**c.-97C>A of Brain-derived Neurotrophic Factor *BDNF* is associated with opium addiction**

Anit Kumar1, Nizamuddin Sheikh2, Niraj Rai2, Biswajit Roy2, Pooja Sauhta3, Jatin Bodwal4, Poonam Rana5, Vineet Kaswan6, Gaurav Gupta7, K. Thangraj2, Amit Kaushik1†

1Amity Institute of Biotechnology, Amity University Uttar Pradesh, India.

2CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India.

3Himalayan Institute of Medical Sciences, Swami Ram Nagar, Jolly Grant, Doiwala, Dehradun, Uttarakhand, India.

4Department of Forensic Medicine, DDU Hospital, Hari Nagar, New Delhi, India.

5NMR division, Institute of Nuclear Medicine and Allied Sciences, DRDO, Timarpur, New Delhi, India.

6Department of Biotechnology, College of basic science and humanities, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujrat, India.

7Genome Foundation, C/o Prasad Hospital, Nacharam, Hyderabad, India.

†Corresponding author

Amit Kaushik

Amity Institute of Biotechnology,

Amity University Uttar Pradesh,

Noida-201303, India

Phone number: +91-9873654752

Email-ID: kaushikamit77@gmail.com/akaushik@amity.edu

**Abstract**

**Aims**

Opium addiction or use disorder has been widely investigated and is genetic in nature. The present study aims to investigate the effect of putative functional variants in brain-derived neurotrophic factor (*BDNF*), Opioid Receptor Mu 1 (*OPRM1*) and dopamine receptor D2 (*DRD2*) on opium addiction.

**Methods**

In this study, 331 opium addicted male subjects and 100 ethnically matched controls were selected from North India (Haryana and Punjab) which are having higher rate of addiction. Within the range of ±10kb each for *BDNF*, *OPRM1* and *DRD2*, 17 putative function variants (minor allele frequency > 0.05) were identified by annotating DNA sequence data of 182 Indo-Europeans (PJL and BEB) using Annovar tool. The variants thus identified were genotyped in our cohort with SequenomiPLEX assay and MassARRAY system. Quantitative trait association analysis was performed to find the effect of variation on gene expression level, using Plink software.

**Findings**

In total, 3 SNPs (rs4314511, p-value = 1.65×10-3; rs7755659, p-value = 4.77×10-11 and rs56164415, p-value = 4.27×10-2) were not in Hardy-Weinberg equilibrium and hence excluded from the analysis. We observed that the promoter variation rs7944119/c.-97C>A of *BDNF* is associated with opium addiction using dominant genetic model (p-value = 9.86×10-3). We also observed that c.-97C>A significantly decreases expression of *BDNF* transcript 2 (NM\_170732.2) (p-value = 8.443×10-3).

**Conclusion**

The promoter variant rs7944119/c.-97C>A of *BDNF* is a genetic risk factor for opium addiction in North-Indians. Also, the variant c.-97C>A of *BDNF* increases the risk of opium addiction in North-Indians by lowering expression level of *BDNF*.

**Introduction**

Addiction is an enduring struggle and considered as one of the most severe health problem throughout the world. Addiction to psychotropic drugs like Opioids (afeem, poppy husk, Heroin, smack, Black Tar, Brown, Sugar), Cannabis (hashish/hash, charas, ganja) and coca alkaloids (cocaine) is a persistent turmoil. Carving and relapse are most important aspect in controlling this despair. Even after many months of abstinence from drugs, addicts can relapse into drug use when acutely exposed to the drug itself, drug-associated cues or stress. Extensive drug use and relapse may reflect the ability of drugs/substance to weaken or takeover the brain systems that mediate reward learning (1).This causes the neurons to become responsive to psychotropic substances and relapsing events more persistent.

