Interactive effect of MTHFR and ADRA2A gene polymorphisms on pathogenesis of schizophrenia

Jan Lochman¹, Jiří Plesník¹, Vladimír Janout², Jana Povová², Ivan Míšek³, Dagmar Dvořáková⁴, Omar Šerý¹,³

¹ Laboratory of Neurobiology and Molecular Psychiatry, Laboratory of Molecular Physiology, Department of Biochemistry, Faculty of Science, Masaryk University, Brno, Czech Republic
² Department of Epidemiology and Public Health, Faculty of Medicine, University of Ostrava, Czech Republic
³ Institute of Animal Physiology and Genetics, Academy of Science, Brno, Czech Republic
⁴ Psychiatric Hospital Jihlava, Jihlava, Czech Republic

Correspondence to: Assoc Prof. RNDr. Omar Šerý, PhD.
Laboratory of Neurobiology and Molecular Psychiatry, Department of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic.
tel: +420 549 497 312; fax: +420 542 213 827; e-mail: omarsery@sci.muni.cz

Submitted: 2013-11-20 Accepted: 2013-12-12 Published online: 2014-01-15

Key words: MTHFR; COMT; ADRA2A; schizophrenia; association; polymorphism

Abstract

OBJECTIVES Objectives: Increasing evidences support the importance of epigenetic control in schizophrenia pathogenesis. One of the enzymes involved in DNA methylation process through homocysteine metabolism is methylenetetrahydrofolate reductase (MTHFR). The most extensively studied variant in the MTHFR gene is the C677T polymorphism, resulting in reduced enzyme activity and elevated homocysteine level.

Methods: In sample of 192 schizophrenics and 213 healthy controls an increasing risk of schizophrenia associated with MTHFR 677 CT+TT genotype was found (OR=1.6, p=0.021). Association was also evaluated by considering the C677T polymorphism as an interaction with COMT Val158Met and ADRA2A C-1291G polymorphisms previously associated with schizophrenia risk using a logistic regression analysis.

RESULTS: Previous studies of MTHFR*COMT (C677T*Val158Met) interaction in relation to schizophrenia resulted in inconsistent results. In our sample this interaction did not significantly differ between schizophrenics and control subjects. On the other hand analysis of MTHFR*ADRA2A (C677T*C-1291G) interaction revealed significant association between ADRA2A CC+CG genotype in the MTHFR TC+TT carriers (p=0.008).

CONCLUSIONS: Our results support role of noradrenergic functions as well as previously proposed role of epigenetic control in the pathogenesis of schizophrenia. Further relevant studies including larger sample size and more markers are needed to prove our results.
INTRODUCTION

Today, schizophrenia is believed to be a group of diseases with similar symptoms and different molecular causes. This fact greatly complicates the research of genetic dispositions to the schizophrenia. Recent explanation of schizophrenia pathogenesis assumes hypothesis of brain developmental malfunction. In our previous studies we found a relationship between schizophrenia and polymorphisms in OPRM1, DRD3 and SNAP-25 genes (Lochman et al. 2013; Šerý et al. 2010).

The relationship between methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms and schizophrenia has been described by many authors (Kempisty et al. 2006; Peerbooms et al. 2011; Saetre et al. 2011; Yoshimi et al. 2010). MTHFR is an enzyme influencing intracellular and consequently also plasma level of homocysteine. Homocystine is a sulfur-containing amino acid absent in naturally occurring dietary sources formed intracellularly from the demethylation of dietary methionine via two intermediate compounds, S-adenosylmethionine and S-adenosylhomocysteine (Castro et al. 2006). S-adenosylmethionine is the methyl donor in over 115 different cellular methyltransferase reactions, including those of DNA, RNA, proteins and lipids. DNA methylation is an important epigenetic feature of DNA playing a role in the regulation of gene expression. Frotsst et al. (1995) identified in MTHFR gene a C677T (rs1800544) polymorphism, converting an alanine to a valine residue at position 222 of amino acid sequence, responsible for the synthesis of a thermo labile form of MTHFR showing reduced enzyme activity (Frotsst et al. 1995). In individuals homozygous for the T allele significantly elevated plasma homocysteine levels was found (Frotsst et al. 1995) and in individuals homozygous for the C allele the increased plasma levels of LH was found in our previous study (Šerý et al. 2012).

