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TECHNICAL NOTE

TOXICOLOGY

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Analysis of Dopamine D2 Receptor (DRD2) Gene Polymorphisms in Cannabinoid Addicts*

ABSTRACT: The gene encoding the dopamine D2 receptor (DRD2) has been suggested as a candidate gene for substance dependence. In this study, the possible association between Taq1A and Taq1B DRD2 polymorphisms and cannabinoid dependence was investigated. One hundred and twelve cannabinoid addicted and 130 healthy control subjects were included in this study. The Taq1A and Taq1B genotypes were determined in all subjects by polymerase chain reaction. For each polymorphism (A or B), the subjects were categorized into three groups according to their genotype, that is, the subjects with alleles A1/A1, A1/A2, A2/A2; B1/B1, B1/B2, and B2/B2. A significant association was found between Taq1A gene polymorphism and cannabinoid addicts compared to the control subjects. This finding suggests that polymorphism of the Taq1A, but not the Taq1B, may be associated with the susceptibility to cannabinoid dependence. Further clinical studies are required to be carried out for confirmation and evaluation of these findings.

KEYWORDS: forensic science, dopamine receptor gene, DNA polymorphism, polymerase chain reaction, cannabis, Taq1 polymorphism

Illicit psychoactive substance use, abuse, and dependence are major public health and forensic medicine problems (1). To develop more effective approaches for prevention and treatment, we need to better understand the sources of individual differences in risk. Extensive research efforts suggest that genetic factors play an important role in the development of psychoactive substance use disorder (1,2). Substance dependence, including drug dependence, is described as a cycle of spiraling dysregulation of brain reward systems that progressively increases, resulting in the compulsive use and loss of control over drug-taking (3). The neurobiological mechanism for substance dependence reward has been related to the mesocorticolimbic dopamine reward circuits (4,5). Dysfunction of the central dopaminergic neurotransmission has been suggested to play an important role in the etiology of drug addiction processes (6). It also has been shown that the dopamine D2 receptor (DRD2) gene dysfunction is associated with multidrug addiction (7). Cannabis, one addictive substance, has been shown to increase the release of dopamine from the nucleus accumbens and prefrontal cortex (8). This makes the DRD2 a potential candidate gene for susceptibility to cannabinoid dependence.

The DRD2 gene is located on chromosome 11q23.2 (9). The most studied polymorphisms of this gene are Taq1A restriction fragment length polymorphism (downstream from the polyadenylation signal) (10) and Taq1B restriction fragment length polymorphism (upstream of the initiation codon) (11). Taq1A polymorphism creates the two alleles: A1 (variant) and A2 (10). Genetic backgrounds vary according to ethnicity such as the frequency of the variant allele ranges from 5 to 18% in Caucasians to approximately 36% in African Americans and 37 to 42% in Asians (12). Significant associations between Taq1A polymorphism and substance dependence have been reported (9,13,14). However, many studies have failed to find an association between Taq1A polymorphisms and substance dependence (15,16).

The Taq1B polymorphism consists of two alleles: B1 (Taq1 "absent") and B2 (Taq1 "present") allele (11,17). The prevalence of the B1 allele was reported to be about 10% in Caucasians (18), 15% in Americans (19), 18% in Iranians (20), in 40% Chineses (21), and 42% in Singaporeans (22). Taq1B polymorphism also was associated with the alcoholism (23), cocaine dependence (14), smoking status (24), and polysubstance abuse (15). These findings suggest that Taq1A and Taq1B polymorphisms in the DRD2 gene variants could represent one of the most important gene determinants of susceptibility to cannabinoid dependence.

Therefore, the aim of the current study was to investigate whether the Taq1A and Taq1B polymorpisms of the DRD2 gene is associated with cannabinoid dependence in the Turkish population.

Materials and Methods

A lot of cannabinoid users were sent by the Prosecutors to the Department of Forensic Medicine Medical Faculty Gaziantep University of Turkey for the purpose of assessing the dependence. One hundred and twelve of cannabis users were determined to be dependent based upon the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition criteria (25) and forensic and psychiatric examination.

