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Case-control analysis of DRD2 gene polymorphisms in drug addicted patients

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Summary

Aim. The aim of the study was to determine relationships between the selected DRD2 gene polymorphisms and drug addiction.

Methods. One hundred drug abusers undergoing treatment were recruited from the in-patient psychiatric centers in Poland. All participants were screened by means of the clinical interview SSAGA to describe the clinical picture. In the second part of the study, participants were examined using psychometric tools assessing selected psychopathological features. After that, blood samples were collected for a DNA isolation. The following DRD2 single nucleotide polymorphisms (SNPs) of the dopamine gene were genotyped: rs1800498 polymorphism of DRD2 gene (NC_000011.10:g.113420866G>A, GRCh38.p7); rs1079597 polymorphism of DRD2 gene (NC_000011.10:g.113425564C>T, GRCh38.p7); rs1076560 polymorphism of DRD2 gene (NC_000011.10:g.113412966C>A, GRCh38.p7).

Results. The rs1800498 polymorphism has shown an association with drug abuse in which a higher frequency of the allelic T form was observed in the whole group of patients and selected subgroups with concomitant opiates or cannabis abuse history when compared with the controls.

Conclusions. In the presented study, one of selected polymorphisms of DRD2 gene, revealed to be correlated with substance use disorder (at the limit of statistical significance), which could suggest its impact on dependence endophenotype. The presented research was a pilot study, so it requires replication on a larger group of patients to verify and confirm obtained outcomes.

Key words: DRD2 gene, reward

Introduction

The research carried out so far have shown that a lot of people have contact with drugs through their lives. About 60% of the American population took an illicit drug at least once in their lifetime and a percentage of the population exposed to addictive factors, including alcohol, exceeds 90%, but only few display a clinically significant dependence syndrome. Even in the case of very addictive drugs, including cocaine, only 15–16% display addiction [1]. Addiction is defined as a compulsive pattern of drug-seeking and drug-taking behavior expanding on all human activity [2].

The data presented below prompt reflection on a potential basis of pathogenesis of addiction. The widespread use of substances has led to intensive scientific research into the issue of mechanisms involved in abuse. Moreover, it has been well known for a long time that one drug abuse often leads to yet another concomitant abuse. The experience of the recent decades of research elucidates a common underlying mechanism shared by different abusive substances [3].

It appears that brain circuits connecting the globus pallidus, limbic system and nucleus accumbens seem to be a crucial factor for the expression of reward in the central nervous system (CNS) [4]. However, while each drug of abuse seems to affect this system in a different way, the ultimate result is the same and consists in dopamine release, which is the most important messenger of reward in such reward sites as the nucleus accumbens and the hippocampus [5]. Dopaminergic transmission through the D2 receptor in the above-described sites in the brain determines expression of reward [3]. As it was observed in animal studies, an agonist of D2 receptor diminishes alcohol consumption [6]. Its reduced availability in the aforementioned areas has been found to be associated with the tendency to addiction [7].

The rs1079597 polymorphism of the DRD2 gene was associated with a low dopamine receptor density [8]. The influence of the polymorphisms: rs1800497 of the ANKK1 gene and the polymorphism in intron 6 of the DRD2 gene (rs1076560), where the presence of allelic variants with A determined the reduced availability of the receptor, compared with the carriers of the second allelic variant, was also observed [9]. Furthermore, the DRD2 exists in two main splice molecular variants (mRNA) with different length. The D2 short form lacks transcript exon 6 in comparison to D2 long. The results of recent studies suggest an important role of intronic polymorphisms in the proportion of two distinct transcription variants of the D2 receptor gene [10].

The minor variants of SNPs: rs2283265 and rs1076560 have a negative influence on formation of short form of mRNA transcript of the DSD2 gene, which can lead to an increased risk of addition [11]. Indirect impact of intronic polymorphisms of the DRD2 gene suggests connections with mRNA splicing phenomenon, described in previous studies, was a driving force for carrying out this study.

The dopamine receptor D2 gene is located in the chromosome 11q23 and involves area of 65.56 kb. The DRD2 gene includes 8 exons which undergo transcription to mRNA of 2.713 kb, finally translated to D2 receptor protein of 443 amino acids. Skipping of the exon 6 leads to a form of receptor shorter by 29 amino acids [12].

The dopamine D2 receptor is found both in the pre – and postsynaptic area of dopamine neurons in the brain.. The dopamine D2 receptor plays an essential role in rewarding processes in the ventral tegmental area (VTA) and in the nucleus accumbens. The attenuation of dopaminergic transmission in the reward cascade leads to the reward deficiency syndrome [13].

In our study, we selected 3 SNPs located in introns of the DRD2 gene. The rs1079597 polymorphism is located within the first intron of the DRD2 gene. In previous studies some connections with heroin dependence were observed [14, 15]. Moreover, the association with other psychological disorders was also described [16]. The less frequent variant of the rs1079597 polymorphism influenced the D2 receptor binding potential to a similar extent as in the case of the rs1800497 polymorphism of the ANKK1 gene [17].

