ORIGINAL ARTICLE

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

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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.

Abbreviations:

ADRA2A	- α2-adrenergic receptor
ANOVA	- analysis of variance
B_{max}	- maximal binding capacity
bp	- base pair
COMT	- catechol-O-methyltransferase
CSS	- computer statistic software
DNA	- deoxyribonucleic acid
DSMIV	- Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
DRD3	- dopamine D3 receptor
EDTA	- ethylenediaminetetraacetic acid
ICD-10	- 10th revision of the International Statistical Classification of Diseases and Related Health Problems
K_d	- dissociation constant
MTHFR	- methylenetetrahydrofolate reductase
OPRM1	- μ opioid receptor
OR	- odds ratio
PCR	- polymerase chain reaction
RNA	- ribonucleic acid
SNAP-25	- synaptosomal-associated protein of 25kDa
SNP	- single-nucleotide polymorphism

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. Homocystein 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. Frosst *et al.* (1995) identified in *MTHFR* gene a C677T (rs1801133) 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 (Frosst *et al.* 1995). In individuals homozygous for the T allele significantly elevated plasma homocysteine levels was found (Frosst *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, noradrenalin 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 betweenVal158Met 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 aetiology of schizophrenia (Yamamoto and Hornykiewicz 2004). The polymorphism C-1297G (rs1800544) in the regulation region of *ADRA2A* have previously shown significant association with B_{max} and K_d 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 *Taq*I. PCR reaction contained: 5 ng of DNA, Kapa2G Fast ready Mix (Kapa Biosystems), 100 nM primers (F: CATCCCTATTGGCAGGTTA, R: ATGTGTCAGCCTCAAAGAAA). 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 *Taq*I 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 (Ods) 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 of both genotypes 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).

Tab. 1. Genotype frequencies of selected polymorphisms in *COMT* and *MTHFR* genes among cases and controls.

Genes	SNP	Genotype	Controls (*N*=209)		Patiens (*N*=186)		OR	(95% CI)	*p*-value
			Number	(%)	Number	(%)			
MTHFR	rs1801133	CC	105	50.2	72	38.7	1.00	–	–
		CT	86	41.2	90	48.4	1.53	(1.00–2.33)	0.049
		TT	18	8.6	24	12.9	1.94	(0.98–3.84)	0.054
		CT+TT	104	49.8	114	61.3	1.60	(1.07–2.39)	0.021
ADRA2A	rs1800544	CC	123	58.9	131	70.4	1.00	–	–
		CG	63	30.1	42	22.6	0.63	(0.39–0.99)	0.046
		GG	11	5.3	6	3.2	0.51	(0.18–1.43)	0.19
		CG+GG	74	35.4	48	25.8	0.61	(0.39–0.95)	0.027
COMT	rs4680	GG	42	20.1	41	22.0	1.00	–	–
		GA	81	38.7	86	46.2	0.92	(0.54–1.56)	0.75
		AA	63	30.1	47	25.3	1.31	(0.74–2.32)	0.36
		GA+AA	144	68.9	133	71.5	0.95	(0.58–1.55)	0.83

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=0.63 (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 (χ^2=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, χ^2=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

Tab. 2. Interaction of *MTHFR* and *ADRA2A* polymorphisms under a codominant model for the *ADRA2A* in controls and patients.

***ADRA2A* Genotype**	**Risk allele (T)**			***MTHFR* Risk genotype (CT+TT)**		
	Controls	**Patients**	***p*-value**	**Controls**	**Patients**	***p*-value**
-1291CC	37 (19.7)	13 (10.2)	0.020	32 (34.0)	12 (18.8)	0.032
-1291GC	40 (24.7)	26 (15.3)	0.032	34 (42.0)	23 (27.1)	0.043
-1291GG	6 (18.8)	12 (25.0)	0.509	6 (37.5)	11 (45.8)	0.600

Genotype frequencies are presented as percentages (within parentheses). All chi-squared tests are two-tailed. Alpha value is adjusted by Bonferroni correction and statistically significant results (p<0.025) are marked bold.

Tab. 3. Interaction of MTHFR and ADRA2A polymorphisms under a dominant model for the ADRA2A in controls and patients.

	Sample size		***MTHFR* Genotype**				
			677 CC		**677 CT + 677 TT**		***p*-value**
***ADRA2A* Genotype**	**Control**	**Patients**	**Controls**	**Patients**	**Controls**	**Patients**	
-1291CC+CG	175	149	62.3	76.5	37.7	22.8	0.008
-1291GG	16	24	62.5	54.2	37.5	45.8	0.602

All chi-squared tests are two-tailed. Alpha value is adjusted by Bonferroni correction and statistically significant results (p<0.025) are marked bold.

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 CT+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 (Šerý *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 (Šerý *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; Munafo *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.

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