Association and family studies of DRD2 gene polymorphisms in alcohol dependence syndrome*

Badania asocjacyjne, badania rodzin polimorfizmów genu DRD2 w zespole zależności alkoholowej


Department of Psychiatry, Pomeranian Medical University, Szczecin, Poland

Summary

The human dopamine receptor 2 gene DRD2 plays a central role in susceptibility to Alcohol Dependence Syndrome (ADS). The aim of this study was to evaluate 3 single nucleotide polymorphisms: D2 (rs1076560), Tag1D (rs1800498), Tag1B (rs1079597) located in dopamine receptor 2 DRD2 gene and its role in alcohol dependence.

Material and Methods:
DNA was provided from alcohol dependent (AD) patients (n = 171) and healthy control subjects (n = 160) all of Polish descent. The history of alcoholism was obtained using the Polish version of the SSAGA (Semi-Structured Assessment for the Genetics of Alcoholism). We conducted case-control association study and transmission disequilibrium test (TDT). Samples were genotyped using real-time PCR method.

Results:
We did not confirm the association between studied polymorphisms and alcohol dependence syndrome. TDT revealed an adequate transmission of both alleles in the group of alcohol families.

Conclusions:
The lack of association of studied polymorphisms and ADS does not preclude its participation in the pathogenesis. Further research is needed to determine the actual contribution of DRD2 gene in the pathogenesis of alcoholism.

Key words: Alcohol dependence • Case-control study • Transmission disequilibrium test • DRD2 gene

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Author’s address: dr hab. n. zdr. Anna Grzywacz, prof. nadzw. PUM, Department of Psychiatry Pomeranian Medical University, 26 Broniewskiego, 71-460 Szczecin, Poland; e-mail: annagrzywacz@gazeta.pl

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Introduction

Social, psychological and biological factors influencing the etiology of alcohol dependence syndrome (ADS), are the subject of intense studies all over the world. It is believed that the most important risk factors of alcohol dependence are genetic (40-50%) and environmental (50-60%) factors [18,43].

Alcohol Dependence Syndrome is not only multifactorial disease but it is also a polygenic unit. When analyzing alcoholism conditioned by genetics, interactions of many genes from different systems of Central Nervous System (CNS) - dopaminergic, serotonergic and GABAergic should be taken into account as well as their mutual impact on the level of gene – gene, gene – environment.

In the association analysis, suitable selection of both studied and control group is a crucial matter not only in respect of age, sex, but also in respect of ethnical origin. It turns out that specific alleles appear in different populations in a various frequency [21]. It is so called effect of stratification which brings about considerable methodological problems in association studies. Improper selection of studied and control group in respect of ethnical can be the cause of falsification of research results [22]. As association studies based on case – control model bring incoherent results, researches of the whole trios (proband + parents) and using Transmission Disequilibrium Test (TDT) are advisable to use in the analysis of alternative method. This test compares a number of transmitted and not transmitted alleles by parents for their diseased of offspring. Both alleles should be transmitted for offspring with a 50% probability. If one of the allele is transmitted for a diseased person more frequently than it would result from statistical probability then it can be concluded that it has connection with the given trait (disease or disorder) [46]. Therefore, in the presented study there were carried out analysis on the level of both case – control and TDT for the sake of better understanding of selected polymorphisms contribution in ADS. Advisability of the selection of polymorphisms within DRD2 dopamine receptor gene can be found in literature. In 1990, Blum’s team began pioneering research to confirm the important role of dopamine receptor gene in ADS [4].

Analysis of subtypes of dopamine receptors (D1-D5), distinctly showed that (DRD2) dopamine receptor plays a key role in alcoholism [49]. Researches carried out on animals which were given ethanol, turned out to be additional evidence concerning the connection between DRD2 gene and alcohol. Researches on rodents unveiled the reduction in D2 receptor in density of caudate putamen nucleus and in the nucleus accumbens in rats preferring alcohol in comparison to those not – preferring [31]. D2 (DRD2) dopamine receptor has been studied many times for association with alcohol dependence syndrome, addiction to nicotine and schizophrenia. Both of type polymorphisms functional and non – functional were studied. The results turned out to be controversial. Strong association between variants of the DRD2 dopamine receptor gene, ADS and proactive substances were confirmed by many researchers [38,47,48]. There are, however, some reports denying association of the DRD2 gene with alcohol addiction [33,41].

Dopaminergic system

Dopamine in nervous system plays a role of neurotransmitter and is responsible for coordination, hormone production, emotional processes and higher mental activities. Dopaminergic neurons are located in ventral tegmental area and remain in functional communication with nucleus accumbens which is vulnerable to interactions of various addictive substances [24,50]. This considerable role of dopamine in the mechanism of reward system causes that it influences on development of addictions of various types [15].