The rs1076560 polymorphism of the DRD2 gene is located within the 6th intron. It shows association with RNA splicing phenomenon, which can impact receptor density in the brain [13]. In some previous studies carried out on patients suffering from schizophrenia the presence of allelic variant A affected D2 receptor availability in the striatum, which was associated with preponderance of the so-called long form of the receptor in that area [18]. This polymorphism was associated with ineffective excitation of certain areas of the brain, as observed in studies using functional magnetic resonance imaging (fMRI). Both healthy and schizophrenic carriers of the alternative variant of this polymorphism showed similar changes in brain activity [19].

The rs1800498 (T/C) polymorphism of the DRD2 gene is located within 2nd intron. The haplotype analysis revealed association between this polymorphism and different addictions, including heroin [14] and nicotine addiction [20]. Some impact of this polymorphism on prognosis and responsiveness in the treatment of schizophrenia was depicted in previous studies [16].

Material and methods

Our case sample consisted of 100 poly-substance abusers who were recruited from the inpatient psychiatric centers in Poland. It was required that the participants stay free from drugs for at least three months prior to the study. A written consent was obtained after the patients were informed about the aims and process of the research. The presence of other psychiatric disorders apart from addictions was the exclusion criterion. Our control sample consisted of 100 healthy subjects. The presence of any psychiatric disorders was excluded using the Prime MD tool.

Table 1. **Profile of substance use disorder among participants**

Substance dependence	Number of cases
Psychostimulants	100
Opiates	18
Cannabis	67

table continued on the next page

Benzodiazepines	23
MDMA ('Ecstasy')	24

Mean age of treatment onset: 22 years (SD = 2); Mean age of substance use disorder (SUD) onset: 17 years (SD = 2); Mean period of untreated SUD – up to 5 years

Measures

All participants were screened for psychiatric disorders by means of the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA). This tool, used in genetic studies on alcoholism, includes parts devoted to abuse of substances other than alcohol. This tool enables to collect information regarding former conduct and personality disorder [21].

Genetic analysis

After all participants were assessed and screened with psychometric tools, 10 ml blood samples were collected for a DNA extraction using salting out method [22]. For genotyping selected SNPs of the DRD2 gene (rs1800498, rs1079597, rs1076560) Real-time PCR method, involving the use of fluorescent oligonucleotide probes, which bind DNA by hybridization with a specific sequence, known as 'HybProbes', was used.

DRD2 gene polymorphisms analysis was performed using LightCycler 2.0, Roche Diagnostics, and a melting curve analysis for each allele. After the reaction was completed, genotyping results were analyzed by means of LightCycler System Software Version 4.1 (Roche).

The paper contains only tables with statistically significant values (rs1800498).

Statistical analysis

1. Potential deviations from the Hardy–Weinberg equilibrium were assessed using an open access HWE calculator [23].
2. Age and gender analysis in the case and control group: Statistica 10 software.
3. Association analysis: case-control comparison of differences in the distribution of alleles or genotypes and comparison in the homogenous subgroups: IBM SPSS Statistics 20 software.

In the study, a significance level was set at $p \leq 0.05$.

Results and discussion

Table 2. **Age characteristic of the control and study group**

	Study group (n = 100)		Control group (n = 100)	
	Men (n = 80)	Women (n = 20)	Men (n = 77)	Women (n = 23)
Mean age/SD	25.0 (SD = 4.0)	26.0 (SD = 4.0)	25.0 (SD = 2.0)	24.0 (SD = 4.0)
Min.-max.	18–35	18–33	18–37	24–34

SD – standard deviation

The study as well as control group was matched according to gender and age.

Table 3. **Assessment of the Hardy–Weinberg equilibrium**

SNPs	Hardy-Weinberg equilibrium	
	Study group	Control group
D2 rs1076560	0.9341	0.2458
D2 rs1800498	0.3163	0.7619
D2 rs1079597	0.9341	0.743

In both the study group (n = 100) and the control group (n = 100), there were no deviations from the Hardy–Weinberg equilibrium.

Analysis of genotypes and alleles

Table 4. **Frequency of genotypes and alleles of the rs1800498 polymorphism of the DRD2 gene in patients with opiate dependence syndrome and in the control group**

Group	n	Genotypes			p	Alleles		p	HWE
		C/C n (%)	C/T n (%)	T/T n (%)		C n (%)	T n (%)		
Study	18	0 (0.0)	10 (0.56)	8 (0.44)	0.09	10 (0.28)	26 (0.72)	0.05	0.103
Control	100	21 (0.21)	48 (0.48)	31 (0.31)		90 (0.45)	110 (0.55)		0.762

p – statistical significance; n – number of subjects; HWE – Hardy–Weinberg equilibrium

A significantly more frequent occurrence of the allelic variant T was found among patients diagnosed with opiate dependence syndrome compared with the control group (p = 0.05).

