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Research Article

The Association of Single-nucleotide Polymorphisms in Dopamine Receptor D2 Gene with Heroin Dependence in an Iranian Population

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Abstract

Drug addiction is a psychiatric condition known to be associated with the dopaminergic system, which has a vital part in the reward mechanism and etiology of drug addictions in the brain. This study's aim is to assess the relationship of single-nucleotide polymorphisms in TaqIB and TaqIA of the DRD2 gene with the risk of drug dependence in a Northwestern Iranian population. 83 drug-dependent subjects and 83 healthy subjects have been recruited in this study. After genomic DNA isolation, two single-nucleotide polymorphisms, TaqIA (rs1800497) and TaqIB (rs1079597), were genotyped using the PCR-RFLP technique. Data analysis has been performed using the Statistical Package for the Social Sciences (SPSS-24) software for the chi-square (X^2) test. No significant difference was detected in the TaqIA polymorphism between the control and case study groups ($p > .05$). In the case group, the TaqIB polymorphism was higher than the control group, showing a statistically significant variation ($p < .05$). The SNPs of TaqIB (rs1079597) in the DRD2 gene are associated with genetic susceptibility to drug abuse in the Iranian-Azeri population.

Keywords

Drug abuse • Polymorphism • DRD2 gene

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Substance addiction causes major health problems and imposes a significant burden on society. Drug addiction is a chronic disease of the brain due to its multiple genetic, environmental, and drug-induced causes that attribute to the growth of drug addiction. Neurobiological research emphasizes that the dopaminergic mesocorticolimbic pathway, which interacts with additional inhibitory as well as stimulatory and neurotransmitter systems, plays a major part in the brain's reward system. Numerous addictive drugs such as cocaine, opiates, nicotine, and cannabis act on distinct parts of these systems. However, in the nucleus accumbens, they all eventually increase extracellular dopamine (Comings & Blum, 2000; Everitt et al., 1999; Robbins & Everitt, 1999), which is the most likely target of drug addiction.

Genetic polymorphisms in the dopamine pathway, such as single nucleotide polymorphisms (SNPs), are candidates for drug-addiction vulnerability. One of these important genes is the dopamine receptor D2 (DRD2), which includes two single-nucleotide polymorphisms, TaqIA and TaqIB, which support controlled digestion in this area through the TaqI enzyme. Initial reports show that one of the risk factors for heroin dependence (Hou & Li, 2009), alcoholism (Blum et al., 1990), and drug addiction is the A1 allele of TaqIA. However, several studies have been unsuccessful at proving this (Gelernter et al., 1991; Noble, 2003). The polymorphism of TaqIA (RFLP) is placed approximately10 kilo-bases down from the DRD2 gene in exon 8 of the ANKK1 (Ankyrin Repeat and Kinase Domain Containing 1) gene (Neville, Johnstone, & Walton, 2004), which is part of the threonine/serine kinase family. TaqIA polymorphism produces a change in amino acids in ANKK1 (Glu713Lys) and appears to have a substantial relation to the outcome of substrate-binding. Furthermore, DRD2 receptor density can be indirectly affected by ANKK1. In addition, TaqIA SNP is likely to be only an indication of additional DRD2 variants that function in association with addiction, such as the powerfully linked TaqIB. The polymorphism TaqIB (restriction fragment length polymorphism [RFLP]; rs17294542, G/A) is positioned in the structural and regulatory gene-coding areas (5' region; Hauge et al., 1991). The current study's aim is the potential involvement DRD2 gene polymorphisms have in heroin addiction among a population sample of Iranian-Azeri adults.

Methods

Patients (Sample Selection)

The study includes 83 addicts (mean age of 40 ± 12) and 83 controls (mean age of 42 ± 16). Blood samples from heroin-addicted patients have been collected from a rehabilitation center in Tabriz. The diagnosis for heroin addiction is based on the DSM-IV criteria (American Psychological Association [APA], 1994; Svenaeus, 2014). A complete set of data including the history of drug addiction and psychiatric evaluation have also been obtained from each addicted patient.

By using an adjusted style of the Composite International Diagnostic Interview – Short Form (CIDI-SF; World Health Organization [WHO], 1990), control subjects were screened using a self-report clinical assessment that checks for psychiatric diseases in adults. Those who self-identified as having had developed psychiatric disorders including alcohol dependence were eliminated from the analysis. Informed written and verbal consent were obtained from all participants. In addition, the Tabriz University of Medical Sciences Ethics Committee accepted the research protocol.

Molecular Techniques

By use of the salting-out method, DNA was removed from the white blood cells, and the TaqIA and TaqIB polymorphisms were amplified through PCR-RFLP using a thermal cycler (*Sensoquest* by GmbH, Germany) in a PCR reaction containing 0.2 µM of reverse and forward primers, 10x PCR buffer, 1.5mM MgCl2, 200 µM dNTPs, and 1 unit of Taq DNA Polymerase (Cinnagen, Iran) in a total volume of 25 µL. PCR conditions for 4 min at 95°C, 30 s at 95°C, 30 s at 60°C and 58° (for TaqIA and TaqIB, respectively), and 30 s at 72°C; then steps 2–4 were repeated for 35 cycles after 3 min at 72°C. Product digestion of PCR was performed using TaqI restriction enzyme (Thermo Sciencetific), and the digested fragments were electrophoresed in agarose gel (Table 1).