Pharmacogenetics of dopamine receptor is a wide area of research of many biological branches. Dopaminergic
receptors transmit both alcohol craving signals as well as negative reinforcement symptoms. As a consequence of it, among genes encoding potential ADS markers, there were also genes of these receptors [27].

D2 receptor occurs in two forms: long D2L (443 amino acids) and short D2S (414 amino acids). The ratio of both these forms shows a large variety of tissue, however, predominant role plays D2L. Both forms are encoded by the same gene and are created as a result of alternative splicing. Literature reports show that this change does not influence on pharmacological properties of the receptor isoforms, but it may affect the interaction of the active receptor with protein G [14]. In hereby study were analyzed such polymorphisms of single nucleotide polymorphism of DRD2 gene as: D2 (rs1076560), Tag1D (rs1800498), Tag1B (rs1079597) and their vulnerability to alcoholism. There were researched frequencies of particular genotypes, allele polymorphism of DRD2 gene in comparison to control group as well as there was analyzed family research association with using TDT (Transmission Disequilibrium Test).

A human gene of dopamine receptor is placed in 11q 22-23 chromosome and consists of 8 exons divided by 7 introns [16,17]. cDNA of DRD2 gene is composed of 2499 bp. During alternative splicing of the precursor mRNA can lead to the loss of a fragment with a length of 87 nucleotides, corresponding to an exon 5. As a consequence there are created two forms of receptor which differs in respect of 29 amino acids. In most studied tissues predominates protein consisting of 443 amino acids, the form composed of 414 amino acids is more uncommon. The change concerns the third cytoplasmic loop and it influences the interaction of the receptor with protein G. There were identified several polymorphic variants in the area of the DRD2 gene, but the possible association with mental disease was shown only in some of them [45]. Genes encoding proteins represent less than 2% of the DNA which is present in every human cell and these are the ones which for the past half of the century have been treated as a store of inherited traits. In recent years, a more careful look has been implemented in the non-coding areas and found that probably some introns can control other genes as a result of which they are related to the inheritance, development and diseases [13]. In this paper we analyzed DRD2 gene polymorphisms, located within the introns, which is considered as a region of non – coding protein so seemingly-less significant.

**Materials and methods**

The study was conducted in the Department of Psychiatry, Pomeranian Medical University in Szczecin in the years 2010-2011, after obtaining the approval of the Bioethics Committee of the Pomeranian Medical and conscious, written consent of the studied. The study involved 171 Caucasian male, with alcohol dependence syndrome and their both parents called trios. The control group comprised 160 unrelated, somatically healthy people without mental disorders, matched to the study group according to age and gender. The demographic data are shown in Table 1. Recruitment and study of each patients were carried out by authorized doctors of the Department of Psychiatry Pomeranian Medical University.

**Genotyping**

Genomic DNA was isolated from peripheral blood leukocytes by salting out method [32]. For genotyping selected DRD2 gene SNPs, Real-time PCR method was used, involving the use of fluorescent oligonucleotide probes, combining the DNA by hybridization with a specific sequence, and are known as „HybProbes„ [6]. DRD2 gene polymorphisms analysis was performed on the LightCycler 2.0 Roche Diagnostics, using a melting curve analysis for each allele (Table 2). After the reaction was completed genotyping results were analyzed by means of software LightCycler System Software ver. 4.1 (Roche).

### Table 1. The mean age of patients in the control group and their parents

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=171)</th>
<th>Control group (n=160)</th>
<th>Parents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean age</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>33.27 (SD=8.49)</td>
<td>38.92 (SD=16.07)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>57.83 (SD=8.29)</td>
<td>60.69 (SD=9.05)</td>
<td></td>
</tr>
</tbody>
</table>

**SD- standard deviation**

### Table 2. Analysis of melting curves for the different alleles of the gene DRD2

<table>
<thead>
<tr>
<th></th>
<th>DRD2 (rs1076560)</th>
<th>DRD2 Tag1D (rs1800498)</th>
<th>DRD2 Tag1B (rs1079597)</th>
</tr>
</thead>
<tbody>
<tr>
<td>For allele A</td>
<td>Tm=57.36 [°C]</td>
<td>Tm=57.87 [°C]</td>
<td>Tm=57.41 [°C]</td>
</tr>
<tr>
<td>For allele C</td>
<td>Tm=64.40 [°C]</td>
<td>Tm=66.34 [°C]</td>
<td>Tm=62.25 [°C]</td>
</tr>
</tbody>
</table>

(Tm) – Melting temperature
Statistical Analysis

Frequencies of genotypes and alleles in patients with ADS and control groups were compared using the Pearson’s chi-square test, for this purpose it was used the computer program IBM SPSS Statistics 20. Compliance genotype frequencies with The Hardy–Weinberg principle was studied separately in the group of probands and controls using statistical package SAS 6.03. The transmission disequilibrium was obtained using software TDT/STDT Version 1.1, which enables the study of transmission of individual alleles and provides information on a number of alleles transmitted and not transmitted in all studied families who meet the criteria for nuclear families (trios). In all tests, the level of significance was p <0.05.

