® Postepy Hig Med Dosw (online), 2014; 68: 1257-1263
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 2014.02.12
Accepted: 2014.07.10
Published: 2014.11.06

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

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

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Iwona Małecka [A, B, C, D, E, F], **Andrzej Jasiewicz** [A, B, C, D, E, F], **Aleksandra Suchanecka** [A, D, F], **Jerzy Samochowiec** [A, D], **Anna Grzywacz** [A, G]

Department of Psychiatry, Pomeranian Medical University, Szczecin, Poland

Summary

Introduction: 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 reveled 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 gen**

Full-text PDF: http://www.phmd.pl/fulltxt.php?ICID=1127883

Word count: 2304
Tables: 6
Figures: 1
References: 51

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

*Studies were carried out under MNiSW Grant NN 402 466540.

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 (probant + 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 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

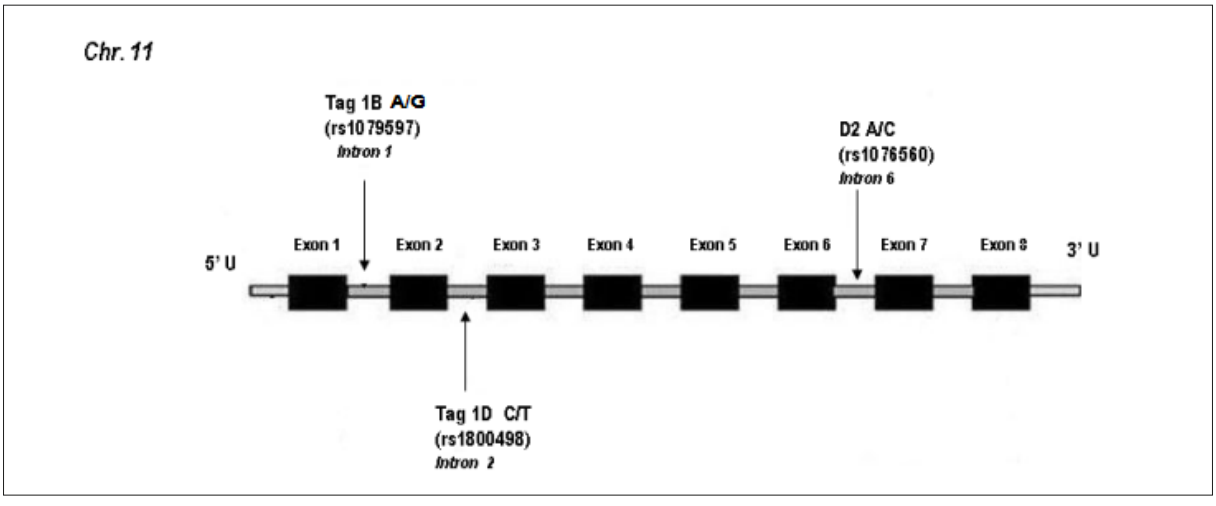

Fig. 1. The structure of the dopamine receptor *DRD2* gene including tested SNP

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

	Patients (n=171)	Control group (n=160)	Parents	
			Mothers	Fathers
Mean age	33.27 (SD=8,49)	38.92 (SD=16.07)	57.83(SD=8.29)	60.69 (SD=9.05)

SD- standard deviation

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

DRD2 (rs1076560)	DRD2 Tag1D (rs1800498)	DRD2 Tag1B (rs1079597)
For allele A Tm=57.36 [°C]	For allele T Tm=57.87 [°C]	For allele G Tm=57.41 [°C]
For allele C Tm=64.40 [°C]	For allele C Tm=66.34 [°C]	For allele A Tm=62.25 [°C]

(Tm) – Melting temperature

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).

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.

Sasabe et al. studied the association of *DRD2* polymorphism (rs1076560) in the group with ADS n= 248 vs controls n=322 in a population of Japanese men. They found that the A allele appeared significantly more often in the patient group compared to the controls. They also found that it is probably important allelic variant of alcohol dependence syndrome [44].

