Analysis of genetic polymorphisms of brain-derived neurotrophic factor and methylenetetrahydrofolate reductase in depressed patients in a Slovak (Caucasian) population

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Abstract. Major depressive disorder (MDD) is a complex neuropsychiatric disorder where both gene-gene and gene-environment interactions play an important role, but the clues are still not fully understood. One carbon metabolism in the CNS plays a critical role in the synthesis and release of neurotransmitters which are relevant to depressive disorder. We studied genetic polymorphisms of the brain derived neurotrophic factor (BDNF) and the methylenetetrahydrofolate reductase (MTHFR) in association with major depressive disorder. We genotyped the *BDNF* G196A, the *MTHFR* C677T, and A1298C polymorphisms in 134 patients diagnosed with major depression and 143 control subjects in Slovak (Caucasian) cohort of patients and probands. We found no significant association of either the *BDNF* G196A or *MTHFR* C677T polymorphisms with major depressive disorder neither in female nor male group of patients. However, the *MTHFR* A1298C genotype distribution was 36.6% (for AA genotype), 48.5% (AC) and 14.9% (CC) for the depressed patients, and 48.9% (AA), 42.7% (AC) and 8.4% (CC), respectively, for the control subjects. Patients with MDD had a higher prevalence of the CC genotype (OR = 2.38; 95% CI = 1.07–5.32; p = 0.032) and the AC + CC genotype (OR = 1.67; 95% CI = 1.03–2.69; p = 0.037) in comparison with the control subjects. This study shows that CC genotype of the *MTHFR* A1298C is associated with higher risk of MDD in Slovak population.

Key words: Depression — Polymorphism — Methylenetetrahydrofolate reductase — Brain-derived neurotrophic factor

Introduction

Many psychiatric disorders including major depressive disorder (MDD) are characterized by structural alterations in specific limbic regions. These changes result from atrophy and loss of neurons and glia (Duman 2004). MDD is a complex neuropsychiatric disorder where both gene-gene and gene-environment interactions play an important role, but still is not fully understood.

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Brain-derived neurotrophic factor (BDNF) is 27-kDa polypeptide, belongs to the family of neurotrophin growth factors which are essential in neuronal development, survival and plasticity. BDNF is synthetized as a precursor protein (proBDNF); mature BDNF is formed when proBDNF is proteolytically cleaved by extracellular proteases (Lessmann et al. 2003). The human *BDNF* gene is located on chromosome 11p13. Egan et al. (2003) have identified G196A single nucleotide polymorphism in the gene encoded BDNF. The *BDNF* G196A polymorphism results in a valine (Val) to a methionine (Met) change at position 66 (Val66Met). This polymorphism markedly alters the intracellular trafficking and packaging of proBDNF. Brain-derived neurotrophic factor widely influences hippocampal neurogenesis and plays an important role in the pathogenesis of the depression (Altar 1999; Duman 2004). In humans, the Met allele has

been associated with poorer episodic memory, abnormal hippocampal activation, and lower hippocampal concentration of N-acetylaspartate (Egan et al. 2003). Geller et al. (2004) found that the Val allele was preferentially identified mainly in Caucasian adult probands with bipolar disorder. Lower plasma concentrations of BDNF in depressive patients were observed by Karege et al. (2002). A decreased content of BDNF was documented in the brain cortex and hippocampus in *post mortem* studies of depressive patients (Dwivedi et al. 2003). But, the association of the *BDNF* gene polymorphism with depression is still ambiguous.

In addition, still is very little known about the metabolic aspects of the disease, such as role of the one carbon metabolism in the pathogenesis of major depressive disorder. Methylenetetrahydrofolate reductase (MTHFR) catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Homocysteine or its metabolites may have an excitotoxic effect to neurons and may inhibit methylation processes in the central nervous system (Bottiglieri et al. 1994). Gene for MTHFR is located on chromosome 1p36.3. A common mutation of the *MTHFR* gene is the C677T change, which converts alanine to valine, results in a thermo-labile enzyme with decreased activity (Ueland et al. 2001). Patients with homozygous 677TT genotype had significantly elevated plasma concentration of homocysteine levels, and decreased serum folate concentration. The heterozygote and homozygous variant of the *MTHFR* C677T were shown to have 65% and 30% of the enzyme activity (Rozen 1996). Another functional polymorphic site was identified in exon 7 by van der Put et al. (1998). An A1298C mutation results in a glutamate to alanine change. This mutation also leads to decreased MTHFR activity, which was more pronounced in the homozygous than heterozygous state (Lievers et al. 2001). Both the C677T and A1298C single nucleotide polymorphisms (SNPs) in the *MTHFR* gene decrease the activity of the enzyme, leading to hyperhomocysteinemia, particularly in folate-deficient states, however this type of study is still missing with Slovak (Caucasian) cohort of patients.