The mu opioid receptors are part of a family of G protein coupled receptors that are expressed in the brain and bind endogenous and exogenous opioids. Opioids activate specific receptors (μ,*δ* and *k*) that couple the G protein (2). The mu1 opioid receptor gene (OPRM1) has been a high- priority candidate for human genetic studies of addiction (3). OPRM1 is the most studied genes in psychoactive substance research(4).OPRM1 is a receptor for opioid analgesic agents and is involved in reward and analgesic pathways (5, 6). Single-nucleotide polymorphism of the gene encoding the μ opioid receptor correlates with an increased likelihood of heroin abuse (7-9). Mice in which different receptors (CB1cannabinoid and D2 dopamine receptors) and transporters (dopamine) have been knocked out have been used to demonstrate the effect of systems other than the opioid on opioid-induced pharmacologic responses (10).

Dopamine is a neurotransmitter is involved variety of brain functions. Low extracellular levels of dopamine may results in a variety of undesirable symptoms. When we are rewarded and due to reward there is a feeling of pleasure, dopamine is released. Any behavior that induces a sense of pleasure such as: betting and winning, alcohol, having sex, eating chocolates, addictive substances – all stimulate the dopaminergic system. A small part in the brain, the nucleus accumbens (NA) is vital for motivation, pleasure, and addiction(11). This cluster of neurons modulates the effects of the neurotransmitter dopamine, on which many neural circuits depend also known as the brain's “pleasure center”. The pleasure associated with the release of dopamine makes certain behaviors addictive. Opioids release dopamine mainly by an indirect mechanism that decreases the activity of GABA inhibitory interneurons in the ventral tegmental area(12). Reward and physical dependence on opioids are mediated by the activation of μ receptors, since reinforcement is blocked by selective receptor antagonists(13). Mice in which the μ receptor has been knocked out do not exhibit place preference or withdrawal signs after the administration of morphine (14). Individuals addicted to certain behaviors or stimuli (e.g. drugs) find to get a temporary boost in dopamine when engaging in the activity. Unfortunately this temporary boost cannot be sustained for a long-term(15). Continuous use of certain drugs may actually lower the endogenous supply of dopamine in the brain; this is seen in those addicted to amphetamines(16). Only stopping the addictive behavior for a long-term will result in dopamine levels to increase.

Neurotrophic factors are involved in neuronal survival and differentiation is well recognized. Neurotrophic factors have been implicated in the modulation of synaptic transmission and in the mechanisms underlying learning and memory, mood disorders, and drug addiction. Neurotrophic factors activate signaling pathways leads to long-term molecular, cellular, and behavioral adaptations associated with drug addiction (17). Large and diverse arrays of neurotrophic factors have been identified (18). They have a significant contribution in the development of the nervous system. Neurotrophic factors are best understood for the role they play in mediating cell growth, survival, and differentiation during nervous system development. First target derived neurotrophic factor, NGF (nerve growth factor) was identified decade ago. NGF belongs to the neurotrophin family of neurotrophic factors, which include brain-derived neurotrophic factor (*BDNF*), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), and neurotrophin-6 (19). The most commonly and abundantly expressed neurotrophin in the nervous system is *BDNF*. [*BDNF*](http://web.stanford.edu/group/hopes/cgi-bin/hopes_test/glossary/bdnf/) has been shown to play a role in [neuroplasticity](http://web.stanford.edu/group/hopes/cgi-bin/hopes_test/glossary/neuroplasticity/), which allows nerve cells in the brain to compensate for injury and new situations or changes in the environment. Graham *et al.*(20)shows *BDNF* in the nucleus accumbens, a brain area critical for the rewarding effects of cocaine, promotes persistent cocaine-seeking behaviors and heightens vulnerability to relapse. *BDNF* is an important component in the signaling pathways that regulate plasticity in brain regions that process reward-related information(21-23).