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.

Group	n	Genotypes			P	Alleles		P	HWE
		A/A n (%)	A/C n (%)	C/C n (%)		A n (%)	C n (%)		
ADS patients	171	8 (0.05)	57 (0.33)	106 (0.66)	0,31	73 (0.21)	269 (0.79)	0.14	0.92
Control group	160	6 (0.04)	42 (0.26)	112 (0.7)		54 (0.17)	266 (0.83)		0.42

p values of x^2 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

Group	n	Genotypes			P	Alleles		P	HWE
		C/C n (%)	C/T n (%)	T/T n (%)		C n (%)	T n (%)		
ADS patients	171	31 (0.18)	89 (0.52)	51 (0.3)	0,92	151 (0.44)	191 (0.56)	0,73	0.47
Control group	160	28(0.17)	81 (0.51)	51 (0.32)		137 (0.43)	183 (0.57)		0.67

p values of x^2 test for genotypes. Figures in parentheses indicate power (p values) or percentages. HWE = Hardy-Weinberg equilibrium

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

Group	n	Genotypes			p	Alleles		p	HWE
		A/A n (%)	A/G n (%)	G/G n (%)		A n (%)	G n (%)		
ADS patients	171	8 (0.05)	54 (0.31)	109 (0.64)	0,17	70 (0.2)	272 (0.8)	0.11	0.69
Control group	160	7 (0.04)	36 (0.23)	117 (0.73)		50 (0,.6)	270 (0.84)		0.06

p-values of x^2 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.

	DRD2 D2 (rs1076560)	DRD2 Tag1D (rs1800498)	DRD2 Tag1B(rs1079597)
A number trio	N= 171	N= 171	N=171
Allele transmitted rarer	C	T	G
Allele transmitted more frequently	A	C	A
Transmitted / not transmitted	54/51 (51/49%)	78/75 (51/49%)	49/48 (51/49%)
X^2	0.09	0.06	0.01
p- values	0.76	0.81	0.92
Statistical significance	SI	SI	SI

SI –statistically insignificant

notype 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 Causasian 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 studied 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].

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.

Piśmiennictwo

[1] Arinami T., Itokawa M., Komiyama T., Mitsushio H., Mori H., Mifune H., Hamaguchi H., Toru M.: Association between severity of alcoholism and the A1 allele of the dopamine D2 receptor gene TaqI A RFLP in Japanese. Biol. Psychiatry., 1993; 33: 108-114

[2] Blomqvist O., Gelernter J., Kranzler H.R.: Family-Based Study of DRD2 Alleles in alcohol and drug dependence. Am. J. Med. Genet., 2000; 96: 659-664

[3] Blum K., Noble E.P, Sheridan P.J, Finley O., Montgomery A., Ritchie T., Ozkaragoz T., Fitch R.J., Sadlack F., Sheffield D.: Association of the A1 allele of the D2 dopamine receptor gene with severe alcoholism. Alcohol, 1991; 8: 409-416

[4] Blum K., Noble E.P., Sheridan P.J., Montgomery A., Ritchie T., Jagadeeswaran P., Nogami H., Briggs A.H., Cohn J.B.: Allelic association of human dopamine D2 receptor gene in alcoholism. JAMA, 1990; 263: 2055-2060

[5] Bolos A.M., Dean M., Lucas-Derse S., Ramsburg M., Brown G.L., Goldman D.: Population and pedigree studies reveal a lack of association between the D2 receptor gene and alcoholism. JAMA, 1990; 264: 3156-3160

[6] Cardullo R.A., Agrawai S., Flores C., Zamecnik P.C., Wolf D.E.: Detection of nucleic acid hybridization by nonradiative fluorescence resonance energy transfer. Proc. Natl. Acad. Sci. USA, 1988; 85: 8790-8794

