001137277.1.p. Val66Met | 11:27,658,369 | Exon 4 | 1 | C > T | Missense | |

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

| SNPs | Primers | Sequences | Primer Length (bp) | PCR products (bp) |
|------|---------|-----------|--------------------|-------------------|
| rs6265 | FO | CTACAGTCCACAGGTGAAAGAGTG | 27 | 400 |
| | RO | ATGGCATATTTGCAGCATCTAGTA | 26 | |
| | FI (c) | TGGTCTCTATCAACAGGCTTCTTATAC | 29 | 253 |
| | RI (t) | TGTCGACTCTTTCCGAAcCA | 22 | 201 |
| rs713729 | FO | CTTTCTAGCGGAACTCGCTACTTG | 24 | 407 |
| | RO | CAGTGAACGGACAAACGAGACACTG | 26 | |
| | FI (a) | CTTCTCATTCTGAGTCCTGACCACA | 27 | 196 |
| | RI (t) | AGGTGGTGAGAAAGCCCTTACA | 23 | 265 |
| rs2076369 | FO | CATGTTGCCAAAGCTGGCTCACAATCT | 27 | 299 |
| | RO | CGGACGCCCACTGCTGCACGACC | 35 | |
| | FI (g) | AGGAGTCTCAGTGCGAACAGTCTCGACG | 29 | 191 |
| | RI (t) | CCTCACACCGGAGGCCACTCTCA | 24 | 165 |

*Expected product size in bps depending on the SNPs. FO: Forward outer primer; FI: Forward inner primer; RI: Reverse inner primer; RO: Reverse outer primer; bp: Base pairs; ARMS-PCR: Amplification refractory mutation system-polymerase chain reaction; SNP: Single nucleotide polymorphism*
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, \( P_{\text{value}} \) 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 \( P_{\text{value}} < 0.016 \) (\( 0.05/3 \) 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 associations between \( BDNF-rs6265 \) and the risk of methamphetamine dependence (SUD). On the contrary, a significant association was observed between two SNPs and the risk of methamphetamine abuse 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 (Table 6). Moreover, haplotype analysis showed that specific haplotypes related to these SNPs were associated with methamphetamine dependence (SUD). In this line, analysis of \( PICK1-rs713729 \) and \( PICK1-rs2076369 \) haplotypes in our population showed that the 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 had a significant association with the risk of methamphetamine dependence SUD) (Table 7).

#### Distribution of single nucleotide polymorphisms (SNPs)

The frequencies of the genotypes with high-quality genotype call were as follows: \( PICK1-rs713729 \) “TT” 174 (77.67%), “AT” 33 (14.73%), and “AA” 17 (7.58%) among cases and “TT” 168 (87.95%), “AT” 17 (8.90%) and “AA” 6 (3.14%) among controls; \( PICK1-rs2076369 \) “GG” 117 (52.23%), “GT” 80 (35.71%) and “TT” 27 (12.05%) among cases and “GG” 117 (52.23%), “GT” 80 (35.71%) and “TT” 27 (12.05%) among cases and 70 (36.08%), 100 (51.54%), 24 (12.37%) and among controls, respectively. Moreover, \( BDNF-rs6265 \) genotype frequencies were: “CC” 161 (72.52%), “CT” 55 (24.77%) and “TT” 6 (2.70%) among cases and 142 (73.57%), 48 (24.87%) and 3 (1.55%) among controls, respectively. Table 7 shows the conferred risk by each haplotype. 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 (Additional file 1: Table 1). We also examined whether the three SNPs were in LD. In this line, there was no strong LD between these SNPs (Additional file 2: Table 1).

### Table 5 The ARMS-PCRs condition for targeted SNPs

| SNPs | Primers | First Denaturation | 35 cycles | Last extension |
|------|---------|-------------------|-----------|---------------|
|      |         | Tm°C Min | Tm°C Min | Tm°C Min | Tm°C Min | Tm°C Min | Tm°C Min | Tm°C Min | Tm°C Min | Tm°C Min | Tm°C Min | Tm°C Min | Tm°C Min |
| rs6265 | FO | 94 °C 7 min | 61.5 25 s | 72 °C 45 s | 72 °C 7 min |
|       | RO |         |          |          |          |          |          |          |          |          |          |          |          |
|       | FI (c) |         |          |          |          |          |          |          |          |          |          |          |          |
|       | 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) | 67 30 s |
|       | FI(g) | 67 30 s |
|       | RO |         |          |          |          |          |          |          |          |          |          |          |          |

FO: Forward outer primer; FI: Forward inner primer; RI: Reverse inner primer; RO: Reverse outer primer; TM: Temperature; Min: Minute; S: Seconds; °C: Centigrade; ARMS-PCR: Amplification refractory mutation system-polymerase chain reaction; SNP: Single nucleotide polymorphism
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 dependence. However, the development of drug dependency is affected via factors, such as pharmacological effects on mental status, environmental and individual factors, such as genetics. 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].

