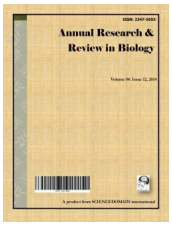

Annual Research & Review in Biology

17(2): 1-5, 2017; Article no.ARRB.36287
ISSN: 2347-565X, NLM ID: 101632869

Study of -141 DRD2 Dopamine Receptor Gene Polymorphism (rs1799732) and Heroin Dependence

L. Mehdizadeh Fanid[1], M. Adampourezare[1*] and P. Sistani[1]

[1]Department of Biology, Faculty of Natural Sciences, University of Tabriz, 29 Bahman Bolvard, Tabriz, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Authors LMF and MA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author PS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/36287
Editor(s):
(1) Giuseppe Lanza Department of Neurology I.C. "Oasi" Institute for Research on Mental Retardation and Brain Aging (I.R.C.C.S.), Troina, Italy and Department "G.F. Ingrassia", Section of Neurosciences, University of Catania, Italy.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Michael Chen, California State University, USA.
(2) Alexandros Sarantopoulos, Aristotle University of Thessaloniki, Greece.
Complete Peer review History: http://www.sciencedomain.org/review-history/21002

Original Research Article

Received 22nd August 2017
Accepted 11th September 2017
Published 15th September 2017

ABSTRACT

Previous studies showed that the dopamine receptor D2 (DRD2) may be associated with drug dependence. This study aimed to determine the role of DRD2 in development of substance dependence in Iranian-Azeri heroin-dependent patients.

Materials and Methods: 160 heroin-dependent subjects and 164 healthy controls were recruited in the North West region of Iran. The single-nucleotide-polymorphisms (SNP) of the DRD2 gene were genotyped in all subjects by PCR-RFLP technique and the data analysis was performed through statistical package for the social sciences software, using Chi-square (X^2) test.

Results: No significant difference was detected in genotypes and alleles of -141 Ins/Del (rs1799732) polymorphism of DRD2 gene between the control and case groups (p-value>0.05).

Conclusions: The results showed that -141 DRD2 gene polymorphism did not relate with heroin dependence in Iranian-Azeri population.

Keywords: Drug abuse; polymorphism; DRD_2 gene.

**Corresponding author: E-mail: adampourezare@gmail.com;*
E-mail: lfanid@yahoo.co.uk;

1. INTRODUCTION

Opiate belongs to group of psychoactive substances (PAS) that are commonly abused and produce highly addictive effect. In any of the form of use (natural, semi synthetic or synthetic) as opium, morphine, kodein or heroin, opioids cause significant health deterioration and its consequences. Opiate dependence is a major social and medical problem and a chronic and relapsing brain disease characterized by drug dependence, tolerance, and compulsive seeking and use despite the harmful consequences [1]. Heroin addiction is a chronic relapsing disease described by compulsive drug seeking, drug abuse, tolerance and physical dependence. It is treated by methadone and behavioral therapy [1]. The relapse rate of heroin dependence is high because of its severe withdrawal symptoms, strong craving induction, and compulsive drug-seeking behavior after repeated use. Hence, preventing relapse of heroin dependence is very challenging. The genetic influence on the development of drug addiction has been shown to be substantial, with an estimated range of 40-60% for the inherited risk of drug addiction [2,3]. The identification of vulnerability genes related to heroin dependence would be useful not only for understanding the pathogenesis of heroin dependence but also for preventing its occurrence and relapse.

Dopamine is an important neurotransmitter involved in reward mechanism in brain and thereby influences development and relapse of Heroin addiction [4]. The dopaminergic system influences/regulates brain reward mechanism [5] and therefore is considered a strong candidate for drug dependence. Animal and human studies of addiction indicate that the D2 dopamine receptor (DRD2) plays a critical role in the mechanism of reward and reinforcement behavior. Promoter polymorphism, −141C insertion/deletion of the DRD2 gene involving the insertion (Ins)/deletion (Del) of a cytosine, is also one of the commonly investigated polymorphisms. The −141C Ins/Del (rs1799732) polymorphism was reported to be associated with DRD2 density [4,5].

2. METHODS

2.1 Participants

We enrolled male Iranian-Azeri heroin-dependent patients receiving methadone replacement therapy at the Sina clinic. The patients were former severe heroin addicts treated at a methadone maintenance treatment program at the time of recruitment. Methadone is a drug that is similar to heroin and subscribed to heroin addicts patients to lessen Withdrawal symptoms. All cases had a history of at least two year of daily multiple uses of heroin. Moreover, all participants were unrelated, and born and living in Tabriz-Iran and were selected based on DSM-5 criteria [6]. The DSM-5 recognizes substance-related disorders resulting from the use of drugs including opioids such as heroin. Criteria for Substance Use Disorders Substance includes: 1. taking the substance in larger amounts or for longer than you're meant to, 2. Wanting to cut down or stop using the substance but not managing to, 3. Spending a lot of time getting, using, or recovering from use of the substance, 4. Cravings and urges to use the substance, 5. Not managing to do what you should at work, home, or school because of substance use, 6. Continuing to use, even when it causes problems in relationships, 7. Giving up important social, occupational, or recreational activities because of substance use, 8. Using substances again and again, even when it puts you in danger, and 9.Continuing to use, even when you know you have a physical or psychological problem that could have been caused or made worse by the substance [6]. A total of 160 heroin addicted patients (mean age 40 ± 12) and 164 controls (mean age 42 ± 16) participated in the study.

