STUDY OF DOPAMINE RECEPTORS GENES POLYMORPHISMS IN BIPOLAR PATIENTS WITH COMORBID ALCOHOL ABUSE

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Abstract — Alcoholism is present in ~40–60% of bipolar patients. This comorbidity between bipolar disorder and alcoholism is high and may result from existence of common genetic factors for the two disorders. In both disorders, dysregulation of the dopaminergic neurotransmission had been implicated. Association analyses revealed several candidate genes acting in the dopaminergic pathway and polymorphisms in those genes that might be associated with both disorders. Aim: The aim of this study was to analyse possible relationship between polymorphisms in the dopaminergic pathway genes (one SNP for each dopamine receptor gene 1–4) and alcohol abuse comorbidity in bipolar patients. Methods: We analysed 317 patients with bipolar disorder. In this group, 42 patients were diagnosed with alcohol abuse. The diagnosis was made for each patient by at least two psychiatrists, using structured clinical interviews for DSM-IV Axis I disorders (SCID). The control group consisted of 350 subjects. We performed RFLP analysis of polymorphisms in four genes: DRD1, DRD2, DRD3, and DRD4. Results: We have not found association of any of the analysed polymorphisms in the dopamine genes in the group of bipolar patients with comorbid alcohol abuse as compared to the control group. In the male group of bipolar patients with comorbid alcohol abuse, we also have not observed any significant differences between the patients and the control subjects. Conclusion: Our findings suggest that the analysed polymorphisms of the dopamine genes polymorphisms may not be involved in the shared genetic vulnerability to both, bipolar disorder, and alcohol abuse.

INTRODUCTION

The lifetime prevalence of alcohol abuse or dependence in bipolar disorder is 43.6% and over 60% for bipolar type I (Regier et al., 1990). For both disorders, co-transmission in families has been reported (Maier and Merikangas, 1996), particularly in subgroups of patients with familial comorbidity (Maier et al., 1995; Winokur et al., 1996). This observation may suggest shared genetic factors being responsible for bipolar disorder and alcohol dependence or abuse. It was supported by several linkage studies which revealed that chromosomal regions associated with bipolar disorder are overlapping, to some extent, with those described for alcohol dependence. The overlapping regions involve chromosomes 1p, 6q, 9p, 14q (Nurnberger, Jr., et al., 2001; Segurado et al., 2003; Hill et al., 2004; Daw et al., 2005; McQueen et al., 2005). Moreover, adoption study performed by Ingraham and Wender (1992) confirmed the genetic contribution revealing that substance abuse was more common in the biological relatives of bipolar adoptees than in controls’ relatives. Therefore, we aimed to investigate whether bipolar disorder and alcohol abuse have any common genetic background that could explain their comorbidity.

The dopaminergic pathway is implicated in the pathogenesis of bipolar disorder and alcohol dependence as in both disorders disturbances in the dopaminergic neurotransmission have been observed. In bipolar disorder, suggestive evidence of the dopaminergic system dysfunction and its role in the pathogenesis of the disease was raised (Goodwin et al., 1970; Taylor and Saint-Cyr, 1990; Willner et al., 1991).

The role of the dopaminergic pathway in the aetiology of bipolar disorder was observed by Murphy who provoked manic states in healthy subjects with psychoactive substances increasing activity of the dopaminergic system such as L-DOPA (L-3,4-dihydroxyphenylalanine, amphetamine, and bromocriptine (Murphy et al., 1971). The dopaminergic system has been also implicated in mediating reward and dependence towards alcohol. Alcohol and other drugs of abuse increase brain dopamine levels and enhance neurotransmission in the nucleus accumbens (Di Chiara and Imperato, 1988; Weiss et al., 1993; Tanda et al., 1997). This activation is involved in the motivational and reward properties of alcohol and other abused drugs (Koob and Le Moal, 1997; Tanda et al., 1997).