In the present study, we examined the associations of the brain derived neurotrophic factor G196A polymorphism, methylenetetrahydrofolate reductase C677T, and A1298C genetic polymorphisms in patients with MDD. Although few studies confirmed the association of *MTHFR* polymorphisms with MDD, studied groups had often less than one hundred patients (van den Put et al. 1998; Reif et al. 2005). Thus the contribution of these polymorphisms to MDD is still questioned. The aim of our study performed on 134 MDD selected Slovak cohort of patients was to examine the possible linkage of these three different polymorphisms with MDD. In addition, we report for the first time a double association analysis on MTHFR polymorphisms in connection with MDD in Slovak population.

Material and Methods

The patient group was comprised of 134 inpatients with average age 48 ± 13.9 (from 21 to 81 years, median 49) of which 60.7% were women with average age 49 ± 14.6 (from 21 to 81 years, median 49) and 39.7% were men with average age 47 ± 12.8 (from 22 to 59 years, median 48) suffered from major depressive disorder with single episode or recurrent, and without other psychiatric comorbidities (as anxiety disorders, alcohol and drug addictions). Patients with bipolar disorder were excluded from the study. Diagnosis was confirmed by two independent psychiatrists at the Psychiatric Clinic of University Hospital Martin (Slovakia) according to DSM-IV-TR (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision) and ICD-10 (The ICD-10 Classification of Mental and Behavioral Disorders) classifications. The core symptoms of depression are depressed mood, and loss of interest or pleasure. At least one of these symptoms (depressed mood or loss of interest or pleasure) is present for at least two weeks according to DSM-IV-TR classification. For the complexity of diagnosis a rating scale (MADRS score) was used too. Patients with primary depression (moderate, severe or psychotic form) were hospitalized at the Psychiatric Clinic of University Hospital Martin (Slovakia) after the failure of ambulance treatment. The patients were hospitalized for an acute treatment from four to seven weeks. After that they were released from hospital to the daily hospital when the MADRS score dropped at least about 50%.

The control group consisted of 143 volunteers blood donors with average age 37 ± 11.3 (from 19 to 63 years, median 36) of which 60.1% were men (average age of 37 ± 11.3 from 19 to 57 years, median 37) and 39.9% were women (average age of 36 ± 12.1, from 20 to 63 years, median 35), as they claimed to be feeling well and intended to donate blood. All subjects gave their informed consent prior to participate in this study. The study was approved by the Bioethics Committee of Jessenius Faculty of Medicine in Martin, Slovakia.

Genomic DNA was extracted from peripheral blood leukocytes according to commercial procedure (Wizard Genomic DNA purification kit, Promega). Analyzed polymorphic variants of the *BDNF* (rs6265) and *MTHFR* (rs1801133, rs1801131) were determined using the PCR-RFLP method. The *BDNF* G196A SNP was genotyped by PCR according to the protocol originally described by Egan et al. (2003). PCR was performed in a final volume of 25 µl with 40 ng of DNA, 25 nmol/l primers and 12.5 µl Readymix REDTaq polymerase (Sigma-Aldrich). PCR products were digested with Fast digest restriction enzyme *PmlI* (Fermentas) at 37°C 5 min. DNA fragments were separated by electrophoresis on 2% agarose gel and visualized by UV illuminator. Three possible genotypes were found: GG 124bp, 180bp; GA 124bp, 180bp, 304bp; AA 304bp.

The *MTHFR* C677T SNP was genotyped by PCR according to the protocol originally described by Frosst et al. (1995). PCR product was digested with Fast digest restriction enzyme *HinfI* (Fermentas) at 37°C 5 min. The C allele corresponded to an undigested 206bp fragment, while the T allele corresponded to 160bp and 46bp digested fragments. The MTHFR A1298C SNP was genotyped by PCR according to the protocol originally described by van der Put et al. (1998). Glutamate allele of the PCR product was digested at 37°C using Fast digest restriction enzyme *MboII* (Fermentas) and separated on 2% agarose gel and genotyped according to the length of the digested PCR products. Homozygous genotypes were identified by the presence of a single 204bp band (CC) or by bands of 150bp and 54bp (AA).