Control subjects were screened using a self-report clinical assessment which screens for adult psychiatric diseases using a modified version of the Composite International Diagnostic Interview – Short Form (CIDI-SF). Medical history of all control subjects were also gathered by a physician. Individuals, who self-identified as having psychiatric disorders, including alcohol dependence, were excluded from this study. Oral and written informed consents were obtained from all participants, and the research protocol was approved by the ethics committee of Tabriz University of Medical sciences.

2.2 Laboratory Evaluation

To determine -141 C Ins/Del DRD2 gene polymorphism, DNA was extracted from white blood cells using saluting out method and the -141 C Ins/Del DRD2 gene was amplified by PCR-RFLP using a thermal cycler (Sensoquest, GmbH, Germany) containing 0.2 Mm of forward and reverse primers, 10x PCR buffer, 1.5 mM MgCl2, 200 mMdNTPs, and 1 unit of Taq DNA Polymerase (Cinnagen, Iran) in a 25 mL total

volume. PCR conditions were 95℃ for 4 min, 95℃ for 30 s, 58° and 60° for 30 s (for Taq I A and B, respectively), 72℃ for 30 s, Steps 2–4 were repeated by 35 cycles followed by 72℃ for 3 min. Product digestion of PCR was done with BstNI restriction enzyme (Thermo Scientific) and the digested fragments were electrophoresed on acrylamide gel (Table 1).

2.3 Statistical Methods

Allele and genotype frequencies were analyzed using statistical package for the social sciences (SPSS) software (v.16; SPSS Inc., USA) with a P<0.05 as being statistically significant (Table 2). To assess Hardy-Weinberg equilibrium, an online HWE calculator, http://www.oege.org/software/hardy-weinberg.html, was applied.

3. RESULTS

To investigate the association between the DRD2 Gene -141 (rs1800497) polymorphism and risk abuse, 160 patients with heroin addiction problems and 164 subjects that healthy participants were selected.

An abnormality from the Hardy–Weinberg equilibrium was not detected in the patient as well as control groups. Ins/Ins and Ins/Del genotypes were 87.5 % and 12.5 % for the case group and 90.2 % and 9.8 % for control group, respectively. Del/Del genotype was not demonstrated in any groups (p>0.05). Statistical substantial difference was not shown (P-values >0.05) (Table 2).

4. DISCUSSION

In relation to drug abuse, the research provides some evidence that genes and their polymorphisms may play an important role in its development [7]. Opioid drugs mediate their reinforcing effects by dopamine-dependent and dopamine-independent mechanisms. The dopaminergic mesocorticolimbic reward pathways have been implicated in the etiology of drug addictions [7,8,9]. Addictive drugs transiently increase extracellular dopamine in the ventral striatum inducing abnormal learning process and promoting compulsive drug abuse [10]. Polymorphisms in genes of the dopamine pathway are candidates for drug addiction vulnerability [11,12].

Hence, the current study was outlined to evaluate the probability of a connotation among single nucleotide polymorphisms of DRD2 gene as well as drug addiction risks in Iranian-Azeri. In this study, a substantial variation was not detected in the frequencies of allele polymorphism of -141 C of DRD2 gene and substance addiction (p>0.05) (Table 2). The Ins/Ins dominant genotyping was lower in the case group than the control group and the Ins/Del heterozygous genotyping was lower in the control group than the case group. The Del/Del recessive homozygous genotyping was not shown in any of groups. The Ins C allele frequency was lower in the case group than the control group and the Del C allele frequency was higher in the case group than the control group and dissimilarities were not substantial (p>0.05) (Table 2). In addition, Wang and colleagues carried out a study on Chinese Han subjects and found that the -141 C Ins/Del polymorphism is associated to heroin abuse [13] and Chinese Han population with Ins allele gene deletion are at lower risk of heroin dependence. However, association between the −141C polymorphism and heroin dependence has not been seriously studied in different populations. Nevertheless, Prasad and his associates have investigated the -141C Ins/Del with alcoholism in India and they reported that this Promoter polymorphism plays an important role in D2 receptor expression [14]. Moreover, an invitro analysis suggested that -141C Ins/Del polymorphism of DRD2 alters its transcriptional activity and thus regulates the expression of DRD2 receptor [15].

However, our statistics was not detected a significant association between the −141C polymorphism and heroin dependence in Iranian-Azeri subjects. This study suggests an extension to the list of susceptibility genes and variants underlying heroin addiction. This results demand further exploration to confirm the tentative associations and to elucidate the involved mechanisms.