Table1
Genotypic Profiles Attained for TaqI DRD2 Polymorphism

Single nucleotide polymorphism	Primers	Annealing temp.	Restrict. enzyme	Fragment size
TaqIA	F: 5'-GCACGTGCCACCATACCC-3' R: 5'-TGCAGAGCAGTCAGGCTG-3'	58°C	TaqI	C = 130, 180 T = 310
TaqIB	F: 5'-GATACCCACTTCAGGAAGTC- 3' R: 5'-GATGTGTAGGAATTAGCCAGG-3'	60°C	TaqI	G= 267, 192 A= 459

Statistical Analysis

Analyses of the genotype and allele frequencies were performed with SPSS-24 software using chi-square (χ^2) with a $p < .05$ for statistical significance (see Table 2). Hardy-Weinberg equilibrium was assessed with an online HWE calculator.

Results

In order to investigate the connection between TaqIA (rs1800497) and TaqIB (rs17294542) polymorphisms of the DRD2 gene with risk abuse, a total of 83 patients with addiction problems and 83 healthy subjects have been selected for this study.

No abnormalities from the Hardy–Weinberg equilibrium were detected in either the patient or control group. The control group's GG genotype in the TaqIB polymorphism was higher than the case group's, while the control group's AG genotype was lower than the case group's, with a statistically significant difference being shown ($p < .05$).

The control group's CC genotype in the TaqIA polymorphism was lower than the case group's, while the case group's CT genotype was lower than the control group's with no significant difference being shown ($p > .05$; see Table 2).

Table 2
Genotypic Profiles Obtained for TaqI Polymorphisms in the DRD2 Gene

	Case group ($N = 83$)	Control group ($N = 83$)	OR (95% CI)	p
Genotypes Taq IB				
GG	56(67.58)	69(83.13)	0.423(0.205-0.868)	
AG	27(32.42)	14(16.87)	2.364(1.152-4.887)	0.008
AA	-	-	-	
Alleles				
G	139(47.8)	152(52.2)	0.474(0.239-0.941)	0.03
A	27(65.9)	14(34.1)		
Genotypes Taq IA				
CC	62(74.69)	55(66.26)	1.503(0.771-2.904)	
CT	21(25.31)	28(33.73)	0.665(0.344-1.283)	0.215
TT	-	-		
Alleles				
C	145	138	1.401(0.790-2.583)	0.279
T	21	28		

Note. OR = Odds ratio, CI = Confidence intervals.

Discussion

Research on experimental animals has shown that chronic abuse of addictive drugs decreases dopamine D2 receptor levels throughout the striatum (Nader et al., 2002). Indeed, the measurement of decreased density in D2 and D3 receptors has already been acknowledged in abstinent stimulant-dependent individuals (SDIs) using positron emission tomography (PET). Hypo-metabolism in the prefrontal cortex (PFC) of the mid-brain is linked with significant declines in D2/3 receptors (Volkow et al., 1993, 2003). This may possibly be due to the cognitive dysfunction and maladaptive behavioral patterns exhibited by persons with substance addiction/dependency (Goldstein & Volkow, 2002).

Therefore, as a possible drug-addiction risk factor, genetic polymorphisms of the genes in the dopaminergic system have been greatly reviewed. However the outcomes have been conflicting. Therefore, we performed the current study to evaluate the probability that the single-nucleotide polymorphisms of DRD2 gene are associated with drug-addiction risks in Iranian Azeris. Significant variations were detected in this study for the frequencies of TaqIB genotype polymorphism and allele in the DRD2 gene with substance addiction ($p < .05$; see Table 2). GG-dominant genotyping

was less frequent in the case group than the control group, and GA-heterozygous genotyping was less frequent in the control group than the case group. AA-recessive homozygous genotyping did not appear in either group. The G-allele frequency was less in the case group than the control group, while the A-allele frequency was higher in the case group than the control group, with significant variations ($p < .05$; see Table 2). Similarly, Vereczkei et al. (2013) carried out a study on Hungarian subjects and found TaqIB polymorphism to be associated with heroin addiction. Also, Xu et al. (2004) detected an association of the D2 dopamine-receptor gene with risk for heroin dependence in Chinese and, to a lesser degree, German populations.

In the current study, the allele and genotype frequencies in the TaqIA polymorphism showed no significant difference in either the case or control group ($p > .05$). This outcome is in agreement with the results of Mehić-Basara, Oruč, Kapur-Pojskić, and Ramić's (2013) study carried out on Bosnian and Herzegovinian populations. It is also consistent with previously published data (de los Cobos et al., 2007; Hou & Li, 2009; Xu et al., 2004).