*BDNF* and its intracellular signaling pathways are also involved in neuroadaptive changes in the dopaminergic or glutamate systems that underlie psychostimulant abuse and dependence (24).Brain-derived neurotrophic factor (*BDNF*) regulates neuronal development, central and peripheral synaptic plasticity. The influence of the *BDNF* pathway (*BDNF* genotype, gene-expression, and protein) may be especially prominent when life stress is present. Such an interaction may also apply to addiction as the condition shares similar patho-physiological mechanisms. *BDNF* and its gene polymorphism may be important in synaptic plasticity and neuron survival, and may become a key target in the physiopathology of drug or substance use. Angelucci et al.(25)reported chronic heroin users had lower serum levels of nerve growth factor and BDNF. Chen et al. (26)hypothesized that the downregulation of brain and circulatory BDNF is highly correlated with the progression of opioid dependence. However, Heberlein et al. (27)reported that serum BDNF levels were significantly higher in opioid-dependent patients. The increase of serum BDNF was found in during heroin. Zhang *et al.*(28) showed that the BDNF serum levels in heroin-dependent patients are lower than those of healthy controls at baseline and increased after 26 weeks of abstinence, although the BDNF serum levels are still lower than those of the healthy controls.

Till now, no precise and effective pharmacological treatment is present or offered. Based on our present knowledge of neurobiology, pharmacological and genomics interventions can be considered at the same time. These approaches can be adopted in conjunction with each other for controlling and treating substance abuse. Inter-individual genetic variability may have a significant effect on diagnosis and treatment. Genetic association studies aim to characterize genetic alterations and polymorphism that trigger addiction and response to treatment. Variation in addiction-related genes (*OPRM1*, *BDNF* and *DRD2)* due to polymorphisms in the genetic sequence may confer susceptibility to continued opioid use.

The current study aims to examine SNP of three different genes variants in *BDNF*, *OPRM1* and *DRD2* to understand their relation with drug addiction. Here, we elucidate a strong association between SNP in the promoter region of *BDNF* gene and addiction.

**Material and methods**

***Subjects***

In total, 331 male subjects were selected from North-India, who are >20 years of age and addicted to opium. To perform genetic association analysis, we utilized 100 subjects from same ethnic group, living at similar geographical location. The present study is approved by ethical committee of Amity University, Noida, India.

***DNA isolation and genotyping***

A total of 10 ml intravenous blood samples of both addicts and controls were collected in EDTA vaccutainer. Genomic DNA was isolated from all the samples using standard protocol. Genotyping was performed using SequenomiPLEX assay and the MassARRAY system (SEQUENOM, San Diego, CA). For iPLEX assay, primers for the PCR and iPLEX reactions were designed (**Table 1**) and custom synthesized (SEQUENOM, San Diego, CA, USA). The genotyping was performed using standard protocol, provided by manufacturer. For the analysis, a pool was designed, which contains 31 variable positions to genotype the targeted positions. Highly variable SNPs, which represent a particular global population, were selected from the HapMap data sets. In total, 3 variants (rs1554819, rs10680447 and rs9383697) were failed due to experimental error.

***Annotation of variants in BDNF, OPRM1 and DRD2***

In order to select, putative functional variants, we extracted variants within ±10 kb flanking region of *BDNF, OPRM1 and DRD2* for Punjabi (PJL) and Bengali (BEB) populations of 1000 genome project. We annotated the variants with Annovar tool and calculated the frequency with VCF tools.

***Statistical analysis***

All statistical analysis was performed using Plink software. To explore effect of promoter variation on gene expression level, we utilized genome expression dataset GSE6536 from GEO (gene expression omnibus) database and genotype data of *BDNF* with ±10 kb flanking region from 1000 genome project. Further, we extracted the population-wise normalized expression value of *BDNF* specific probe from above downloaded GSE6536 dataset and performed quantitative trait association analysis using Plink software.