Results

Tables from 3 to 5 contain the results of the association of DRD2 gene polymorphisms: D2 (rs1076560), Tag1D (rs1800498), Tag1B (rs1079597) in the group of patients with alcohol dependence compared with the control group. Table 6 shows the number and characteristics of the trios tested for DRD2 gene polymorphisms. In our study, in both associative (type: case-control) (Table 3,4,5) and TDT (Table 6), we found no statistically significant differences (p > 0.05). For the entire study group demonstrated compliance frequency distribution of genotypes of the Hardy-Weinberg equilibrium for each polymorphism DRD2 gene.

Discussion

In the present study the association between DRD2 gene polymorphisms and alcohol dependence syndrome has not been confirmed. Differences may result from a smaller number of the control group or differences in population. Literature reports tell about the inconsistent results concerning dependence DRD2 gene and alcohol dependence.

ADS with DRD2 gene polymorphism of the studied association has been frequently reported.

It was not confirmed in our study. The reason of this was supposed to be both a lower number of a study group as well as studies on another population. However, Chinese population was studied by Lu et al. They conducted the association analysis of Tag 1B polymorphism (rs1079597) in the Chinese population, in the group of 97 patients with ADS (including 34 with behavioral disorder and 63 without the disorder) for the control group-85 subjects without psychiatric disorders. They found that the ge-

### Table 3. The frequency of genotypes and alleles of each DRD2 gene polymorphism (rs1076560) in patients with alcohol dependence syndrome and the control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Genotypes</th>
<th>Alleles</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A/A</td>
<td>A/C</td>
<td>C/C</td>
</tr>
<tr>
<td>ADS patients</td>
<td>171</td>
<td>8 (0.05)</td>
<td>57 (0.33)</td>
<td>106 (0.66)</td>
</tr>
<tr>
<td>Control group</td>
<td>160</td>
<td>6 (0.04)</td>
<td>42 (0.26)</td>
<td>112 (0.7)</td>
</tr>
</tbody>
</table>

p values of x² test for genotypes. Figures in parentheses indicate power (p values) or percentages. HWE = Hardy-Weinberg equilibrium.

### Table 4. The frequency of genotypes and alleles of each gene polymorphism Tag1D DRD2 (rs1800498) in patients with alcohol dependence syndrome and the control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Genotypes</th>
<th>Alleles</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C/C</td>
<td>C/T</td>
<td>T/T</td>
</tr>
<tr>
<td>ADS patients</td>
<td>171</td>
<td>31 (0.18)</td>
<td>89 (0.52)</td>
<td>51 (0.3)</td>
</tr>
<tr>
<td>Control group</td>
<td>160</td>
<td>28 (0.17)</td>
<td>81 (0.51)</td>
<td>51 (0.32)</td>
</tr>
</tbody>
</table>

p values of x² test for genotypes. Figures in parentheses indicate power (p values) or percentages. HWE = Hardy-Weinberg equilibrium.
A/A and A allele appeared more frequently in the group of alcoholics with behavioral disorder than in the control group of healthy people [30].

There are available studies concerning Caucasian population. Preuss et al. have studied in the German population Tag 1D polymorphism (rs1800498) in subjects addicted to alcohol and tobacco. The study group was n=265. The study group consisted of addicted to both alcohol and nicotine, were divided into mild and strong (heavy smokers and less smoking and hard-drinking and low drinkers). The results did not show any statistical significance in both groups [42]. These studies are in compliance with our results - they have been performed on the same population, however, on a larger group.

On the other hand Konishi et al. studied the risk factors for alcoholism among Mexican Americans living in Los Angeles. Association studies of the rs1079597 and rs1076560 polymorphism conducted on a group of 200 ADS patients and 251 controls. Also, no statistical significance observed in the aforementioned study analysis of the polymorphisms [23].

