

Haplotype analysis of DRD2 and ANKK1 gene polymorphisms in alcohol dependence

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Summary

Aim. The aim of the study was to prove the hypothesis that the DRD2 and ANKK1 gene haplotypes, containing the polymorphic variant associated with reduced DRD2 availability predisposes to a severe form of alcohol dependence.

Methods. The authors studied haplotypes of 3 SNPs (rs 1799732, rs 6276, rs 1800497) and one DRD2 intron 2 STRP (dinucleotide microsatellite polymorphism) in 85 male alcoholics.

Results. Data have shown that the haplotypes are more common in the withdrawal without complications subgroup and more common in the withdrawal with complications (delirium or/and seizures) subgroup.

Conclusion. The results do not confirm the initial hypothesis but suggest a discriminative role of STRP in the severity of alcohol dependence.

STRP / haplotype / alcohol dependence / dopamine / genes

INTRODUCTION

It is a well known fact that conditioned alcohol cues cause dopamine firing [1] and can elicit craving [2], whilst the blocking of the D2 receptors can influence craving [3]. The detailed role of the DRD 2 and ANKK1 genes in alcoholism is still being investigated with different results depending on the type of alcoholic. Tarantino et al. [4] found linkage between the gene and alcohol

preference. Although Blum et al. [5] suggested an association between the gene and severe alcoholism, some groups failed to observe this association [6, 7, 8]. Huang et al. [9] did not confirm this association between the DRD2 gene and pure alcohol dependence, but managed to confirm it in anxious-depressive alcoholics whilst studying the Chinese population. In alcohol – preferring mice one single nucleotide polymorphism (SNP) in the 3' UTR (untranslated region) of the DRD2 gene was identified [10].

The haplotype analysis results of the DRD2 and ANKK1 gene polymorphisms are not conclusive. Yang et al. [11] found that the genes' role in alcohol dependence was only of minor importance, as opposed to Luo et al. [12], who confirmed a significantly higher prevalence of the H1+ haplotype (H1/H1 and H1/Hn genotypes) in non-Caucasian (Mexican American) group of alcoholics and in subgroups (including early onset subgroup), whilst investigating 20 (H1... H20) haplotypes where the H1 haplotype con-

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sists of I-B1-T-C-A-A1 polymorphic variants of the DRD2 gene.

However, the linkage disequilibrium between the -141C allele and five other *loci*, has only been found in a non-alcoholic group. Lu et al. [13] observed associations between Taq1A and Taq1B at the DRD2 locus (tested both individually and as haplotypes) and alcoholism with conduct disorder in the Chinese population. Noble et al. [14] found that in Caucasians there is a strong association of the frequency of minor alleles at the 3'-untranslated site (Taq1A) and two intronic sites (Taq1B and intron 6) in the DRD2 gene with severe alcohol dependence and higher frequency of the minor/major allele heterozygote haplotype combination (A1/A2 B1/B2 T/G) than the major allele homozygote haplotype combination (A2/A2 B2/B2 G/G) as compared with the controls. Galeeva et al. [15] described a decrease in the frequency of the locus Taq1A genotypes carrying the A1 allele and a loss of the A1N1N2, A1A2N2 haplotypes of Taq1A and NcoI loci in the DRD2 gene in Russian men with acute alcoholic psychosis as compared with the controls. Gelernter et al. [16] found no relationship between DRD2 polymorphisms and the phenotypes of alcohol dependence using haplotype analysis.

We tried to depict the doubts regarding the role of the DRD2 and ANKK1 genes polymorphisms in the risk of alcoholism, using another STRP marker within the DRD2 gene. Jönsson et al. [17], whilst investigating healthy volunteers, have found no significant relationship between the silent intronic DRD2 STRP and striatal dopamine D2 receptor density. This STRP has already been investigated in alcohol dependent subjects by Gorwood et al. [18], but without using haplotype analysis.