Interactive effect of Val158Met polymorphism (rs4680) of catechol-O-methyltransferase (COMT) gene and C677T polymorphism of MTHFR gene on schizophrenia risk was described (Muntjewerff et al. 2008; Roffman et al. 2008). COMT catalyses the transfer of a methyl group from S-adenosylmethionine to catecholamines including dopamine, noradrenaline and adrenaline, thereby inactivating these neurotransmitters. Hence, dysfunctional MTHFR might act synergistically with a less functional COMT enzyme due to Val158Met polymorphism and influencing the methylation of neurotransmitters. Šerý described the association between Val158Met polymorphism of the COMT gene and alcoholism in male subjects in Czech population (Šerý et al. 2006).

Dysregulation of the noradrenergic system has been implicated in the etiology of schizophrenia (Yamamoto and Hornykiewicz 2004). The polymorphism C-1297G (rs1800544) in the regulation region of ADRA2A have previously shown significant association with Bmax and Kd phenotype (Deupree et al. 2006) and in our previous studies hardly significant association of the C allele with schizophrenia was found (Lochman et al. 2013).

In the present study we investigated the effect of the C677T variant in the MTHFR gene alone and in combination with the COMT Val158Met polymorphism and ADRA2A C-1291G polymorphism on the risk of schizophrenia. Considering the prior evidence of differential associations to schizophrenia by gender (Leung & Chue 2000, Hoenicka et al. 2010), in the present study we focused only on males.

MATERIALS & METHODS

Subjects

A sample of 405 males of Czech nationality was studied. The group of patients with schizophrenia included 192 males (mean age 35.5±10.9) hospitalized for schizophrenia at the Department of Psychiatry, Faculty Hospital, Brno and the Psychiatric Hospital, Jihlava. The patients were diagnosed according to ICD-10 criteria (International Classification of Diseases, 10th Edition) and according DSMIV criteria (APA 1994). All patients underwent structured interview with psychiatrists Dr. Radovan Prikryl and Dr. Dagmar Dvorakova. Patients with psychiatric comorbidities were excluded from the study. The study was approved by the Ethical Committee of the Faculty Hospital, Brno.

The control group included 213 males (mean age 48.2±13.8). Control persons were recruited from blood donors at Blood bank Brno, patients treated for erectile dysfunction at Trauma hospital Brno, employees of some companies in Brno city, employees of agriculture farms in area around Brno, university teachers and employees of National theatre in Brno. The Mini-International Neuropsychiatric Interview (M.I.N.I.) was performed with each member of the control group (Lecrubier et al. 1997) followed by an interview with a psychiatrist Dr.
Radovan Prikryl or Dr. Dagmar Dvorakova. All individuals with any mental illness were excluded from the control group. Patients and controls were not examined for neurological disorders. Genotypes of participants were analysed after interviews with psychiatrists. All participants signed an informed consent to participate in the study.

Genotyping
DNA was extracted from 200 ul of EDTA anticoagulated whole blood using the UltraClean Blood DNA Isolation Kit (Mobio, USA).

The MTHFR C677T SNP was analyzed using restriction analysis with restriction endonuclease TaqI. PCR reaction contained: 5 ng of DNA, Kapa2G Fast ready Mix (Kapa Biosystems), 100 nM primers (F: CATCCCTATTGGCAGGTTA, R: ATGTGT-CAGCCTCAAAAGAAA). Reaction were incubated at 95°C for 3 min, then cycled 30 times at (95°C 15 s, 60°C 20 s, 72°C 20 s) followed by 72°C for 5 min. The amplified PCR product was digested with Fast TaqI endonuclease (Fermentas) at 65°C for 10 min and restriction fragments were analyzed on the 3100 DNA Fragment Analysis System (Applied Biosystems, USA) in 36 cm capillary array with POP7 polymer with size standard LIZ 120 (Applied Biosystems, USA). The COMT Val158Met SNP and ADRA2A C-1291G SNP were analyzed as previously by TaqMan and SNaPshot assay, respectively (Hosak et al. 2011; Lochman et al. 2013).