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The total sample was composed of 112 unrelated cannabinoid addicts (12 women and 100 men; mean age: 30 years, range: 17–56), and 130 unselected healthy controls (32 women and 98 men; mean age: 35 years, range: 17–64), all from southeastern region of Turkey and of the same Turkish ethnic origin. There was no relationship between any of the cannabionid and control group. Control subjects were selected among healthy volunteers who did not have a history of cannabis or other narcotic drugs usage. Blood samples were obtained from the subjects for the molecular analysis of the DRD2 gene polymorphism. Written informed consent was obtained from the subjects, and the local ethics committee of the Gaziantep University approved the study.

Genomic DNA was purified from leukocytes using standard methods (26). The genomic sequence of 310 bp spanning the polymorphic Taq1A site of the DRD2 gene was amplified by polymerase chain reaction (PCR) with the primer pair 5′-CCG TCG ACG GCT GGC CAA GTT GTC TA-3′ and 5′-CCG TCG ACC CTT CCT GAG TGT CAT CA-3′ (27). The oligonucleotide primers 5′-GAT ACC CAC TTC AGG AAG TC- 3′ and 5′- GAT GTG TAG GAA TTA GCC AGG- 3′ were used to amplify a 459 bp fragment that included the Taq1B polymorphism in the DRD2 gene (17). The PCR products were digested with Taq1 restriction enzyme (TaKaRa; Otsu, Shiga, Japan) for the Taq1A and Taq1B polymorphisms.

Hardy–Weinberg equilibrium (HWE) was calculated using De-finetti program (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). The chi-square (χ^2) test was used for the statistical analysis of allele frequencies and the distribution of genotype in the case and control groups. The odds ratio (OR) and 95% confidence limit (CL) were used as estimates of the relative risk. A level of $p < 0.05$ was considered statistically significant. Statistical calculations were performed using GraphPad InStat version 3.05 (GraphPad Software, San Diego, CA).

Results

Tables 1 and 2 summarize the frequencies of the Taq1A and Taq1B genotype and alleles in cannabinoid addicts and control subjects. The distribution of A1/A1, A1/A2, and A2/A2 genotypes for Taq1A polymorphism was 8.9%, 27.7%, and 64.4% in cases compared with 3.1%, 25.4%, and 71.5% in controls. The incidence of A1/A1 genotype was found to be significantly higher in cannabinoid addicts than control subjects ($p = 0.048$). The allele frequency of A1 and A2 was 0.228 and 0.782 in cases compared with 0.158 and 0.842 in controls.

Similarly, the frequency of the allele A1 was found to be significantly higher in cannabinoid addicts group as compared to control subjects ($p = 0.033$). No significant difference was found in frequencies of A1/A2 and A2/A2 genotypes between the same groups ($p > 0.05$). The observed genotype counts were deviated significantly from those expected according to the HWE ($\chi^2 = 5.080$ $p = 0.02$).

TABLE 1—*The distrubution of Taq1A polymorphism allele and genotype between cannabinoid addicts and control subjects.*

	†Cannabinoid Addicts *n* (%)	‡Control *n*(%)	Odds Ratio (CL %)*	*p*	x^2
Genotype					
A1/A1	10 (8.9)	4 (3.1)	3.1 (0.94–10.14)	0.048*	2.782
A1/A2	31 (27.7)	33 (25.4)	1.1 (0.63–1.99)	0.399	0.066
A2/A2	71 (64.4)	93 (71.5)	0.7 (0.40–1.18)	0.112	1.474
Allele					
A1	51 (22.8)	41 (15.8)	1.6 (0.99–2.49)	0.033*	3.387
A2	173 (78.2)	219 (84.2)	0.6 (0.40–1.00)	0.033*	3.387

CL, confidence limit.
*Significant differences in genotype and allele between cannabinoid addicts and control group.
†HWE $p = 0.02$ $x^2 = 5.080$.
‡HWE $p = 0.612$ $x^2 = 0.257$.

TABLE 2—*The distrubution of Taq1B polymorphism allele and genotype between cannabinoid addicts and control.*

	†Cannabinoid Addicts *n* (%)	‡Control *n* (%)	Odds Ratio (CL %)*	*p*	x^2
Genotype					
B1/B1	8 (7.1)	4 (3.1)	2.4 (0.71–8.28)	0.124	1.336
B1/B2	31 (27.7)	35 (26.9)	1.0 (0.59–1.83)	0.448	0.017
B2/B2	73 (65.2)	91 (70.0)	0.8 (0.46–1.38)	0.254	0.439
Allele					
B1	47 (21.0)	43 (16.5)	1.3 (0.85–2.12)	0.128	1.290
B2	177 (79.0)	217 (83.5)	0.7 (0.47–1.18)	0.128	1.290

CL, confidence limit.
*Significant differences in genotype and allele between cannabinoid addicts and control group.
†HWE $p = 0.080$ $x^2 = 3.060$.
‡HWE $p = 0.777$ $x^2 = 0.080$.