The χ^2 method was used to test frequencies of alleles and genotypes among depressed patients and controls. The odds ratios (OR), which estimate relative risk, with 95% confidence intervals (CI) were calculated to assign the strengths of association of the genotypes with MDD. For the double association analysis the χ^2 method was used, too. Differences resulting in a *p* value of less or equal to 0.05 were considered statistically significant. Distribution of genotypes was tested for deviation from Hardy-Weinberg equilibrium.

Results

The distribution of the *BDNF* G198A, *MTHFR* C677T, and *MTHFR* A1298C allele and genotype frequencies among the group of control subjects and cohort of the depressed patients from Slovak (Caucasian) population are shown in Tables 1 and 2. As a measure for quality group, distribution of genotypes in three SNPs was analyzed and has found to fit in Hardy-Weinberg equilibrium (results not shown). To assess the variant allele positivity, we analyzed heterozygous and polymorphic genotypes together.

As shown in the Table 1, we have analyzed the association of the *BDNF* Val/Met (G196A) polymorphism with the incidence of MDD. We genotyped 134 patients with confirmed MDD, 75.4% of which had the GG genotype, 20.9% had the GA genotype, and 3.7% had the AA genotype. Genotype frequencies in the control Slovak probands were as follows: 69.9% for the GG genotype, 26.6% for the GA genotype and 3.5% for the AA genotype. As seen from the Table 1, the GG genotype and G allele was present more frequently in patients with MDD than in healthy Slovak volunteers. We have observed no statistically significant differences in the genotype or allele frequencies of *BDNF* G196A polymorphism in the cohort of the MDD patients and control subjects neither for the G allele nor for the A allele of *BDNF*.

However, the genotype distribution according to gender showed slightly different results, but they were not statistically significant. In men, we observed 1.15-fold increased risk (95% CI = 0.62 – 2.16) for the A allele in depressed group (19.6%) compared with controls (17.4%). On the contrary, in women we found lowered risk (OR = 0.65, 95% CI = 0.32 – 1.31) for the A allele among the depressed group (10.8%) and controls (15.8%).

The genotype and allele frequency distribution of *MTHFR* C677T polymorphism in Slovak MDD patients and control subjects is shown in Table 2. We have analyzed cohort of 134 depressed patients for the *MTHFR* C677T polymorphism. As can be seen from results, the frequency of the CC genotype in MDD group was 52.2%, the heterozygous CT genotype was 40.3%, and the TT genotype was 7.5%. In healthy volunteers, the distribution of genotypes was found as follows: 40.5% for the CC genotype, 51.1% for the CT genotype and 8.4% for the TT genotype. We did not observe a significant difference between the heterozygous CT (OR = 0.61, 95% CI = 0.37 – 1.01, *p* = 0.052) and variant TT (OR = 0.69, 95% CI = 0.28 – 1.71, *p* = 0.42) genotypes in comparison to the CC genotype in this group of probands. In addition, there were not statistically significant differences in the incidence of genotypes and alleles. However, the values for the heterozygous CT genotype in comparison to the wild CC genotype as a potentially protective genotype to MDD were boundary (*p* = 0.052).

Table 1. Genotype frequencies of the *BDNF* G196A polymorphism in the cohort of patients with major depressive disorder (MDD) and in control subjects

BDNF G196A genotype	Controls (*n* = 143)	Patients (*n* = 134)	OR (95% CI)	*p*	χ^2
GG	100	101	1.00		
GA/GG	38	28	0.73 (0.42 – 1.28)	0.270	1.22
AA/GG	5	5	0.99 (0.28 – 3.53)	0.988	0.00
GA+AA/GG	43	33	0.76 (0.45 – 1.29)	0.310	1.03
Allele G	238	230	1.22 (0.77 – 1.94)	0.398	0.72
Allele A	48	38	0.82 (0.52 – 1.30)	0.398	0.72

n, number of probands; OR, odd ratio; CI, confidence interval.