**Results and discussion**

***Selection of genetic variants for genotyping***

In the present study, we selected 3 genes *BDNF*, *OPRM1* and *DRD2*. To explore these genes, initially we extract putative functional variants which are present within 10,000 base pairs (bps) of gene and have minor allele frequency (maf) > 0.05. Since, subjects from our study are North-Indians/Indo-Europeans, we consider only 96 and 86 subjects of Punjabi (PJL) and Bengali (BEB) populations respectively from 1000 genome project. Both PJL and BEB are Indo-Europeans. To identify putative function non-synonymous variants, we utilized scores of SIFT, PolyPhen2-HDIV, PolyPhen2-Hvar, LRT, Mutation Taster, Mutation Assessor, FATHMM, Meta-SVM and Meta-LR, from dbNSFP (version 30a). Any non-synonymous variations which are predicted as deleterious in >6 tests are considered as putative functional non-synonymous variants. Besides this, we also annotated the non-coding variation which disrupt promoter, using chromHMM predicted ENCODE datasets.

We conducted a case control study on opium addicts on north Indian population and aim of the study was to investigate the correlation between opium addiction and polymorphism in BDNF gene. BDNF influences neuronal differentiation, neuronal survival and synaptic plasticity (21). In recessive model analysis, we found very strong association of polymorphism in promotor region of BDNF gene (rs7944119). An increasing number of studies on genetic risk factor for opium addiction have been published suggesting significant role of BDNF in opium or other substance abuse dependence. Neurotransmitters serotonin and dopamine are highly linked to addiction and influenced by BDNF (29-32). Heroin addiction in people of various ethnicities is associated with polymorphism in BDNF (33). An article reported that BDNF polymorphism is involved in susceptibility of addiction (34). However, the susceptibility to opium addiction exactly on genetic basis is not very clear but a study also hypothesized the association BDNF with smoking (35). *BDNF* mediates synaptic plasticity and is associated with cocaine induced behavior & cocaine dependence. BDNF involves in regulation of drug induced long–term neuroadaptations that encompass alterations in synaptic molecular components, gene expression changes and modifications of behavioral output (36, 37). BDNF mRNA expression negatively involves in regulation of alcohol induced behaviors and alcohol consumption (37, 38). In this study, we have investigated that polymorphism of BDNF gene may provide an effect for opium addiction risk, indicating that this gene may be concerned in the development of opium addiction.

We selected (1) 5 variations present within poised promoter of *BDNF*; (2) 10 variations present within active promoter and 1 stop-gained variation of *OPRM1*; and (3) 1 variation present within poised promoter of *DRD2*, for genotyping (**Table 2**).

***Genetic association analysis using various models***

To identify the genetic risk factors associated with opium addiction, we performed association analysis using allelic, genotype, recessive and dominant model. In total, 3 SNPs were not in hardy-Weinberg equilibrium (HWE), (1) rs4314511, p-value = 1.65×10-3, (2) rs7755659, p-value = 4.77×10-11 and (3) rs56164415, p-value = 4.27×10-2(**Table 2**). These SNPs were excluded from further analysis. In allelic association analysis, we identified that rs13306221 is marginally associated with addiction (p-value = 0.0397). While in genotype and dominant model, we identified rs7944119 in significant association (p-value = 0.024 and 0.009863, respectively) (**Table 3** and **4**).AA+AC was 61% in addicted subjects, while 44.74% in controls. It suggests that mutant “A” allele acts as dominant way and increases risk of addiction.

***Expression analysis of rs7944119***

Since, rs7944119 was in the promoter region of *BDNF* gene, we predicted effect of this variation on mRNA expression level. To explore it, we utilized the genotype data from 1000 genome project for the same subjects, for which, the expression data was available on gene expression omnibus dataset GSM232560. We observed 179 subjects with expression and genotype data. In total, 7 probes (GI\_34106706-I, GI\_34106707-I, GI\_34106708-I, GI\_34106709-A, GI\_34106709-I, GI\_34106711-I and GI\_34170263-I) were utilized to find out the expression level of mRNA transcripts of *BDNF*. In quantitative trait association analysis, we observed that rs7944119 was significantly associated with GI\_34106707-I (p-value = 8.443×10-3) (**Figure 1**).The probe GI\_34106707-I is for *BDNF* transcript 2 (NM\_170732.2). The normalized expression level of NM\_170732.2 mRNA was 5.889±0.064, 5.948±0.06955 and 5.955±0.05526, for AA, AC and CC genotype, respectively. It suggests that lower expression level of *BDNF* caused by mutation C>A, is the reason of addiction in North-Indians. It has been reported that heroin, Cocaine addicts have lower serum level of *BDNF*, our results find correlation.