Statistics
The CSS Statistica software (StatSoft, USA) was used for statistical evaluation of the results. The chi-square test was used for the comparison of genotype frequencies in the studied groups. Odds ratios (Odds) and 95% confidence intervals (95% CI) as estimates relative risk for the schizophrenia associated with different genotypes were calculated with logistic regression. To avoid false-positive results due to multiple testing, we applied the Bonferroni correction for three independent loci genotyped. Significant p-values were raised to p=0.017. In addition, a stepwise logistic regression model was used to calculate the independent association between each loci and the presence of the schizophrenia, as well as interaction effects between the genes.

RESULTS
Risk of schizophrenia in relationship with MTHFR, COMT and ADRA2A gene polymorphisms
All genotype frequencies of the three analyzed SNPs, MTHFR C677T (rs18001133), COMT Val158Met (rs4680) and ADRA2A C-1291G (rs1800544) in the control and patients group were consistent with the Hardy-Weinberg equilibrium (p>0.05).

The frequency of the MTHFR 677T allele for the control group and patient group was 0.29 and 0.37, respectively. MTHFR 677CT genotype and marginally 677TT genotype were associated with an increased risk of schizophrenia compared to the MTHFR 677CC genotype (OR 1.53 (95%CI: 1.00–2.33, p=0.05) and 1.94 (95%CI: 1.07–2.39, p=0.05), Table 1). Combined group of subjects with 677CT and 677TT genotypes showed statistically significant association with schizophrenia, OR 1.60 (95%CI: 1.07–2.39, p=0.02). However, none of the found associations remained significant after an application of Bonferroni correction (p<0.017, Table 1).

<table>
<thead>
<tr>
<th>Genes</th>
<th>SNP</th>
<th>Genotype</th>
<th>Controls (N=209)</th>
<th>Patients (N=186)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Number (%)</td>
<td>Number (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTHFR</td>
<td>rs1801133</td>
<td>CC</td>
<td>105 50.2</td>
<td>72 38.7</td>
<td>1.00 – –</td>
<td>– –</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT</td>
<td>86 41.2</td>
<td>90 48.4</td>
<td>1.53 (1.00–2.33)</td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>18 8.6</td>
<td>24 12.9</td>
<td>1.94 (0.98–3.84)</td>
<td>0.054</td>
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<tr>
<td></td>
<td></td>
<td>CT+TT</td>
<td>104 49.8</td>
<td>114 61.3</td>
<td>1.60 (1.07–2.39)</td>
<td>0.021</td>
</tr>
<tr>
<td>ADRA2A</td>
<td>rs1800544</td>
<td>CC</td>
<td>123 58.9</td>
<td>131 70.4</td>
<td>1.00 – –</td>
<td>– –</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CG</td>
<td>63 30.1</td>
<td>42 22.6</td>
<td>0.63 (0.39–0.99)</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>11 5.3</td>
<td>6 3.2</td>
<td>0.51 (0.18–1.43)</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CG+GG</td>
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<td>48 25.8</td>
<td>0.61 (0.39–0.95)</td>
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</tr>
<tr>
<td>COMT</td>
<td>rs4680</td>
<td>GG</td>
<td>42 20.1</td>
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<td>1.00 – –</td>
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<td></td>
<td></td>
<td>GA</td>
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<td>86 46.2</td>
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<tr>
<td></td>
<td></td>
<td>AA</td>
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<td>47 25.3</td>
<td>1.31 (0.74–2.32)</td>
<td>0.36</td>
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<tr>
<td></td>
<td></td>
<td>GA+AA</td>
<td>144 68.9</td>
<td>133 71.5</td>
<td>0.95 (0.58–1.55)</td>
<td>0.83</td>
</tr>
</tbody>
</table>

All chi-squared tests are two-tailed. Alpha value is adjusted by Bonferroni correction and statistically significant results (p<0.017) are marked bold.
The frequency of the ADRA2A-1291G allele for the control group and patient group was 0.21 and 0.13, respectively. The ADRA2A-1291CG genotype was associated with significantly decreased risk of schizophrenia compared to the -1291CC genotype, OR of 0.95 (95%CI: 0.39–0.99, p=0.05). When subjects with -1291CG and -1291GG genotypes were combined, an OR of 0.61 (95%CI: 0.39–0.95, p=0.03) with protecting effect was found. However none of the found associations remained significant after Bonferroni correction was applied (p<0.017, Table 1).