An interesting issue is the study of Foley et al who studied Taq 1B (rs1079597) in post-mortem brain tissue of Caucasian ADS subjects n=74 (including 34% of confirmed liver cirrhosis) and 64 controls. Research showed that genotype A/A is much more common in case group than in controls which may indicate a predisposition to ADS of this polymorphism [11]. Researchers are also interested in gene region DRD2/ANKK1 (Ankyrin Repeat and Kinase Domain containing 1) associated with ADS.

Dick et al. conducted a family study on 219 Caucasian families of the region DRD2/ANKK1 genes 26 SNP (including 10 of ANKK1 and 16 of the DRD2). Association was discovered in the area of ANKK1, suggesting that this gene is involved in signal transduction pathways and is likely biological candidate in alcohol dependence [9]. They also found that the involvement of many genes near DRD2/ANKK1 can explain such conflicting reports in the literature on the effect of DRD2 on ADS.

What also is interesting is other researchers results on the of TDT analysis in refer for our analysis. Blomqvist et al. examined the transmission disequilibrium in three DRD2 gene polymorphisms: Tag 1A, Tag 1D, -141C Ins/Del in the European population of Americans with alcohol and/or drug addiction. Our studies showed similar results concerning TDT Tag1D. The transmission disequilibrium was not found [2].

### Table 5. The frequency of genotypes and alleles of each gene polymorphism Tag1B DRD2 (rs1079597) in patients with alcohol dependence syndrome and the control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Genotypes</th>
<th>Alleles</th>
<th>p-value</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A/A</td>
<td>A/G</td>
<td>G/G</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>ADS patients</td>
<td>171</td>
<td>8 (0.05)</td>
<td>54 (0.31)</td>
<td>109 (0.64)</td>
<td>0.17</td>
</tr>
<tr>
<td>Control group</td>
<td>160</td>
<td>7 (0.04)</td>
<td>36 (0.23)</td>
<td>117 (0.73)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

p-values of x² test for genotypes. Figures in parentheses indicate power (p values) or percentages. HWE = Hardy-Weinberg equilibrium.

### Table 6. Transmission frequencies of D2 (rs1076560), Tag1D (rs1800498) and Tag1B (rs1079597) polymorphisms alleles.

<table>
<thead>
<tr>
<th>A number trio</th>
<th>N= 171</th>
<th>N= 171</th>
<th>N=171</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele transmitted rarer</td>
<td>C</td>
<td>T</td>
<td>G</td>
</tr>
<tr>
<td>Allele transmitted more frequently</td>
<td>A</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>Transmitted / not transmitted</td>
<td>54/51 (51/49%)</td>
<td>78/75 (51/49%)</td>
<td>49/48 (51/49%)</td>
</tr>
<tr>
<td>X²</td>
<td>0.09</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>p-values</td>
<td>0.76</td>
<td>0.81</td>
<td>0.92</td>
</tr>
</tbody>
</table>

SI – statistically insignificant.
Other researchers [3,25,35] suggest that the association between DRD2 and alcohol dependence is limited mainly to the “heavy” states of ADS.

As it can be seen from the data presented in the literature, there are conflicting reports and they certainly require further studies in larger populations. Discrepancies in the literature result from various factors: the severity of alcohol consumption, drinking length, the effect of stratification and different ADS phenotypes.

Studies of other scientists are still controversial. At the level of both case-control studies and TDT. The association between alcohol dependence and DRD2 gene polymorphisms is confirmed by many researchers [3,7,11,19,34,36,37,40]. But others did not confirm this relationship [1,8,10,12,26,28,29,39,51].

As for familial association study, none of the five publications revealed association between DRD2 and ADS: [2,5,10,34,40]. However, some researchers have found evidence for linkage of alcohol addiction and DRD2 gene [20], suggesting the participation of DRD2 in the alcohol addictions.

**Conclusions**

In our study we found no association in studied polymorphisms with alcohol dependence syndrome. Both associative study of the case-control type and family studies performed by TDT revealed no association DRD2 gene with alcohol dependence syndrome. The demonstration of the lack of association of studied polymorphisms does not preclude effective participation in the pathogenesis of the disease. The fact that the studied groups were not well matched for the disease course and its symptoms, can cause false positive or negative results. It should be remembered that ADS is not a homogeneous disease entity, which is why it is advisable for subsequent studies to extract homogeneous subgroups of patients, increase the population of subjects, and perform haplotype analysis. The pathogenesis of ADS, as well as other complex diseases are also influenced by environmental factors, so it is advisable to examine a number of genes with a very small genetic effect. Further research is needed to determine the actual contribution of dopaminergic receptors, especially DRD2, in the pathogenesis of alcoholism.

### References


[51] Waldman I.D., Robinson B.F., Rhee S.H.: A logistic regression ex-

The authors have no potential conflicts of interest to declare.