For instance, an important limitation of this study is the number of patients; consequently, the result of this paper can't explain the whole situation of this region. Therefore, further studies on larger population samples will be required for explaining the association between polymorphisms of −141C of DRD2 gene and risk of abuse.

Table 1. Genotypic profile attained for -141 C Ins/Del polymorphismof DRD2 gene

Single nucleotide polymorphism	Primers	Annealing temperature	Restriction enzyme	Fragment size
−141C insertion/deletion (rs1799732)	F:5'GACCCAGCCTGCAATCAC3' R:5'AGGAGCTGTACCTCCTCGG3'	57℃/	Bst NI	Ins C = 124, 32 Del C = 156

Table 2. Genotypic profiles obtained for -141 C Ins/Del polymorphismof DRD2 gene

	Case group (N=160)	Control group (N=164)	OR (95% CI)	P-value
Genotypes -141 C Ins/Del				
Ins/Ins	140(87.5%)	148(90.2%)	1.321(0.493-3.54)	0.578
Ins/Del	20(12.5%)	16(9.8%)		
Del/Del	0	0		
Alleles				
Ins C	300	312	0.769(0.391-1.513)	0.446
Del C	20	16		

OR: Odds ratio, CI: Confidence intervals

5. CONCLUSIONS

The results showed that -141 DRD2 gene polymorphism did not relate with heroin dependence in Iranian-Azeri population. However, our results have to be viewed in the perspective of potential limitation posed by small sample size and it warrants replication in larger sample sets.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Van den Bree MB, Johnson EO, Neale MC, Pickens RW. Genetic and environmental influences on drug use and abuse/dependence in male and female twins. Drug Alcohol Depend. 1998;52:231-241.
2. Uhl GR. Molecular genetics of substance abuse vulnerability: Remarkable recent convergence of genome scan results. Ann. N Y Acad. Sci. 2004;1025:1-13.
3. Uhl GR, Drgon T, Johnson C, Fatusin OO, et al. ''Higher order'' addiction molecular genetics: Convergent data from genome-wide association in humans and mice. Pharmacol. Res. 2008;75:98-111.
4. Arinami T, Gao M, Hamaguchi H, et al. A functionalpolymorphism in the promoter region of the dopamine D2 receptor gene is associated with schizophrenia. Hum Mol Genet. 1997;6:577–82.
5. Jonsson EG, Nöthen MM, Grunhage F, et al. Polymorphismsin the dopamine D2 receptor gene and their relationships to striataldopamine receptor density of healthy volunteers. Mol Psychiatry. 1999; 4:290–96.
6. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th Ed. New York: American Psychiatric Association; 2015.
7. Le Foll B, Gallo A, Le Strat Y, Lu L, Gorwood P. Genetics of dopamine receptors and drug addiction: A comprehensive review. Behav. Pharmacol. 2009;20(1):1–17.
8. Volkow ND, Wang GJ, Fowler JS, Tomasi D, Telang F. Addiction: Beyond dopamine reward circuitry. Proc. Natl acad. Sci. Usa. 2011;108(37):15037–15042.
9. Hauge XY, Grandy DK, Eubanks JH, Evans GA, Civelli O, Litt M. Detection and characterization of additional DNA polymorphisms in the dopamine d2 receptor gene. Genomics. 1991;10(3): 527-30.
10. Girault JA. Signaling in striatal neurons. Thephosphoproteins of reward, addiction, and dyskinesia. Prog. Mol. Biol. Transl. Sci. 2012;106:33–62.
11. Bertolino A, Fazio L, Caforio G, Blasi G, Rampino A, Romano R, Di Giorgio A, Taurisano P, Papp A, Pinsonneault J,

Wang D, Nardini M, Popolizio T, Sadee W. Functional variants of the dopamine receptor d2 gene modulate prefronto-striatal phenotypes in schizophrenia. Brain. 2009;132:417–425.

12. Bertolino A, Taurisano P, Pisciotta NM, Blasi G, Fazio L, Romano R, Gelao B, Lo Bianco L, Lozupone M, Di Giorgio A, Caforio G, Sambataro F, Niccoli-Asabella A, Papp A, Ursini G, Sinibaldi L, Popolizio T, Sadee W, Rubini G. Genetically determined measures of striatal d2 signaling predict prefrontal activity during working memory performance. Plos One. 2010;5:e9348.
13. Wang N, Zhang JB, Zhao J, Cai XT, Zhu YS, Li SB. Association between dopamine d2 receptor gene polymorphisms and the risk of heroin dependence. Genet. Mol. Res. 2016;15(4).
14. Pushplata Prasad, Atul Ambekar, Meera Vaswani. Dopamine receptor d2 polymorphisms and susceptibility to alcohol dependence in Indian males: A preliminary study. National Drug Dependence Treatment Centre, All India Institute of Medical Sciences, New Delhi – 110029; India; 2008.
15. Arinami T, Gao M, Hamaguchi H, Toru M. A functional polymorphism in the promoter region of the dopamine d2 receptor gene is associated with schizophrenia. Hum Mol Genet. 1997;6:577-82.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21002