The frequency of the COMT 324A allele for the control group and patient group was 0.44 and 0.48, respectively. We found no association between COMT polymorphism Val158Met and schizophrenia. The COMT 324AA and 324GA genotypes were not associated with a significantly increased risk of the schizophrenia in the comparison with the COMT 324GG genotype (Table 1). When subjects homozogyous or heterozygous for the COMT G324A polymorphisms were combined, OR of 0.95 (95%CI: 0.58–1.55, p=0.83) was found (Table 1).

**Risk of schizophrenia in relation to MTHFR-COMT and MTHFR-ADRA2A interactive effect**

The interactive effect was assessed for all possible genotype combinations of the MTHFR SNP with the ADRA2A and COMT SNPs. Using a logistic regression analysis we found an evidence of a statistically significant interaction between the C677T polymorphism of MTHFR gene and C-1291G polymorphism of ADRA2A gene (p=0.02 without correction, Table 2). We found no interaction between the MTHFR and COMT gene polymorphisms (p=0.46). In order to examine the interactive effect of MTHFR polymorphism C677T and ADRA2A polymorphism C-1291G, we studied MTHFR genotypes in the groups stratified according to the C-1291G ADRA2A genotypes using codominant model. An interactive effect between ADRA2A and MTHFR genotypes was detected in the group of -1291CC homozygous persons, where MTHFR polymorphism showed a significant association with the schizophrenia. Allele 677T of MTHFR polymorphism was present among 19.7% controls vs 10.2% among patients (χ²=5.74; p=0.02; OR value of 0.47, 95% CI=0.24–0.90; Table 2). We found also an interactive effect in the group of -1291CG heterozygous persons, where the T allele and CT+TT genotype of C677T polymorphism of MTHFR gene showed a significant association with schizophrenia (for T allele p=0.032; OR value of 0.55, 95% CI=0.33–0.93; for CT and TT genotypes p=0.043; OR value of 0.51, 95% CI=0.28–0.93; Table 2). We did not find any association between schizophrenia and MTHFR polymorphism in the ADRA2A GG genotype group.

Further, we stratified the samples according to the ADRA2A genotypes following dominant model. The C677T MTHFR SNP analysis showed a significant trend-level association (T carriers, χ²=7.69, p=0.008; Table 3) when MTHFR CT+TT genotype had protective effect in combined group of subjects with ADRA2A CC+CG genotype (Table 3).

**DISCUSSION**

In this study, we investigated two functional SNPs in MTHFR and COMT genes, and one SNP in regulation region of ADRA2A gene in association with schizophrenia risk. A number of studies covering recent huge meta-analysis have suggested moderate but statistically significant association between schizophrenia and...
MTHFR C677T SNP (Gilbody et al. 2007; Okochi et al. 2009; Peerbooms et al. 2011; Sazci et al. 2005). Our study showed significantly increased risk of schizophrenia associated with 677T allele and 677 TT genotype, even though both associations did not survive Bonferroni correction for multiple analyses (Table 1).