The above mentioned studies seem to suggest that the DRD2 and ANKK1 genes may confer vulnerability to alcohol dependence. Due to controversial results from previous studies, the aim of this study was to prove the hypothesis that the DRD2 and ANKK1 gene haplotypes, containing a polymorphic variant associated with reduced DRD2 availability, predispose to the severe form of alcohol dependence.

MATERIAL AND METHODS

We investigated a group of 85 Caucasian males, of Polish descent, recruited from the Department

of Psychiatry at the Pomeranian Medical University of Szczecin and also from the Addiction Unit in Stanomino, mean age 35±9, who fulfilled the ICD-10 alcohol dependence criteria. Their alcohol and family history was assessed by means of a structured interview, based on SSAGA (Semi-Structured Assessment for the Genetics of Alcoholism) [19]. The mean alcohol consumption was 178±96 g per day, mean age at onset was 25.1±7.4 years. A written informed consent was obtained from all the participants. The study protocol was approved by the Ethical Committee at the Pomeranian Medical University of Szczecin.

Genotyping

Genomic DNA was extracted from uncoagulated venous blood samples using a salting out method [20]. SNPs were tested using PCR and STRP was tested using Gene Scan (ABI PRISM 310). The following polymorphisms were investigated (dbSNP ID): -141 Ins (I)/del (D) (rs 1799732), exon 8: A1385G (rs 6276), and Taq 1A (rs 1800497). The PCR procedures for the gene polymorphisms under examination are described in the following literature: DRD2 (actually ANKK1) Taq1A by Grandy et al. [21], -141C Ins/del in the promoter region by Arinami [22], exon 8 A/G by Finckh et al. [23] and intron 2 (STRP) alleles by Hauge et al. [24].

Haplotype analysis

We investigated a bi-allelic restriction fragment length polymorphism (RFLPs) and one short tandem repeat polymorphism (STRP). Three SNPs and one STRP in the alcoholics group and also in the following subgroups were analysed: (i) those with a history of delirium tremens or/and seizures during withdrawal and (ii) those without the complications of alcohol withdrawal.

Haplo Stats (version 1.2.2) suite of statistical program running the R routines (version 2.3.0 <http://www.r-project.org>) was used for the statistical analysis of data.

The genotype distribution in the polymorphisms was in the Hardy-Weinberg equilibrium (calculated using the SAS for Windows application, version 6.03). The creation of homoge-

nous subgroups of alcoholics with substantial predisposition to severe alcoholism has a good theoretical background. The authors of the text took into consideration the possibility of applying the Bonferroni correction. However, the idea was abandoned since we wished to report those associations that were based on a prior hypothesis.

Linkage disequilibrium

Linkage disequilibrium (LD) refers to the fact that particular allele at nearby sites can co-occur in the same haplotypes more often than it is expected by chance.

LD is determined by D' and has a value in the range 0 (no disequilibrium) to 1 (complete disequilibrium).

RESULTS

The frequency of the intron 2 STRP alleles for the DRD2 gene in the group of 85 alcoholics was as follow: allele 1–0.01, allele 5–0.17, allele 6–0.09, allele 7–0.49, allele 8–0.24.

Table 1. Alleles and genotype frequencies for – 141C ins/del and exon 8 polymorphisms for the DRD2 gene and the Taq1A polymorphism of the ANKK1 gene for the whole group (n=85)

	Genotypes			Alleles	
	II n(%)	DD n(%)	ID n(%)	I (%)	D (%)
-141C ins/del	72 (84.7)	1 (1.18)	12 (14.12)	92 %	8 %
Exon8 (A/G)	AG	AA	GG	A	G
	44 51.76 %	35 41.18 %	6 7.06 %	67 %	33 %
Taq1A	A1A1	A1A2	A2A2	A1	A2
	1 1.18 %	35 41.18 %	49 57.65 %	22 %	78 %

Legend: Alleles of –141 C DRD2 gene polymorphism: I–insertion allele, D–deletion allele
 Alleles of Exon8 DRD2 gene polymorphism: G–guanine allele, A–adenine allele
 Alleles of ANKK1 gene polymorphism, A1–T allele, A2–C allele

No statistically significant differences of the allele or genotype frequencies between subjects with and without withdrawal complications were found.