Table 2. Genotype frequencies of the *MTHFR* C677T and A1298C polymorphisms in the cohort of patients with major depressive disorder (MDD) and in control subjects

	Controls (n = 143)	Patients (n = 134)	OR (95% CI)	p	χ^2
MTHFR C677T genotype					
CC	58	70	1.00		
CT/CC	73	54	0.61 (0.37 – 1.00)	0.052	3.78
TT/CC	12	10	0.69 (0.28 – 1.71)	0.423	0.64
CT+TT/CC	85	64	0.62 (0.39 – 1.00)	0.051	3.80
Allele C	189	194	1.35 (0.94 – 1.93)	0.108	2.58
Allele T	97	74	0.74 (0.52 – 1.07)	0.108	2.58
MTHFR A1298C genotype					
AA	70	49	1.00		
AC/AA	61	65	1.52 (0.92 – 2.52)	0.106	2.67
CC/AA	12	20	2.38 (1.07 - 5.32)*	0.032*	4.62
AC+CC/AA	73	84	1.66 (1.03 – 2.69) *	0.037*	4.33
Allele A	201	163	0.66 (0.46 – 0.93)	0.019	5.49
Allele C	85	105	1.52 (1.07 – 2.17) *	0.019*	5.49

* statistically significant results ($p < 0.05$). For abbreviations, see Table 1.

Consequently, when we analyzed genotype distribution according to gender, the distribution of genotypes was similar as it is shown in the Table 2. We did not detect statistically significant association of heterozygous CT genotype as a protective genotype to MDD neither for men nor for women ($p > 0.05$).

Unlike no association of *MTHFR* C677T polymorphism with MDD, the analysis of the same patients for another *MTHFR* polymorphism: *MTHFR* Glu/Ala (A1298C*)* showed statistically significant association with MDD. Distribution of genotypes in depressed group of patients was as follows: 36.6% for the AA genotype, 48.5% for the AC genotype, and 14.9% for the CC genotype. In control Slovak subjects we observed the following distribution of genotypes: 48.9% for the AA genotype, 42.7% for the AC genotype, and 8.4% for the CC genotype. The genotype and allele frequency distribution of *MTHFR* A1298C polymorphism in patients and control subjects is shown in Table 2. Interestingly, we found out a 2.38-fold increased risk of MDD incidence in individuals with the AA and CC genotype (OR = 2.38; 95% CI = 1.07–5.32; p = 0.032). The result was also statistically significant when we compared the AC and CC genotype against the AA genotype, but the OR was lower (OR = 1.66; 95% CI = 1.03–2.69; p = 0.038). Allelic frequencies in depressed patients were 60.8% for the A allele and 39.2% for the risk C allele in comparison with healthy volunteers 70.3% for the A allele and 29.7% for the C allele. We have also observed a statistically significant association of the C allele with the increased risk of MDD (OR = 1.52; 95% CI = 1.07–2.17; p = 0.019). Genotype distribution according to gender showed similar tendency as the results for whole group. In men,

we found statistically significant association of the risk CC genotype carriers (OR = 3.1, 95% CI = 1.01–9.6, p = 0.042) compared to AA genotype carriers among the depressed group (17.7%) and controls (7.4%).

In women, we observed higher frequency of the risk CC genotype (OR = 1.60, 95% CI = 0.81–2.38) and the C allele (OR = 1.38, 95% CI = 0.48–5.3) in depressed group (12.5%) compared to controls (10.4%), but the difference was not statistically significant.

In addition, we also tried to uncover whether combination of two polymorphic sites in the *MTHFR* gene is associated with the incidence of MDD in Slovak population. We performed χ^2 method to test frequency of coexistence of genotypes (CC, CT, TT) and (AA, AC, CC), to verify whether exists characteristic combination of the *C677T* and *A1298C* genotypes in patients suffering from MDD compared to healthy individuals. We have observed that the TT *C677T* and the AC *A1298C* genotypes are considerably associated with the higher risk of major depression disorder in Slovak population. Likewise, the OR analysis showed that persons with such a genotype combination are more than five times prone to develop depression than persons with other genotype combinations. However, detailed analysis showed that the result was not statistically significant (Table 3).