**Conclusion**

In conclusion, we identified promoter variation rs7944119/c.-97C>A of *BDNF* as genetic risk factor for opium addiction. The subjects having genotype AA and AC have the lower expression of *BDNF*, comparative to wild-type CC genotype. Lower expression level of *BDNF* caused by mutation C>A, is the genetic risk of addiction in North-Indians.

**Acknowledgments**

We acknowledge the participants who provided samples for the present study. SN acknowledge ICMR-SRF (Senior Research Fellowship) program.

**Conflict of interest**

Authors declare no conflict of interest to disclose.

**Figure legends**

**Figure 1**.Quantitative trait association analysis of rs7944119/c.-97C>A with mRNA expression of *BDNF*. Middle blue bar represents the mean of normalized expression level while black bars represent its standard deviation.



**Table 1.**Details of the primer utilized in IPLEX assay

|  |  |  |
| --- | --- | --- |
| **SNP\_ID** | **2nd-PCRP** | **1st-PCRP** |
| rs7944119 | ACGTTGGATGTGAGGCTGGGGCTGGAACAC | ACGTTGGATGACATCGCCCTGCGAGTCCT |
| rs1800498 | ACGTTGGATGAAGGAATGATGCCTGGATGC | ACGTTGGATGTAGTAGCAGAGGAAGGAGTG |
| rs56164415 | ACGTTGGATGATTCCCAGCGCTTGCCTAC | ACGTTGGATGAATCGGAACCACGATGTGAC |
| rs7741417 | ACGTTGGATGTCAAATGTTGTCTAAGCACG | ACGTTGGATGTTTGCATTGGCGACTGTCAC |
| rs1554817 | ACGTTGGATGCAGTGACTTTTTGTGAATCCC | ACGTTGGATGCTCCTCTTTGGGTTCCATAA |
| rs7755659 | ACGTTGGATGATTCTTAGAAAGTGTGCTG | ACGTTGGATGCGTCTCCTCATTCGACATTC |
| rs677830 | ACGTTGGATGTTGAACCTGGACTGTCACTG | ACGTTGGATGTCTTCCTGGGAAGGGAAATG |
| rs7116768 | ACGTTGGATGGAGCTGGAAGCCTCAAGCA | ACGTTGGATGTGCTTACCTTCAAGCCATAG |
| rs4314511 | ACGTTGGATGGCTTGGGTCTTATTTCACAG | ACGTTGGATGTGTGAGGGAAAAAAGGCTAC |
| rs13306221 | ACGTTGGATGTGACCTCTCTAGAGTTTGCC | ACGTTGGATGTTGGTGTAACGTTATCTGGG |
| rs9397698 | ACGTTGGATGGCCATCACTTAACATGGCAC | ACGTTGGATGCACCCTTGTAGGTCGTGAAG |
| rs9479798 | ACGTTGGATGCGCTCCAATTCCCAGAAATC | ACGTTGGATGTCCCAGCCGTTTTAATGAGG |