Genes for the dopamine receptors involved in the dopaminergic neurotransmission and signalling were analysed in both bipolar disorder and alcoholism. The results concerning particular genes in both disorders are inconsistent which is probably due to heterogeneity in methods used and populations analysed. Molecular genetics analyses for the dopamine receptors genes revealed polymorphisms that may increase vulnerability to bipolar disorder as well as alcoholism. As for the DRD1 gene, the –48 G/A polymorphism was found to be associated with sensation seeking in alcohol dependence in men (Limosin et al., 2003). Moreover, several linkage studies revealed that the risk locus for bipolar disorder is localized on the 5q chromosome, in the proximity to the DRD1 gene (Garner et al., 2001; Shinika et al., 2002) and it was observed that this polymorphism is associated with bipolar disorder in a Sardinian population (Severino et al., 2005) as well as in Polish sample (Dmitrzak-Weglarz et al., 2006). For the DRD2 gene, apart from numerous studies focusing on the TaqA1 polymorphism, studies on relationship between promoter polymorphism and alcoholism have been conducted, demonstrating both positive
et al., 1998; Samochowiec et al., 2000; Wodorz et al., 2003) and negative associations (Sander et al., 1999; Noble et al., 2000). For bipolar disorder, a positive association was found in Chinese bipolar patients (Li et al., 1999), however this result was not confirmed in other studies, including sample from our centre (Furlong et al., 1998; Stober et al., 1998; Leszczynska-Rodziewicz et al., 2005). For the DRD3 gene, research performed in this field resulted in positive association studies between 9 Ser/Gly and alcoholism (Sander et al., 1995; Thome et al., 1999; Eichhammer et al., 2003; Limosin et al., 2005). This supported possible involvement of the dopamine receptor D3 gene polymorphism in the development of addiction to alcohol. For bipolar disorder, the association studies involving this polymorphism were considered as suggestive (Parsian et al., 1995) or negative (Rietschel et al., 1993; Piccardi et al., 1997; Elvidge et al., 2001; Leszczynska-Rodziewicz et al., 2005). For the DRD4 gene, strong association between novelty seeking, alcohol and drug abuse and DRD4 –521 C/T promoter polymorphism has been suggested (Schinka et al., 2002). Indirect support for a role for DRD4 in alcohol dependence is the location of this gene in one of the possible linkage regions in chromosome 11 (Long et al., 1998). However, these findings were followed by both positive and negative replication studies (Lusher et al., 2001; Soyka et al., 2002). For bipolar disorder, the recent meta-analysis performed by Leon et al. (Lopez Leon et al., 2005) revealed the association of bipolar illness and the DRD4 gene polymorphism.

In our study, we investigated the possible relationship between polymorphisms in the four dopamine receptor genes (−48 A/G in DRD1, −141 C ins/del in DRD2, 9 Ser/Gly in DRD3, −521 C/T in DRD4) and the comorbidity of alcohol abuse in bipolar patients. We hypothesized that presence of bipolar illness and alcohol abuse considered as a secondary diagnosis may result from common genetic background for these two disorders.

SUBJECTS AND METHODS

Subjects

The study was performed on 317 patients with bipolar disorder (n = 269 for BPI and n = 48 for BPII). The group of patients consisted of 131 males with a mean age of 30 years, SD = 11 and 186 females with a mean age of 33 years, SD = 12. Subgroup of 42 patients with coexistent alcohol abuse without any other addictive disorders was distinguished (n = 34 males and n = 8 females). Patients were recruited from inpatients from Wielkopolska region, treated at the Department of Psychiatry, University of Medical Sciences in Poznan, Department of Psychiatry and Psychiatric Hospital in Koscián. Consensus diagnosis by at least two psychiatrists, according to DSM-IV criteria was made for each patient using structured clinical interview for DSM-IV Axis I disorders (SCID) (First et al., 1996).

Control group consisted of 350 subjects (139 males with a mean age of 41 years, SD = 12; 211 females with a mean age of 40 years, SD = 11). Control subjects were recruited from the group of blood donors, hospital staff, and students of University of Medical Sciences in Poznan. Although they were not psychiatrically screened, hospital staff and students with psychiatric disorders concerning them and their families were excluded from the study. Blood donors had routine clinical examinations and exclusion criteria for this group were chronic diseases including psychiatric disorders and alcohol dependence.

All participants have given written informed consent. Local ethics committee accepted the project.

Genotyping

We analysed the following polymorphisms in the dopamine receptors genes: DRD1 (polymorphism −48 A/G), DRD2 (polymorphism −141 C ins/del), DRD3 (polymorphism Ser9Gly), DRD4 (polymorphism −521 C/T). The DNA was extracted from 10 ml of EDTA anticoagulated whole blood using the salting out method (Miller et al., 1988).

For the DRD1 gene, a 207 bp fragment of the gene was amplified by PCR with the set of primers described by Cichon et al. (1994). PCR product was then digested with Ddel restriction endonuclease. The uncut PCR product size was 207 bp. After RFLP analysis, the following alleles were observed: for A allele the cut bands of 61 bp and for G allele the cut bands of 42 and 19 bp. Additionally, in all analysed subjects we observed non-polymorphic restriction site for Ddel enzyme which produced 146 bp band.

For the DRD2 gene, a 304 bp fragment of the gene was amplified by PCR with the set of primers described by Arinami et al. (1997). PCR product was then digested with Mval restriction endonuclease. The uncut PCR product size was 304 bp. After RFLP analysis, the following alleles were observed: for insertion of C allele the cut bands of 304 bp and for deleted C allele the cut bands of 160 and 144 bp.