[7] Comings D.E., Comings B.G., Muhleman D., Dietz G., Shahbahrami B., Tast D., Knell E., Kocsis P., Baumgarten R., Kovacs B.W.: The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders. JAMA, 1991; 266: 1793-1800

[8] Cruz C., Camarena B., Mejia J.M., Paez F., Eroza V., de la Fuente J.R., Kershenobich D., Nicolini H.: The dopamine D2 receptor gene TaqI A1 polymorphism and alcoholism in a Mexican population. Arch. Med. Res., 1995; 26: 421-426

[9] Dick D.M., Wang J.C., Plunkett J., Aliev F., Hinrichs A., Bertelsen S., Budde J.P., Goldstein E.L., Kaplan D., Edenberg H.J., Nurnberger J., Hesselbrock V., Schuckit M., Kuperman S., Tischfield J., Porjesz B., Begleiter H., Bierut L.J., Goate A.: Family-based association analyses of alcohol dependence phenotypes across DRD2 and neighboring gene ANKK1. Alcohol Clin. Exp. Res., 2007; 31: 1645-1653

[10] Edenberg H.J., Foroud T., Koller D.L., Goate A., Rice J., Van Eerdewegh P., Reich T., Cloninger C.R., Nurnberger J.I., Kowalczuk M., Wu B., Li T.K., Conneally P.M., Tischfield J.A., Wu W., Shears S., Crowe R., Hesselbrock V., Schuckit M., Porjesz B., Begleiter H.: A family-based analysis of the association of the dopamine D2 receptor (DRD2) with alcoholism. Alcohol Clin. Exp. Res., 1998; 22: 505-512

[11] Foley P.F., Loh E.W., Innes D.J., Williams S.M., Tannenberg A.E., Harper C.G., Dodd P.R.: Association studies of neurotransmitter gene polymorphisms in alcoholic Caucasians. Ann. N.Y. Acad. Sci., 2004; 1025: 39-46

[12] Gelernter J., Kranzler H.R.: D2 dopamine receptor gene (DRD2) allele and haplotype frequencies in alcohol dependent and control subjects: no association with phenotype or severity of phenotype. Neuropsychopharmacology, 1999; 20: 640-649

[13] Gibbs W.W.: The unseen genome: gems among the junk. Sci. Am., 2003; 289: 26-33

[14] Giros B., Sokoloff P., Martres M.P., Riou J.F., Emorine L.J., Schwartz J.C: Alternative splicing directs the expression of two D^2 dopamine receptor isoforms. Nature, 1989; 342: 923-926

[15] Gonzales R.A., Job M.O., Doyon W.M.: The role of mesolimbic dopamine in the development and maintenance of ethanol reinforcement. Pharmacol. Ther., 2004; 103: 121-146

[16] Grandy D.K., Litt M., Allen L., Bunzow J.R., Marchionni M., Makam H., Reed L., Magenis R.E., Civelli O.: The human dopamine D2 receptor gene is located on chromosome 11 at q22-q23 and identifies a Tag RFLP. Am. J. Hum. Genet., 1989; 45: 778-785

[17] Grandy D.K., Marchionni M.A., Makam H., Stofko R.E., Alfano M., Frothingham L., Fischer J.B., Burke-Howie K.J., Bunzow J.R., Seryer A.C.: Cloning of the cDNA and gene for a human D2 dopamine receptor. Proc. Natl. Acad. Sci. USA, 1989; 86: 9762-9766

[18] Hauser J.: The typological analysis of male alcoholics. Psychiatr. Pol., 1996; 30: 835-846

[19] Hietala J., Pohjalainen T., Heikkila-Kallio U., West C., Salaspuro M., Syvalahti E.: Allelic association between D2 but not D1 dopamine receptor gene and alcoholism in Finland. Psychiatr. Genet., 1997; 7: 19-25