In conclusion, our statistics support a connection between DRD2 gene polymorphisms and abuse. For an explanation of the association between polymorphisms of the DRD2 gene and risk of abuse, additional studies need to be performed on larger population samples.

References

American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders* (4th ed.). Washington, DC: Author.

Blum, K., Noble, E. P., Sheridan, P. J., Montgomery, A., Ritchie, T., Jagadeeswaran, P. … Cohn, J. B. (1990). Allelic association of human dopamine D2 receptor gene in alcoholism. *JAMA*, *263*(15), 2055–260.

Comings, D. E., & Blum, K. (2000). Reward deficiency syndrome: Genetic aspects of behavioral disorders. *Progress in Brain Research*, *126*, 325–341.

De los Cobos, J. P., Baiget, M., Trujols, J., Sinol, N., Volpini, V., Banuls, E. … Alvarez, E. (2007). Allelic and genotypic associations of DRD2 Taq IA polymorphism with heroin dependence in Spanish subjects: A case control study. *Behavioral and Brain Functions*, *3*(1), 25. https://doi.org/10.1186/1744-9081-3-25

Everitt, B. J., Parkinson, J. A., Olmstead, M. C., Arroyo, M., Robledo, P., & Robbins, T. W. (1999). Associative processes in addiction and reward the role of amygdala–ventral striatal subsystems. *Annals of the New York Academy of Sciences*, *877*(1), 412–438.

Gelernter, J., O'Malley, S., Risch, N., Kranzler, H. R., Krystal, J., Merikangas, K., … Kidd, K. K. (1991). No association between an allele at the D2 dopamine receptor gene (DRD2) and alcoholism. *JAMA*, *266*(13), 1801–1807. http://dx.doi.org/10.1001/jama.1991.03470130081033

Goldstein, R. Z., & Volkow, N. D. (2002). Drug addiction and its underlying neurobiological basis: Neuroimaging evidence for the involvement of the frontal cortex. *American Journal of Psychiatry*, *159*(10), 1642–1652.

Hauge, X. Y., Grandy, D. K., Eubanks, J. H., Evans, G. A., Civelli, O., & Litt, M. (1991). Detection and characterization of additional DNA polymorphisms in the dopamine D2 receptor gene. *Genomics*, *10*(3), 527–530.

Hou, Q.-F., & Li, S.-B. (2009). Potential association of DRD2 and DAT1 genetic variation with heroin dependence. *Neuroscience Letters, 464*(2), 127–130.

Mehić-Basara, N., Oruč, L., Kapur-Pojskić, L., & Ramić, J. (2013). Association of dopamine receptor gene polymorphism and psychological personality traits in liability for opioid addiction. *Bosnian Journal of Basic Medical Sciences*, *13*(3), 158–162.

Nader, M. A., Daunais, J. B., Moore, T., Nader, S. H., Moore, R. J., Smith, H. R., … Porrino, L. J. (2002). Effects of cocaine self-administration on striatal dopamine systems in rhesus monkeys: Initial and chronic exposure. *Neuropsychopharmacology*, *27*(1), 35–46.

Neville, M. J., Johnstone, E. C., & Walton, R. T. (2004). Identification and characterization of ANKK1: A novel kinase gene closely linked to DRD2 on chromosome band 11q23. *Human Mutation, 23*(6), 540–545.

Noble, E. P. (2003). D2 dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *116*(1), 103–125.

Robbins, T. W., & Everitt, B. J. (1999). Drug addiction: Bad habits add up. *Nature*, *398*(6728), 567–570.

Svenaeus, F. (2014). Diagnosing mental disorders and saving the normal. *Medicine, Health Care and Philosophy*, *17*(2), 241–244.

Vereczkei, A., Demetrovics, Z., Szekely, A., Sarkozy, P., Antal, P., Szilagyi, A., … Barta, C. (2013). Multivariate analysis of dopaminergic gene variants as risk factors of heroin dependence. *PLoS One*, *8*(6). https://doi.org/10.1371/journal.pone.0066592

Volkow, N. D., Chang, L., Wang, G.-J., Fowler, J. S., Ding, Y.-S., Sedler, M., … Pappas, N. (2003). Low level of brain dopamine D2 receptors in methamphetamine abusers: Association with metabolism in the orbitofrontal cortex. *Focus*, *1*(2), 150–157.

Volkow, N. D., Fowler, J. S., Wang, G. J., Hitzemann, R., Logan, J., Schlyer, D. J., … Wolf, A. P. (1993). Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. *Synapse*, *14*(2), 169–177.

Xu, K., Lichtermann, D., Lipsky, R. H., Franke, P., Liu, X., Hu, Y., … Goldman, D. (2004). Association of specific haplotypes of D2 dopamine receptor gene with vulnerability to heroin dependence in 2 distinct populations. *Archives of General Psychiatry, 61*(6), 597–606.

World Health Organization. (1990). *Composite international diagnostic interview, version 1.0.* Geneva: Author.