Table 2. Linkage disequilibrium for the four loci of ANKK1 and DRD2 genes (-141C ins/del, Taq1A, Exon 8, STRP). Values of D' – standardized linkage factor (Levotin) (n=85)

locus/allele	1	5	6	7	8
-141C ins/del	0.41	0.23	0.33	0.79	0.16
exon8 (A/G)	0.99	0.81	0.85	0.44	0.67
Taq1A	0.99	0.99	0.20	0.75	0.69

1, 5, 6, 7, 8 – STRP alleles

Table 3. Linkage disequilibrium for three loci of the DRD2 and ANKK1 genes (-141C ins/del, Taq1A, exon 8)

locus	-141C ins/del	Taq 1 A	Exon 8 (A/G)
-141C ins/del	—	P=0.00068	P=3.668 x 10 ⁻⁷
Taq 1 A	D'=0.9976	—	P=1.778 x 10 ⁻¹¹
Exon 8 (A/G)	D'=0.5360	D'=0.9211879	—

D' – standardized linkage factor (Levotin)

No statistically significant differences between the three polymorphisms (-141 C ins/del, Exon 8, Taq 1A) haplotype of frequencies in the whole alcoholics group and the categorised subgroups were found. Tab. 4 – on next page.

The I-A2-A-8 haplotype was observed more frequently in the subgroup containing alcoholics without the severe withdrawal complications. The I-A2-A-6 and D-A2-A-7 haplotypes were found more frequently in the subgroup containing alcoholics with withdrawal complications. No statistically significant differences were observed in the examined subgroup with seizures.

DISCUSSION

As mentioned in the introduction, there are several polymorphic variants in the DRD2 and ANKK1 genes which have with a putative functional impact on receptor availability. Evaluating the contradictory results based on the analysis of single SNPs suggested that the different haplotypes might determine the inconsistencies. Therefore, we examined the association of

Table 4. DRD2 (-141 C ins/del, exon 8 (A/G) and intron 2 STRP) and ANKK1 (Taq1A (A1/A2) haplotype combinations in the group of 85 alcoholics and in the subgroups with and without severe withdrawal complications.

Haplotypes	Frequency in group			p	Frequency in group		p	Frequency in group		p
	whole group n=85	with seizures and/or delirium n=33	without seizures and/or delirium n=52		with delirium n=19	without delirium n=66		with seizures n=21	without seizures n=64	
DA1A5	0.006	0.000	0.010	0.42	0.000	0.008	0.59	0.000	0.007	0.58
DA2A1	0.006	0.000	0.010	0.42	0.000	0.008	0.59	0.000	0.008	0.58
DA2A7	0.007	0.018	0.000	0.19	0.035	0.000	0.03	0.000	0.009	0.54
DA2G5	0.023	0.032	0.019	0.56	0.048	0.016	0.19	0.000	0.029	0.32
DA2G6	0.034	0.041	0.029	0.60	0.049	0.030	0.47	0.050	0.030	0.63
DA2G8	0.006	0.000	0.010	0.42	0.000	0.008	0.58	0.000	0.008	0.57
IA1A5	0.006	0.018	0.000	0.13	0.026	0.000	0.07	0.000	0.008	0.73
IA1A7	0.026	0.021	0.015	0.71	0.000	0.032	0.26	0.043	0.012	0.23
IA1A8	0.167	0.167	0.145	0.90	0.158	0.167	0.79	0.175	0.164	0.96
IA1G6	0.011	0.000	0.029	0.27	0.000	0.018	0.50	0.000	0.018	0.49
IA1G7	0.001	NA	0.007	NA	NA	0.002	NA	NA	0.005	NA
IA1G8	0.000	NA	0.016	NA	NA	NA	NA	NA	0.000	NA
IA2A1	0.006	0.000	0.010	0.42	0.000	0.008	0.59	0.000	0.008	0.58
IA2A5	0.007	0.000	0.011	0.46	0.000	0.008	0.64	0.000	0.009	0.57
IA2A6	0.006	0.016	0.000	0.20	0.029	0.000	0.05	0.000	0.009	0.56
IA2A7	0.377	0.443	0.355	0.20	0.357	0.384	0.82	0.457	0.361	0.28
IA2A8	0.056	0.000	0.109	0.02	0.000	0.075	0.13	0.000	0.073	0.13
IA2G5	0.128	0.126	0.123	0.87	0.110	0.135	0.64	0.193	0.108	0.12
IA2G6	0.037	0.049	0.019	0.56	0.080	0.020	0.11	0.050	0.027	0.50
IA2G7	0.083	0.064	0.085	0.43	0.108	0.074	0.54	0.025	0.096	0.13
IA2G8	0.006	0.000	0.000	0.41	0.000	0.008	0.59	0.000	0.008	0.56