Table 5. **Frequency of genotypes and alleles of the rs1800498 polymorphism of the DRD2 gene in patients with cannabis dependence syndrome and in the control group**

Group	n	Genotypes			p	Alleles		p	HWE
		C/C n (%)	C/T n (%)	T/T n (%)		C n (%)	T n (%)		
Study	67	6 (0.09)	34 (0.51)	27 (0.4)	0.09	46 (0.34)	88 (0.66)	0.05	0.304
Control	100	21 (0.21)	48 (0.48)	31 (0.31)		90 (0.45)	110 (0.55)		0.762

p – statistical significance; n – number of subjects; HWE – Hardy–Weinberg equilibrium

It was found that the allelic variant T was significantly more frequent among patients diagnosed with dependence on cannabis preparations compared with the control group ($p = 0.05$).

The remaining polymorphisms examined in the presented study did not show statistically significant differences in the frequency of occurrence of both genotypes and alleles between the study and control group.

Discussion

Dopamine D2 receptor is widely present in the striatum and the prefrontal cortex (PFC), areas engaged in the cognitive processes [24]. The results indicate that intronic polymorphisms of the DRD2 gene determinate, at least partially, the risk of displaying substance abuse in a lifetime [11]. In animal studies (monkeys), the effect of an environmentally-induced dopaminergic transmission deficit on cocaine self-administration was observed [25]. Inversely, a high D2 receptor expression reduced alcohol self-administration in rats [26].

In family studies, the subjects with abuse history showed a lower D2 receptor density when compared with unaffected relatives [27].

The dopamine D2 receptor is one of the main target for many antipsychotic medicines. The rs1076560 polymorphism in intron 6, similarly to the rs1800497 polymorphism of the ANKK1 gene, influences D2 receptor availability in the CNS [11]. The altered availability of the receptor may lead to the development of substance abuse and behavioral addictions [28]. In one study, benzamide, which is a D2 receptor agonist, displayed a 2.4-fold lower affinity to the long form of D2 receptor as compared to the short form of D2 receptor. The predominance of the long form of D2 receptor determinates a lower availability of its protein, which results in fewer presynaptic terminals where D2 short receptors are commonly located. This may be a ground for development of dopamine super-sensitivity evidenced both in schizophrenia [18] and as result of psychostimulants administration [29].

In post-mortem studies of the brain tissue of cocaine addicts genomic mRNA and DNA were extracted. The proportions of the DRD2 mRNA transcript between the long

and short form in the collected samples of the CNS tissue from the prefrontal cortex and the putamen were evaluated. The selected SNPs were also determined: rs1076560 and rs2283265. Significant association between rs2283265 and rs1076560 polymorphisms and reducing formation of the short form of D2 receptor in relation to the long form of D2 was evidenced. The minor gene variant was significantly more frequent among cocaine abusers compared with the control group [10].

In another study, the authors did not find the rs1076560 polymorphism to be associated with cocaine dependence, however, they did observe the trend that the minor allele was increased in cocaine addicts compared with the controls. The rs1076560 polymorphism was found to be strongly associated with opioid dependence [30].

The rs1076560 and rs2283265 polymorphisms exhibited an association with a lowered expression of the short form of D2 receptor, whereas the rs12364283 (T/C) polymorphism in the promoter region of the gene showed an association with a higher level of receptor mRNA. The decreased expression of the DRD2 gene caused an increased firing of the striatal medium spiny neurons. The electrophysiological investigations results demonstrated the influence of the rs1076560 and rs2283265 polymorphisms on the increased activity in the striatum and other regions associated with working memory. This was manifested by lower results in tests evaluating cognitive processes [31].

The rs1079597 polymorphism was investigated in heroin dependent patients. The allelic variant T of the rs1079597 polymorphism exhibited an association with heroin dependence, which remained significant after the Bonferroni's correction [14]. In other studies the rs1079597 polymorphism appeared to be associated with nicotine addiction [32, 33], cocaine abuse [34] and addiction to other psychostimulants [35].

Conclusions

In our study, an allelic variant T of the rs1800498 polymorphism of the DRD2 gene appeared to be significantly more frequent in opiate ($p = 0.05$) and cannabis ($p = 0.05$) addicts when compared with the controls. The rs1076560 polymorphism of the DRD2 gene, described in previous data as having impact on cutting introns out (and related dopamine D2 receptor availability in the brain) did not show any significant association with drug abuse in the presented study.

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***Conflict of interest**: The authors have declared that no competing interests exist.*

***Bioethical statement:** The study was conducted in accordance with the worldwide Good Clinical Practice (GCP) standards and ethical standards as outlined by the Declaration of Helsinki (1989). All experiments were done with approval of the Bioethics Committee of the Pomeranian Medical University in Szczecin.*

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