**Table 2.** Hardy-Weinberg equilibrium for the variations selected in present study

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Chr | Physical position(hg19) | Gene | rsIDs | Annotation | Frequency ofminor allele inPJL+BEB | Allele | Genotype in controlsAA/AB/BB | Heterozygosity | HWEP-value |
| Major(B) | Minor(A) | Observed | Expected |
| 6 | 154428666 | *OPRM1* | rs677830 | Stop gain | 0.15 | T | C | 2/15/60 | 0.1948 | 0.2163 | 0.3094 |
| 6 | 154565335 | rs4314511 | Promoter | 0.2776 | G | A | 1/40/27 | 0.5882 | 0.4269 | 0.001647 |
| 6 | 154566421 | rs1554817 | Promoter | 0.355 | A | G | 7/22/32 | 0.3607 | 0.416 | 0.3541 |
| 6 | 154566991 | rs7755659 | Promoter | 0.2897 | C | T | 1/63/12 | 0.8289 | 0.4895 | 4.77E-10 |
| 6 | 154567145 | rs7741417 | Promoter | 0.2897 | T | C | 9/22/32 | 0.3492 | 0.4334 | 0.1451 |
| 6 | 154567362 | rs9397698 | Promoter | 0.2662 | A | C | 7/30/34 | 0.4225 | 0.4277 | 1 |
| 6 | 154567666 | rs9479798 | Promoter | 0.2839 | G | T | 3/32/34 | 0.4638 | 0.3991 | 0.2382 |
| 11 | 27721735 | *BDNF* | rs56164415 | Promoter | 0.1008 | A | G | 5/16/51 | 0.2222 | 0.2959 | 0.04271 |
| 11 | 27722298 | rs7944119 | Promoter | 0.2169 | T | G | 9/25/42 | 0.3289 | 0.4057 | 0.09709 |
| 11 | 27722689 | rs13306221 | Promoter | 0.1008 | T | C | 3/16/56 | 0.2133 | 0.2503 | 0.1808 |
| 11 | 113345818 | *DRD2* | rs7116768 | Promoter | 0.2426 | G | C | 1/13/24 | 0.3421 | 0.3168 | 1 |

**Table 3.** Association analysis of variants, under allelic and genotype model

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| rsIDs | Physical Position(hg19) | MinorAllele (A) | Allelic | Genotype: AA/AB/BB |
| Addicted | Control | P-value | Addicted | Control | P-value |
| rs677830 | 154107531 | T | 0.1307 | 0.1234 | 0.8074 | 7/72/250 | 2/15/60 | - |
| rs1554817 | 154245287 | G | 0.3277 | 0.2951 | 0.4863 | 41/93/133 | 7/22/32 | 0.7402 |
| rs7741417 | 154246011 | T | 0.2836 | 0.3175 | 0.4504 | 31/94/150 | 9/22/32 | 0.7652 |
| rs9397698 | 154246228 | A | 0.3538 | 0.3099 | 0.3198 | 47/131/140 | 7/30/34 | 0.5429 |
| rs9479798 | 154246532 | G | 0.2614 | 0.2754 | 0.7375 | 22/116/168 | 3/32/34 | - |
| rs7944119 | 27700751 | T | 0.3632 | 0.2829 | 0.06178 | 37/157/124 | 9/25/42 | 0.02398 |
| rs13306221 | 27701142 | T | 0.2228 | 0.1467 | 0.03966 | 13/109/181 | 3/16/56 | - |
| rs7116768 | 113475096 | G | 0.1724 | 0.1974 | 0.6124 | 4/42/99 | 1/13/24 | - |

**Table 4**. Association analysis of variants, using recessive and dominant genetic model

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| rsIDs | Physical position (hg19) | MinorAllele (A) | Dominant: AA+AB/BB | Recessive: AA/AB+BB |
| Addicted | Control | P-value | Addicted | Control | P-value |
| rs677830 | 154107531 | T | 79/250 | 17/60 | - | 7/322 | 2/75 | - |
| rs1554817 | 154245287 | G | 134/133 | 29/32 | 0.7092 | 41/226 | 7/54 | 0.4391 |
| rs7741417 | 154246011 | T | 125/150 | 31/32 | 0.59 | 31/244 | 9/54 | 0.5043 |
| rs9397698 | 154246228 | A | 178/140 | 37/34 | 0.554 | 47/271 | 7/64 | 0.2783 |
| rs9479798 | 154246532 | G | 138/168 | 35/34 | - | 22/284 | 3/66 | - |
| rs7944119 | 27700751 | T | 194/124 | 34/42 | **0.009863** | 37/281 | 9/67 | 0.9598 |
| rs13306221 | 27701142 | T | 122/181 | 19/56 | - | 13/290 | 3/72 | - |
| rs7116768 | 113475096 | G | 46/99 | 14/24 | - | 4/141 | 1/37 | - |

**References**

1. Milton A. L., Everitt B. J. The persistence of maladaptive memory: addiction, drug memories and anti-relapse treatments, Neurosci Biobehav Rev 2012: 36: 1119-1139.