Although a number of studies have evaluated the association between the functional SNP Val158Met of the COMT gene and the schizophrenia, the results are not consistent regarding the involvement of the Val or Met allele in the aetiology of schizophrenia. In our previous studies we investigated the relationship between Val158Met polymorphism of COMT gene and alcoholism (Serý et al. 2006) and methamphetamine dependence (Hosak et al. 2011) in which inconsistencies were found as well. While the association between Val allele and alcoholism was observed in some studies (Serý et al. 2006; Vandenbergh et al. 1997), in other ones there was observed association of Met allele to the risk of the alcoholism (Tiihonen et al. 1999; Wang et al. 2001). Several authors suggested an association of schizophrenia with the more active Val allele (Kremer et al. 2003), but recent meta-analyses found no evidence for a association between Val158Met polymorphism of the COMT gene and schizophrenia (Barnett et al. 2007; Munafò et al. 2005; Okochi et al. 2009). Similarly, in our study genotype and allele frequencies of the Val158Met polymorphism of COMT gene did not significantly differed between schizophrenics and control subjects (Table 1).

Further we analyzed the relationship of the schizophrenia risk to the interaction between Val158Met polymorphism of COMT gene and the C677T polymorphism of MTHFR gene. Both polymorphisms exert their influence on the neurotransmission through the homocysteine and methylation pathway and thus influencing dopamine levels. Roffman et al. (2008) found significant MTHFR X COMT genotype interactions. In this study, reduced prefrontal activation was associated with the 677T and 158Val alleles in schizophrenic patients but with 677C/C and 158Met/Met genotype in controls. In our sample we found no evidence for an interaction between both SNPs within control and patient group in the relationship to the schizophrenia risk. However, our finding is consistent with previous study that did not find an association between interactive effect of these polymorphisms and schizophrenia risk (Kang et al. 2010; Muntjewerff et al. 2008).

In the last decade, dysregulation of the noradrenergic system has been implicated in the etiology of schizophrenia (Yamamoto and Hornykiewicz 2004). Moreover, many of the successful antipsychotics demonstrate affinity to subtypes α-1A and α-2A. In case of ADRA1A gene a significant association with schizophrenia risk has been found (Clark et al. 2005). Our previous results showed statistically significant association between the schizophrenia and ADRA2A polymorphism (Lochman et al. 2013). Moreover, the present study is the first to show interactive effect of the C-1291G polymorphism of ADRA2A gene and C677T polymorphism of MTHFR gene on the risk to develop schizophrenia. The analysis of interaction between ADRA2A and MTHFR genes polymorphisms revealed significant association (p<0.008) with schizophrenia risk when protective effect of ADRA2A CC+CG genotypes in the MTHFR TC+TT carriers was observed. This is opposite to observation that G allele of ADRA2A polymorphism and C allele of MTHFR polymorphism has protective effect. The explanation of this interesting finding might be in physiological roles of both genes and function of studied polymorphisms. The T allele of MTHFR polymorphism causes higher homocysteine level and higher risk of the schizophrenia. Higher level of homocysteine leads to an inhibition of the catecholamines degradation and it causes higher levels of catecholamines on synapses. In ADRA2A polymorphism, G allele was associated with better response on methamphetamine treatment (Cheon et al. 2009; Polanczyk et al. 2007). It could be assumed that C allele of ADRA2A polymorphism leading to lower response to catecholamines in combination with risk T allele of MTHFR polymorphism (and higher level of catecholamines) finally results in equalize catecholamine level on synapses and thus in protective effect to schizophrenia risk.

To validate our results, ADRA2A and MTHFR gene analyses should be performed in independent samples and in other populations. One of the limitations of this study is small size of some subgroups. Therefore, the positive findings in this study need to be investigated further in enlarged samples size with enough power. Despite these limitations our analysis supports the role of MTHFR gene in the pathogenesis of schizophrenia and suggests interaction between functional SNP in MTHFR gene and ADRA2A gene and schizophrenia risk.

In summary, the present results demonstrate protective effect of ADRA2A CC+CG genotypes in the MTHFR TC+TT carriers in schizophrenia risk. They support the importance of epigenetic control in schizophrenia pathogenesis, and call for other studies examining genes associated with the methylation pathway and their synergistic effects on schizophrenia risk.

ACKNOWLEDGMENTS

We would like to acknowledge Dr. Radovan Prikryl for his assistance in sample collection. This work has been supported by the internal grant agency of the Ministry of Health of the Czech Republic (IGA MZCR) No. NT/14504-3.

REFERENCES