Showing a possible association between \(PICK1\)-rs713729 and \(PICK1\)-rs2076369 in the \(PICK1\) gene promoter with methamphetamine dependence were the key findings of the present study. Consistent with our study, Matsuzawa et al. showed that \(PICK1\)-rs713729 and \(PICK1\)-rs2076369 were significantly associated with methamphetamine abuse in a Japanese population (Table 8). 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

| SNP number  | Gene    | Position          | Genetic models | OR      | 95% CI     | \(P\)-value \* | Bonferroni correction \(P\)-value |
|-------------|---------|-------------------|----------------|---------|------------|----------------|----------------------------------|
| rs713729    | PICK1   | 22:38,059,462     | 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  | 0.021         | 0.061                             |
| rs2076369   | PICK1   | 22:38,067,645     | 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    | 11:27,658,369     | 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; SNP: Single nucleotide polymorphism. OR: Odds ratio; CI: Confidence interval; \(P\)-values were obtained from Chi-square tests; \* Significant of \(P\)-value < 0.05; The association between methamphetamine, risk factors and alleles/genotypes was evaluated by binary logistic regression, estimating ORs and also CIs. Note: Significant \(P\)-value in bold; Bonferroni corrected \(P\)-value is 0.016

| Genetic models | Haplotype | \(P\)-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    |
| Reccessive     | 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; OR: Odds ratio; CI: Confidence interval; \(P\)-values were obtained from Chi-square tests. \* Significant of \(P\)-value < 0.05; Significant \(P\)-value in bold

### 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 dependence. However, the development of drug dependency
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 on the cell surface [18, 38]. This clustering leads to an increase of dopamine transporter uptake activity. Thus, PICK1 expression levels can alter the role of the dopamine transporter and can affect the pathogenesis of methamphetamine abuse/dependency [16]. Moreover, the interactions of PICK1 with α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors, metabotropic glutamate receptors and D-serine synthesizing enzyme have been shown and might be implicated in the pathogenesis of drug-related disorders [39–42]. Further studies are still required to show the direct association of these proteins in the pathogenesis of methamphetamine abuse.

Based on previous studies, it was reported that gene variations, which are associated with glutamatergic and serotonin systems explain differences in SUD and/or dependency risk between individuals. It is indicated that glutamate-related genes influence 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 is involved in the expansion, maintenance and survival of dopaminergic neurons in CNS [43].

In the present study, no association was found between BDNF-rs6265 and the risk of methamphetamine dependence (SUD) in our 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) [44]. However, Cheng et al. showed that there was an association between methamphetamine-dependency and BDNF gene in 103

### Table 8 The association studies regarding methamphetamine and BDNF/PICK1 gene polymorphisms in the methamphetamine abusers and controls

| Gene-SNP                  | Ethnicity | Sample size | Result                                                                 | Refs   |
|---------------------------|-----------|-------------|----------------------------------------------------------------------|--------|
| BDNF-Val66Met             | Chinese   | 200         | No significant differences in genotype and allele. The Met allele was associated with earlier age onset of METH use | [52]   |
| BDNF-rs16917204, rs16917234, rs2030324 | Chinese | 200         | No significant differences in genotype and allele distributions       | [53]   |
| BDNF-Val66Met             | Taiwanese | 103         | Significant differences in BDNF Val66Met genotype distribution        | [21]   |
| BDNF-Val66Met (rs6265)    | Thai      | 100         | Significant differences in the distribution of rs6265 genotype       | [20]   |
| BDNF promoter methylation | Chinese   | 30          | BDNF promoter methylation is associated with drug addiction          | [54]   |
| BDNF-Val66Met             | Chinese   | 194         | No significant differences in genotype and allele distributions      | [55]   |
| BDNF-Val66Met             | Japanese  | 189         | No significant differences were found in the frequency of the genotype or allele | [56]   |
| BDNF-Val66Met             | Chinese   | 24          | No significant differences in genotype and allele distribution       | [22]   |
|                           | Malay     | 59          | A significant distribution of the BDNF Val66Met genotype             |        |
|                           | Kadazan-Dusun | 50       | with METH dependence and METH psychosis in Chinese                    |        |
|                           | Bajau     | 53          | A significant positive correlation between serum BDNF and the delayed memory index score |        |
|                           | Total     | 186         | No significant differences in genotype and allele distribution       |        |
| BDNF-Val66Met             | Caucasian | 60          | Significantly more pretreatment days with METH use in females than males | [57]   |
| PICK1- s737622, rs3026682, rs713729, rs2076369 | Japanese | 208         | A significantly association between: rs713729 and METH abusers       | [16]   |
|                           |           | 218         | rs713729 and rs2076369 with spontaneous relapse of psychosis         |        |

SNP: Single nucleotide polymorphism; PICK1: Protein that interact with C-kinase-1; BDNF: Brain-derived neurotrophic factor; Val66Met (also called rs6265): Met: Methionine and Val: Valine; METH: Methamphetamine; Ref: References
methamphetamine abusers and 122 normal controls. They noted that the lower 66Met carriers were linked to substance abuse [21]. Furthermore, 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 (Table 8). It is indicated that ethnic differences can affect the frequency of BDNF Val66Met [45, 46].