For the DRD3 gene, a 462 bp fragment of the gene was amplified by PCR with the set of primers described by Lannfelt et al. (1992). PCR product was then digested with Mls1 restriction endonuclease. The uncut PCR product size was 462 bp. After RFLP analysis, the following alleles were observed: for Ser allele the cut bands of 304 bp and for Gly allele the cut bands of 206 and 98 bp.

For the DRD4 gene, a 244 bp fragment of the gene was amplified by PCR with the set of primers described by Jönsson et al. (2001). PCR product was then digested with FspI restriction endonuclease. The uncut PCR product size was 244 bp. After RFLP analysis, the following alleles were observed: for allele 1 the cut bands of 244 bp and for allele 2 the cut bands of 176 and 68 bp.

Statistical analysis

The Pearson’s chi-square (χ²) test and Fisher’s exact test were applied to test the differences in the genotypic and allelic (respectively) distribution in the group of bipolar patients with comorbid alcohol abuse vs bipolar patients without alcoholism as well as bipolar patients with comorbid alcohol abuse vs control group. Calculations were performed using the computer program SPSS version 12.

RESULTS

The genotype distribution in our sample was in Hardy–Weinberg equilibrium for all studied polymorphisms.
We have not found any significant differences in genotype distribution of the polymorphisms of four dopamine genes analysed between patients with bipolar disorder and comorbid alcohol abuse in comparison to the control group (for DRD1 \( P = 0.220 \); for DRD2 \( P = 0.447 \); for DRD3 \( P = 0.773 \); for DRD4 \( P = 0.219 \)). Allele frequencies for those genes were not significantly different between cases and controls (for DRD1 \( P = 0.110 \); for DRD2 \( P = 0.446 \); for DRD3 \( P = 0.672 \); for DRD4 \( P = 0.442 \) (Table 1).

Similarly, we have not found any association of the four analysed SNPs in the group of bipolar patients without alcohol abuse, neither for genotypes (for DRD1 \( P = 0.996 \); for DRD3 \( P = 1.000 \); for DRD4 \( P = 0.897 \)) nor for alleles (for DRD1 \( P = 0.109 \); for DRD2 \( P = 1.000 \); for DRD3 \( P = 1.000 \); for DRD4 \( P = 0.691 \)).

In statistical analysis comparing bipolar patients with and without comorbid alcohol abuse, we found no differences in genotype distribution (DRD1 \( P = 0.410 \), DRD2 \( P = 0.334 \), DRD3 \( P = 0.561 \), DRD4 \( P = 0.252 \)).

We also analyzed bipolar I patients with comorbid alcohol abuse \((n = 35)\); however, we did not observe significant differences between the patients and the control group. We did not analysed the bipolar II patients as there were too few of them \((n = 9)\) to perform powerful statistical analysis.

When we analysed groups by gender, we have not found any significant differences in genotypes distribution and allele frequencies in the male group of bipolar patients with the comorbid alcohol abuse.

**DISCUSSION**

In our study, we have not found any relationship between the analysed polymorphisms and the bipolar patients (types I and II analysed together and separately) with comorbid alcohol abuse. We also have not observed any significant associations in the male group of bipolar patients with comorbid alcohol abuse. Our results have not confirmed that comorbidity of bipolar disorder and alcohol abuse may be related to analysed polymorphisms of genes for dopamine receptors.

Our finding considering the DRD1 gene \(-48\ A/G\) polymorphism is lack of association in the group of bipolar patients with comorbid alcohol abuse. The \(-48\ A/G\) polymorphism of the DRD1 gene is localized in the 5’-UTR region of the DRD1 gene and is unlikely to affect translation of the receptor (Cichon et al., 1996). However, it cannot be excluded that this polymorphism may influence regulation of the DRD1 gene transcription which may play role in both disorders.

Our finding considering the DRD2 \(-141\ C\ ins/del\) gene polymorphism is lack of significant differences between the group of bipolar patients with comorbid alcohol abuse and the control group. It has been shown that the deleted allele of \(-141\ C\ polymorphism is responsible for decrease in promoter strength (Arinami et al., 1997), which subsequently might lead to aberrant expression of the DRD2 receptor and, therefore, be associated with bipolar disorder and alcohol dependence. Moreover, this SNP influences clinical phenotype of alcoholics (Samochowiec et al., 2000). We chose this SNP as more suitable for analysis of our small group as it is more frequent than TaqIA polymorphism, which has been extensively studied with alcoholism. However, on the basis of obtained results we cannot confirm the relationship between this polymorphism and bipolar disorder with comorbid alcohol abuse.