[20] Hill S.Y., Zezza N., Wipprecht G., Zu J., Neiswanger K.: Linkage studies of D2 and D4 receptor genes and alcoholism. Am. J. Med. Genet., 1999; 88: 676-685

[21] Kang A.M., Palmatier M.A., Kidd K.K.: Global variation of a 40-bp VNTR in the 3'-untranslated region of the dopamine transporter gene (SLC6A3). Biol. Psychiatry, 1999; 46: 151-160

[22] Kapelski P.,Hauser J., Skibińska M., Szczepankiewicz A., Dmitrzak--Węglarz M.,Gorzkowska K., Pawlak J., Czerski P.: Family based association study of DRD1, DRD2, DRD3, DRD4, DAT, COMT gene polymorphism in schizophrenia. Psychiatr. Pol., 2010; 44: 405-413

[23] Konishi T., Luo H.R., Calvillo M., Mayo M.S., Lin K.M., Wan Y.J.: ADH1B, ADH1C, DRD2 (-141C Ins), and 5-HTTLPR are associated with alcoholism in Mexican American men living in Los Angeles. Alcohol Clin. Exp. Res., 2004; 28: 1145-1152

[24] Lajtha A., Sershen H.: Nicotine: alcohol reward interactions. Neurochem. Res., 2010; 35: 1248-1258

[25] Lawford B.R., Young R.M., Rowell J.A., Gibson J.N., Feeney G.F., Ritchie T.L., Syndulko K., Noble E.P.: Association of the D2 dopamine receptor A1 allele with alcoholism: medical severity of alcoholism and type of controls. Biol. Psychiatry, 1997; 41: 386-393

[26] Lee J.F., Lu R.B., Ko H.C., Chang F.M., Yin S.J., Pakstis A.J., Kidd K.K.: No association between DRD2 locus and alcoholism after controlling the ADH and ALDH genotypes in Chinese Han population. Alcohol Clin. Exp. Res., 1999; 23: 592-599

[27] Lesch O., Dietzel M., Musalek W., Walter H., Zeiler K.: The course of alcoholism. long- term prognosis in different types. Forensic. Sci. 1988; 36: 121-138

[28] Lobos E.A., Todd R.D.: Association analysis in an evolutionary context: cladistic analysis of the DRD2 locus to test for association with alcoholism. Am. J. Med. Genet., 1998; 81: 411-419

[29] Lu R.B., Ko H.C., Chang F.M., Castiglione C.M., Schoolfield G., Pakstis A.J., Kidd J.R., Kidd K.K.: No association between alcoholism and multiple polymorphisms at the dopamine D2 receptor gene (DRD2) in three distinct Taiwanese populations. Biol. Psychiatry, 1996; 39: 419-429

[30] Lu R.B., Lee J.F., Ko H.C., Lin W.W.: Dopamine D2 receptor gene (DRD2) is associated with alcoholism with conduct disorder. Alcohol Clin. Exp. Res., 2001; 25: 177-184

[31] McBride W.J., Chernet E., Dyr W., Lumeng L., Li T.K.: Densities of dopamine D2 receptors are reduced in CNS regions of alcohol-preferring P rats. Alcohol, 1993; 10: 387-390

[32] Miller S.A., Dykes D., Plesky H.F.: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res., 1988; 16: 1215

[33] Neiswagner K., Hill S.Y., Kaplan B.B.: Association between alcoholism and the Taql A RFLP of the dopamine D2 receptor gene in the absence of linkage. Psychiatr. Genet., 1995; 3: 130

[34] Neiswanger K., Hill S.Y., Kaplan B.B.: Association and linkage studies of the TAQI A1 allele at the dopamine D2 receptor gene in samples of female and male alcoholics. Am. J. Med. Genet., 1995; 60: 267-271

[35] Noble E.P.: The DRD2 gene in psychiatric and neurological disorders and its phenotypes. Pharmacogenetics, 2000; 1: 309-333

[36] Noble E.P.: D2 dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes. Am. J. Med. Genet. B Neuropsychiatr. Genet., 2003; 116: 103-125