Legend: Alleles of -141 C DRD2 gene polymorphism: I-insertion allele, D-deletion allele

Alleles of exon8 DRD2 gene polymorphism: G – guanine allele, A–adenine allele

Alleles of ANKK1 gene polymorphism, A1–T allele, A2–C allele Alleles of intron 2 STRP: 1, 5, 6, 7, 8

the severity of alcohol dependence with distinct haplotypes. Our hypothesis was that haplotypes containing SNPs, shown to be associated with reduced DRD2 availability, are associated with a severe form of alcohol dependence.

The investigated genes have been among the stronger candidate genes implicated in substance use disorders. Taq1A RFLP (rs1800497) has been reported to be located in the 3' flanking region of the DRD2 gene, which was subsequently recognized as a functional coding polymorphism of the adjacent functionally unrelated gene ANKK1.

The A1 allele of Taq1A has been associated with substance use disorders [25, 26], but its role

is controversial; due to the less potent dopamine binding it could be linked to the seeking of stimulation and the reduction of alcohol consumption [27].

The impact of the A1 allele on the course and complications of alcohol dependence is biased by the level of exposure to ethanol [28]. According to Heinz et al. [29] the A2 allele could be linked with severe alcoholism. The following study has revealed that the A2 allele was present in the haplotypes of both groups: with and without severe complications, whereas the discriminative effect had the STRP variations. Versions 6 and 7 of STRP were present in patients with de-

lirium, whereas version 8 of STRP was protective against alcohol withdrawal complications.

The A/A homozygotes of exon 8 DRD2 polymorphism were reported to be associated with a more severe form of alcoholism (owing to higher CIWAA scores during withdrawal) [22], whilst the A allele occurs more frequently in the haplotypes for the subgroup with severe alcoholism. We found that the A allele was also present in the most common haplotypes in both subgroups (with and without complications).

A deletion polymorphism at position –141 was found to be associated with a lower transcription rate *in vitro* [1] and a higher striatal dopamine receptor density *in vivo* [17]. Several association studies concerning this polymorphism and alcoholism revealed conflicting results [30, 31, 32]. In our results we observed both insertion and deletion alleles present in the more common haplotypes of subjects with delirium.

To sum up, the protective haplotype was IA2A8, whereas: IA2A6 and DA2A7 were predisposing to withdrawal complications. The results do not confirm the initial hypothesis but suggest the discriminative role of STRP on the severity of alcohol dependence.

The limitation of the present study may lie in the fact that: the sample was relatively small, it was restricted to one region of Poland, a limited set of gene polymorphisms was analyzed, no control group was used to compare the frequencies.

Further studies are necessary to replicate the present findings in greater populations. However, the results confirmed the postulated partial role of DRD2 in the development of alcohol dependence, especially on the severity of the dependency. The knowledge of genetic relationships between the DRD2 and ANKK1 gene haplotypes and alcohol dependence could provide a more accurate selection of genetic markers for further studies into alcoholism and in the support of therapeutic decisions.

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