2. Al-Hasani R., Bruchas M. R. Molecular mechanisms of opioid receptor-dependent signaling and behavior, Anesthesiology 2011: 115: 1363-1381.

3. Schwantes-An T. H., Zhang J., Chen L. S., Hartz S. M., Culverhouse R. C., Chen X. et al. Association of the OPRM1 Variant rs1799971 (A118G) with Non-Specific Liability to Substance Dependence in a Collaborative de novo Meta-Analysis of European-Ancestry Cohorts, Behav Genet 2016: 46: 151-169.

4. Jones J. D., Comer S. D. A review of pharmacogenetic studies of substance-related disorders, Drug Alcohol Depend 2015: 152: 1-14.

5. Pecina M., Love T., Stohler C. S., Goldman D., Zubieta J. K. Effects of the Mu opioid receptor polymorphism (OPRM1 A118G) on pain regulation, placebo effects and associated personality trait measures, Neuropsychopharmacology 2015: 40: 957-965.

6. Kreek M. J., Koob G. F. Drug dependence: stress and dysregulation of brain reward pathways, Drug Alcohol Depend 1998: 51: 23-47.

7. Kreek M. J. Drug addictions. Molecular and cellular endpoints, Ann N Y Acad Sci 2001: 937: 27-49.

8. Teh LK B. Z., Zakaria ZA, Fazleen HMH, Salleh MZ. Single Nucleotide Polymorphism (SNPs) analysis of Mu-opioid receptors (OPRM1) using Denaturing High Performance Liquid Chrom.atography (DHPLC) among the intravenous drug users., International Journal of Pharmacy and Pharmaceutical Sciences 2014: 6: 7.

9. Mistry C. J., Bawor M., Desai D., Marsh D. C., Samaan Z. Genetics of Opioid Dependence: A Review of the Genetic Contribution to Opioid Dependence, Curr Psychiatry Rev 2014: 10: 156-167.

10. Kieffer B. L., Gaveriaux-Ruff C. Exploring the opioid system by gene knockout, Prog Neurobiol 2002: 66: 285-306.

11. Shirayama Y., Chaki S. Neurochemistry of the nucleus accumbens and its relevance to depression and antidepressant action in rodents, Curr Neuropharmacol 2006: 4: 277-291.

12. Matsui A., Williams J. T. Opioid-sensitive GABA inputs from rostromedial tegmental nucleus synapse onto midbrain dopamine neurons, J Neurosci 2011: 31: 17729-17735.

13. Feng Y., He X., Yang Y., Chao D., Lazarus L. H., Xia Y. Current research on opioid receptor function, Curr Drug Targets 2012: 13: 230-246.

14. De Vries T. J., Shippenberg T. S. Neural systems underlying opiate addiction, J Neurosci 2002: 22: 3321-3325.

15. Satel S., Lilienfeld S. O. Addiction and the brain-disease fallacy, Front Psychiatry 2013: 4: 141.

16. Koob G. F., Volkow N. D. Neurocircuitry of addiction, Neuropsychopharmacology 2010: 35: 217-238.

17. Bolanos C. A., Nestler E. J. Neurotrophic mechanisms in drug addiction, Neuromolecular Med 2004: 5: 69-83.

18. Bibel M., Barde Y. A. Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system, Genes Dev 2000: 14: 2919-2937.

19. Dechant G., Neumann H. Neurotrophins, Adv Exp Med Biol 2002: 513: 303-334.

20. Graham D. L., Edwards S., Bachtell R. K., DiLeone R. J., Rios M., Self D. W. Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse, Nat Neurosci 2007: 10: 1029-1037.