[37] Noble E.P., Syndulko K., Fitch R.J., Ritchie T., Bohlman M.C., Guth P., Sheridan P.J., Montgomery A., Heinzmann C., Sparkes R.S.: D2 dopamine receptor TagI A alleles in medically ill alcoholic and nonalcoholic patients. Alcohol, 1994; 29: 729-744

[38] O'Hara B.F., Smith S.S., Bird G., Persico A.M., Suarez B.K., Cutting G.R., Uhl G.R.: Dopamine D2 receptor RFLPs, haplotypes and their association with substance use in black and Caucasian research volunteers. Hum. Hered., 1993; 43: 209-218

[39] Parsian A., Cloninger C.R., Zhang Z.H.: Functional variant in the DRD2 receptor promoter region and subtypes of alcoholism. Am. J. Med. Genet., 2000; 96: 407-411

[40] Parsian A., Todd R.D., Devor E.J., O'Malley K.L., Suarez B.K., Reich T., Cloninger C.R.: Alcoholism and alleles of the human D2 dopamine receptor locus. Arch. Gen. Psychiatry, 1991; 48: 655-663

[41] Parsian A., Todd R.D., O'Malley K.L., Suarez B.K., Cloninger C.R.: Association and linkage studies of new human dopamine D2 receptor polymorphisms (RFLPs) in alcoholism. Clin. Neuropharmacol., 1992; 15 (suppl. 1), Pt. B

[42] Preuss U.W., Zill P., Koller G., Bondy B., Sokya M.: D2 dopamine receptor gene haplotypes and their influence on alcohol and tobacco consumption magnitude in alcohol-dependent individuals. Alcohol Alcohol, 2007; 42: 258-266

[43] Samochowiec J.: Molekularno-biologiczne mechanizmy zespołu zależności alkoholowej. Aachen: ShakerVerlag; 1999; 10-11

[44] Sasabe T., Furukawa A., Matsusita S., Higuchi S., Ishiura S.: Association analysis of the dopamine receptor D2 (DRD2) SNP rs1076560 in alcoholic patients. Neurosci. Lett., 2007; 29: 139-142

[45] Shi J., Gershon E.S., Liu C.: Genetic associations with schizophrenia: meta-analyses of 12 candidate genes. Schizophr. Res., 2008; 104: 96-107

[46] Spielman R.S., McGinnis R.E., Ewens W.J.: Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am. J. Hum. Genet., 1993; 52: 506-516

[47] Suarez B.K., Parsian A., Hampe C.L., Todd R.D., Reich T., Cloninger C.R.: Linkage disequilibria at the D2 dopamine receptor locus (DRD2) in alcoholics and controls. Genomics, 1994; 19: 12-20

[48] Thanos P.K.,Volkow N.D., Freimuth P., Umegaki H., Ikari H., Roth G., Ingram D.K., Hitzemann R.: Overexpression of dopamine D2 receptors reduces alcohol self-administration. J. Neurochem., 2001; 78: 1094-1103

[49] Tupala E., Hall H., Bergstrom K., Mantere T., Rasanen P., Sarkioja T., Tiihonen J.: Dopamine D2 receptors and transporters in type 1 and 2 alcoholics measured with human whole hemisphere autoradiography. Hum. Brain. Mapp., 2003; 20: 91-102

[50] Tupala E., Tiihonen J.: Dopamine and alcoholism: neurobiological basis of ethanol abuse. Prog. Neuropsychopharmacol. Biol. Psychiatry, 2004; 28: 1221-1247

[51] Waldman I.D., Robinson B.F., Rhee S.H.: A logistic regression extension of the transmission disequilibrium test for continuous traits: application to linkage disequilibrium between alcoholism and the candidate genes DRD2 and ADH3. Genet. Epidemiol., 1999; 17: S379-S384

The authors have no potential conflicts of interest to declare.