On the other hand, the frequencies of the B1/B1, B1/B2, and B2/B2 genotypes for Taq1B polymorphism were 7.1%, 27.7%, and 65.2% in cases compared with 3.1%, 26.9%, and 70% in controls. The allele frequency of B1 and B2 was 0.210 and 0.790 in cases compared with 0.165 and 0.835 in controls ($p = 0.128$). No significant differences were found between cases and controls ($p > 0.05$). Genotype distribution did not differ significantly from those predicted by the HWE distribution ($p > 0.05$).

Discussion

Cannabis exerts its effect by binding to cannabinoid receptor 1 (CNR1) and cannabinoid receptor 2 (CNR2) (28). CNR1 is expressed mainly in the brain (29) and CNR2 is mainly expressed in the immune system and in hematopoietic cells (30). It has been shown that the the CNR1 regulates mesolimbic dopaminergic transmission in brain areas known to be involved in the reinforcing effects of abused drugs (31). Besides, cannabis mimics the actions of endogenous cannabinoids and influences the action of dopamine (32).

The neurobiological mechanisms underlying the actions of cannabis and other abused drugs are primarily associated with the activated dopamine pathway (33). Many genes are considered to be involved in the regulation of the dopamine system, and the DRD2 gene is one of the most frequently studied in addictive disorders (34).

DRD2 Taq1A and Taq1B polymorphisms can affect human habits that are related to dopaminergic neurotransmission (13,14, 23,24). A strong correlation between Taq1A1 allele of the DRD2 gene and substance abuse, such as alcoholism (35), cocaine dependence (14), polysubstance abuse (36), and nicotine dependence (13,37), has been reported. The A1 allele of Taq1A DRD2 polymorphism has been shown to be associated with reduced DRD2 (9,38) and diminished function of DRD2 (14) in the central nervous system.

Bice et al. (39) showed that high alcohol-preferring mice exhibited lower levels of DRD2 mRNA expression in the nucleus accumbens and the hippocampus compared to low alcohol-preferring mice. On the other hand, many studies have demonstrated the influence of Taq1B polymorphism on substance dependence. For example, Taq1B polymorphism was found to be associated with the alcoholism (23), cocaine dependence (14), smoking status (24), and polysubstance abuse (15).

However, the role of DRD2 Taq1A and Taq1B polymorphisms in cannabinoid dependence is still unclear. Therefore, in this study, we investigated whether DRD2 polymorphisms affect the risk of developing cannabinoid dependence in the Turkish population. To our best knowledge, this is the first study that demonstrates an association of polymorphisms Taq1A and Taq1B with risk of cannabinoid dependence.

In this study, we found significant association of the DRD2 Taq1A polymorphism in our cannabinoid addict samples. In cannabinoid addicts group, both A1 allele and A1/A1 genotype were higher than in control group. These findings indicate an association between the Taq1A1 allele, which is associated with reduced DRD2 presence in the central nervous system and cannabinoid dependence. We therefore suggest that the A1 allele may increase the risk of cannabinoid dependence.

In contrast to the results of DRD2 Taq1A polymorphism, we did not observe any significant association of the DRD2 Taq1B polymorphism in our cannabinoid addict samples. In fact, the allele and genotype frequencies of both of these polymorphisms were almost identical in both cases and controls. This polymorphism, therefore, do not seem to be implicated in the genetic liability to cannabinoid dependence. However, this study is the first report on prevalence of DRD2 Taq1B polymorphism in Turkish population.

In conclusion, our results suggest that the presence of the A1 allele may be a risk factor for susceptibility to cannabinoid dependence and could be an important marker of genetic susceptibility to cannabinoid dependence. But DRD2 Taq1B polymorphism was not associated with cannabinoid dependence. Further studies with different ethnic groups and functional analysis of this polymorphism will be required to clarify this issue.

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