Long-term methamphetamine-induced brain changes are significantly dependent on BDNF genetic variation [47]. Val66Met might enhance the risk of suicide behavior [45]. Moreover, BDNF variations might be involved in methamphetamine withdrawal. BDNF levels, equal or less than 1,251.0 pg/ml has been stated to be linked to depression symptoms during methamphetamine withdrawal [48]. BDNF Val66Met had an important effect on the Treatment Effectiveness Score (TES), methamphetamine-negative urine drug screens during treatment, which was higher among Val/Val Caucasian carriers [49]. Interestingly, there were more pretreatment days with methamphetamine use in females with Val/Val genotype than males [10]. Estrogen improves BDNF expression, which has implications on the release of striatal dopamine caused by methamphetamine and protects against neurotoxicity caused by methamphetamine [50, 51]. Thus, females can be able to use methamphetamine more frequently [10].

Furthermore, there was no association between PICK1 and BDNF gene polymorphisms in our study. That may be attributed to the different positions of the gene polymorphisms on different chromosomes [PICK1 (22q13.1) and BDNF (11p13–15)].

There were some limitations in our study. We did not investigate all the SNPs in these genes. Although, the sample size was large enough to detect an association between these SNPs and SUD in comparison with previous studies, larger sample size would strengthen the results. We therefore recommend further replication studies with larger sample size in order to validate and explain this association with conclusive findings.

Conclusions
Collectively, the variation in the PICK1 gene was associated with methamphetamine dependence (SUD), reflecting the underlying biological mechanisms, which can make a bridge between pathways and methamphetamine dependence (SUD). Our findings suggest that the PICK1 gene might be involved in susceptibility to SUD in our 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 individuals susceptible to SUD. All in all, understanding genetic variations might help to understand the biological mechanisms of progression, and suppression of methamphetamine. In addition, our findings provide the basis for future genetic research on the use of methamphetamine dependence and related neurological side effects.

Supplementary information
is available for this paper at https://doi.org/10.1186/s12920-021-00873-7.

Additional file 1. Haplotype frequencies in population.
Additional file 2. LD map and LD block.

Abbreviations
AKT1: V-akt murine thymoma viral oncogene homolog 1; AMPA: α-Amino-3-hydroxy-5-methyl-4-isoxazole propionate; ARMS-PCR: Amplification refractory mutation system-polymerase chain reaction; ARR82: Arrestin beta 2; BDNF: Brain-derived neurotrophic factor; Cis: Confidence intervals; CNS: Central nervous system; COMT: Catechole-O-methyltransferase; CYP2D6: Cytochrome P450 2D6; DRD4: Dopamine receptor D4; EDTA: Ethylene diamine tetra acetic acid; GABRA1: Gamma-aminobutyric acid type A receptor subunit alpha1; GABRG2: Gamma-aminobutyric acid receptor subunit gamma-2; GLYT1: Glycine transporter-1; GSTM1: Glutathione S-transferase mu 1; GSTP1: Glutathione S-transferase P1; HWE: Hardy–Weinberg equilibrium; LD: Linkage disequilibrium; MAF: Minor allele frequency; Nm: Nanometres; ORs: Odds ratios; PDIYN: Prodynorphin; PICK1: Protein interacting with C kinase; SLC22A3: Solute carrier 22 member 3; SNP: Single nucleotide polymorphism; SUD: Substance use disorder; TES: Treatment Effectiveness Score; TrKB: BDNF-tropomyosin kinase B; WASP: Web-based allele-specific primer designing tool.

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Authors’ contributions
AT: Conception and design, sample and data collection, extraction of genomic DNA, genotyping, data analysis and writing the manuscript; MA1: Sample and data collection, extraction of genomic DNA, genotyping and data analysis; MA2: Sample and data collection, extraction of genomic DNA, genotyping and data analysis; MA3: Sample and data collection, extraction of genomic DNA, and genome genotyping; SSKh: Performing required interviews, completing questionnaires, sample and data collection and analysis; ANB: Sample and data collection, extraction of genomic DNA, and data analysis; FA: Sample and data collection, and data analysis; MA3: Sample and data collection, extraction of genomic DNA, and data analysis; AS: Data analysis and critical review; MA2: Conception and design, clinical diagnosis, approval of the manuscript, AP: Conception and design, supervision of the project, revising and approval of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets created during the current study are not publicly accessible due to the possibility of compromising the privacy of individuals. According to the written approval forms accepted by the Ethics Committee of the Mashhad University of Medical Sciences (MUMS), the data will only be available to researchers within project. The data would be available upon request from the corresponding authors (according to the MUMS rules and regulations).
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