When considering studies analysing common genetic factors of dopamine receptors pathway as a potential cause of comorbidity of bipolar disorder and alcohol abuse, only study by Gorwood et al. (2000) investigated possible role of dopamine receptor 2 gene (TaqI A1 polymorphism) in the shared vulnerability to both disorders. They analysed four groups (alcohol-dependent patients, bipolar patients, comorbid bipolar and alcohol-dependent patients, and controls) in association with bipolar disorder and comorbid alcoholism. They did not find any significant role for the analysed polymorphism in DRD2 gene in the specific association between bipolar disorder and comorbid alcoholism. In our study, we investigated another SNP of this gene, localized in the promoter region and influencing gene transcription. However, we also did not observe any association of this polymorphism and bipolar disorder with comorbid alcohol abuse.

Although there have been positive association studies concerning the DRD3 gene 9 Ser/Gly polymorphism and addictive properties of ethanol, most results concerning association with bipolar disorder were negative. In this study, we did not observe any significant differences between the analysed groups of patients and the control group.

For the DRD4 gene, we analysed the \(-521\ C/T\) polymorphism as it is localized within the Cpg island and, therefore, affects regulation of gene expression (Ronai et al., 2001). Moreover, high scores of novelty seeking, which are present in alcohol dependence and abuse as well as in bipolar disorder (particularly in relation with manic state), were strongly associated with this SNP in meta-analyses performed by Schinka et al. (2002).

We have chosen to study the dopamine receptors genes in bipolar disorder with comorbid alcohol abuse as they are more specifically implicated in the common susceptibility (Depue et al., 1989; Gorwood et al., 2000). Different studies

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Genotypes and alleles</th>
<th>BD with comorbidity</th>
<th>Control group</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>N %</td>
<td>N % P value</td>
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<tr>
<td>DRD1</td>
<td>–48A/G</td>
<td>AA 3 (7.2) 45 (12.9)</td>
<td>0.220</td>
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<td></td>
<td></td>
<td>AG 18 (42.8) 175 (50.0)</td>
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<td>GG 21 (50.0) 130 (37.1)</td>
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<td></td>
<td></td>
<td>A 24 (28.6) 265 (37.9)</td>
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<tr>
<td></td>
<td></td>
<td>G 60 (71.4) 435 (62.1)</td>
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<tr>
<td>DRD2</td>
<td>–151 C</td>
<td>ins/ins 30 (71.4) 278 (79.4)</td>
<td>0.447</td>
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<td></td>
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<td>ins/del 11 (26.2) 68 (19.4)</td>
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<td>del/del 1 (2.4) 4 (1.1)</td>
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<tr>
<td></td>
<td></td>
<td>ins 71 (84.5) 624 (89.1)</td>
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<tr>
<td></td>
<td></td>
<td>del 13 (15.5) 76 (10.9)</td>
<td>0.204</td>
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<tr>
<td>DRD3</td>
<td>9 Ser/Gly</td>
<td>Ser/Ser 22 (52.4) 181 (51.7)</td>
<td>0.773</td>
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<td></td>
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<td>Ser/Gly 15 (35.7) 138 (39.4)</td>
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<td>Gly/Gly 5 (11.9) 31 (8.9)</td>
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<td>Ser 59 (70.2) 500 (71.4)</td>
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<td></td>
<td></td>
<td>Gly 25 (29.8) 200 (28.6)</td>
<td>0.799</td>
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<tr>
<td>DRD4</td>
<td>–521 C</td>
<td>CC 7 (16.6) 83 (23.7)</td>
<td>0.219</td>
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<tr>
<td></td>
<td></td>
<td>CT 26 (62) 167 (47.7)</td>
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<tr>
<td></td>
<td></td>
<td>TT 9 (21.4) 100 (28.6)</td>
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<td>C 40 (47.6) 333 (47.6)</td>
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<td></td>
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<td>T 44 (52.4) 367 (52.4)</td>
<td>1.000</td>
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have shown that dopamine is involved in bipolar disorder (Goodwin et al., 1970; Murphy et al., 1971; Praag et al., 1975) as well as in alcohol dependence and abuse (Gorwood and Lejoyeux, 1994). Therefore, it seemed justifiable to analyse SNPs within genes encoding for dopamine receptors as potentially involved in both disorders. However, only study by Gorwood et al. (2000), mentioned above, analysed the role of dopamine receptors genes in the shared vulnerability to both disorders. We were not able to find any other paper revising our findings.

Although it is likely that certain genetic components are involved in the susceptibility to bipolar disorder and contribute to development of alcohol abuse, our study has not confirmed that. We excluded from our analysis bipolar patients with other addictive disorders to make the alcohol abuse narrower and more precise phenotype, however, in that way, we might have missed the opportunity to find the relationship of addictive disorders with the dopamine pathway. Moreover, surprisingly low comorbidity observed in our group (13.2%), partially due to predominance of women in our group, may explain lack of association between bipolar disorder and alcohol abuse. However, the results should be confirmed on larger sample size to discover the true effect of polymorphisms in the dopamine receptors genes on comorbidity of bipolar disorder with alcohol abuse.

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REFERENCES