21. Thoenen H. Neurotrophins and neuronal plasticity, Science 1995: 270: 593-598.

22. Bekinschtein P., Cammarota M., Izquierdo I., Medina J. H. BDNF and memory formation and storage, Neuroscientist 2008: 14: 147-156.

23. Lu Y., Christian K., Lu B. BDNF: a key regulator for protein synthesis-dependent LTP and long-term memory?, Neurobiol Learn Mem 2008: 89: 312-323.

24. Corominas M., Roncero C., Ribases M., Castells X., Casas M. Brain-derived neurotrophic factor and its intracellular signaling pathways in cocaine addiction, Neuropsychobiology 2007: 55: 2-13.

25. Angelucci F., Ricci V., Pomponi M., Conte G., Mathe A. A., Attilio Tonali P. et al. Chronic heroin and cocaine abuse is associated with decreased serum concentrations of the nerve growth factor and brain-derived neurotrophic factor, J Psychopharmacol 2007: 21: 820-825.

26. Chen S. L., Lee S. Y., Chang Y. H., Wang T. Y., Chen S. H., Chu C. H. et al. The BDNF Val66Met polymorphism and plasma brain-derived neurotrophic factor levels in Han Chinese heroin-dependent patients, Sci Rep 2015: 5: 8148.

27. Heberlein A., Dursteler-MacFarland K. M., Lenz B., Frieling H., Grosch M., Bonsch D. et al. Serum levels of BDNF are associated with craving in opiate-dependent patients, J Psychopharmacol 2011: 25: 1480-1484.

28. Zhang K., Jiang H., Zhang Q., Du J., Wang Y., Zhao M. Brain-derived neurotrophic factor serum levels in heroin-dependent patients after 26weeks of withdrawal, Compr Psychiatry 2016: 65: 150-155.

29. Dluzen D. E., Story G. M., Xu K., Kucera J., Walro J. M. Alterations in nigrostriatal dopaminergic function within BDNF mutant mice, Exp Neurol 1999: 160: 500-507.

30. Horger B. A., Iyasere C. A., Berhow M. T., Messer C. J., Nestler E. J., Taylor J. R. Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor, J Neurosci 1999: 19: 4110-4122.

31. Kernie S. G., Liebl D. J., Parada L. F. BDNF regulates eating behavior and locomotor activity in mice, EMBO J 2000: 19: 1290-1300.

32. Mamounas L. A., Altar C. A., Blue M. E., Kaplan D. R., Tessarollo L., Lyons W. E. BDNF promotes the regenerative sprouting, but not survival, of injured serotonergic axons in the adult rat brain, J Neurosci 2000: 20: 771-782.

33. de Cid R., Fonseca F., Gratacos M., Gutierrez F., Martin-Santos R., Estivill X. et al. BDNF variability in opioid addicts and response to methadone treatment: preliminary findings, Genes Brain Behav 2008: 7: 515-522.

34. Hou H., Qing Z., Jia S., Zhang X., Hu S., Hu J. Influence of brain-derived neurotrophic factor (val66met) genetic polymorphism on the ages of onset for heroin abuse in males, Brain Res 2010: 1353: 245-248.

35. Lang U. E., Sander T., Lohoff F. W., Hellweg R., Bajbouj M., Winterer G. et al. Association of the met66 allele of brain-derived neurotrophic factor (BDNF) with smoking, Psychopharmacology (Berl) 2007: 190: 433-439.

36. Li X., Wolf M. E. Multiple faces of BDNF in cocaine addiction, Behav Brain Res 2015: 279: 240-254.

37. McGinty J. F., Whitfield T. W., Jr., Berglind W. J. Brain-derived neurotrophic factor and cocaine addiction, Brain Res 2010: 1314: 183-193.

38. Janak P. H., Wolf F. W., Heberlein U., Pandey S. C., Logrip M. L., Ron D. BIG news in alcohol addiction: new findings on growth factor pathways BDNF, insulin, and GDNF, Alcohol Clin Exp Res 2006: 30: 214-221.