Discussion

Many studies proved, that one carbon metabolism in brain has a critical effect on intracellular pathways and neural function, including gene regulation, neurotransmitter

Table 3. Double association analysis of the *MTHFR* C677T and A1298C polymorphisms in the cohort of patients with major depressive disorder (MDD) and in control subjects

MTHFR C677T genotype	MTHFR A1298 genotype	Controls ($n = 143$)	Patients ($n = 134$)	OR (95% CI)	p	χ^2
CC	AA	18	17	1.02 (0.50 – 2.07)	0.550	0.00
CC	AC	27	33	1.40 (0.79 – 2.49)	0.155	1.35
CC	CC	11	18	1.86 (0.85 – 4.11)	0.086	2.43
CT	AA	40	27	0.65 (0.37 – 1.14)	0.084	2.31
CT	AC	33	26	0.80 (0.45 – 1.43)	0.275	0.56
CT	CC	0	1	–	–	–
TT	AA	12	5	0.45 (0.15 – 1.32)	0.107	2.61
TT	AC	1	5	5.50 (0.63 – 47.74)	0.092	–
TT	CC	0	0	–	–	–

For abbreviations, see Table 1.

synthesis and synaptic function. Imbalances in one carbon metabolism therefore initiate complex cascades which appear to be related to a variety of neuropsychiatric disorders, such as chronic fatigue, depression, schizophrenia, psychosis and dementia (Coppen et al. 2000; Saetre et al. 2011; Suchanek et al. 2011).

Several authors reported positive association of *BDNF* polymorphism with affective disorders (Sen et al. 2003, 2008; Chen et al. 2006; Kaufman et al. 2006), but the results are inconsistent. Sen et al. (2003) published that allele A of *BDNF* is associated with lower neuroticism in the common population, while Chen et al. (2006) observed in mouse models that with exprimed allele A number and length of apical dendrites in hippocampus and prefrontal cortex was lowered. *BDNF* G196A polymorphism was reported to be associated with bipolar disorder by Egan et al. (2003). Most of studies did not confirm the association of the *BDNF* gene polymorphism with MDD (Schumacher et al. 2005; Green et al. 2006; Kunugi et al. 2004). Similarly to the study of Schumacher et al. (2005) which examined 465 subjects with major depression also Surtees et al. (2007) did not find significant association of MDD with *BDNF* G196A polymorphism in 1214 subjects with a history of major depression. Suchanek et al. (2011) found GG genotype was more frequent in patients with recurrent major depressive disorder in studied cohort of Polish patients compared to the control group. In Slovak population with MDD, we did not observe a statistically significant association between *BDNF* G/A polymorphism and MDD. The frequency of the combined variant AA and heterozygous GA genotypes and the wild type GG genotype was not significantly different in our cohort of depressed patients and control probands. Likewise, genotype and allelic distribution between men and women did not show statistically significant results, too. Thus, our study did not prove any association of *BDNF* G196A polymorphism with MDD, and it is not likely that there is a direct relationship

between the *BDNF* G196A polymorphism and vulnerability to major depressive disorder.

The association for the polymorphisms of the second studied gene, the *MTHFR* C677T with MDD, was first reported in 1998 by Arinami et al. on the group of Japanese subjects. Authors found that depressed patients have significantly higher prevalence of TT genotype; however, results can be questioned because studied group of patients comprised only 32 patients. Larger study including 71 patients was also accomplished on Japanese population with unipolar depression. In line of previous, this study did not prove any association between MTHFR polymorphisms and unipolar depression, too (Kunugi et al. 1998).

Bjelland et al. (2003) examined the association between folate, total homocysteine, vitamin B12, and the *MTHFR* C677T polymorphism and anxiety and depression in a large population-based study. Hyperhomocysteinemia and the TT genotype were significantly related to depression without comorbid anxiety disorder (Bjelland et al. 2003). They concluded that the results provided evidence for impaired 1-carbon metabolism in depression. The largest European women study on *MTHFR* polymorphism was realized in 2006 by Lewis et al. in the British Women´s Heart and Health study. The study have included 545 women and found an evidence of an increased risk of depression in *MTHFR* TT polymorphic individuals compared with CC individuals with the OR = 1.35, 95% CI = 1.1–1.8 (Lewis et al. 2006).

Later meta-analyses of genetic studies associated with major depressive disorder (Gilbody et al. 2007; Lewis et al. 2006; Zintzaras 2006; Lopez-Leon et al. 2008; Gaysina et al. 2008; Peerbooms et al. 2011) also reported the *MTHFR* 677T (OR = 1.20) polymorphism as one of the six positively associated polymorphisms with MDD.

Recently, several case-control studies have analyzed cohorts of patient with the European origin, diagnosed with unipolar depression. All patients were based on DSM-IV

or ICD 10 criteria, which have also been used in our study. Gaysina et al. (2008) published a powerful study in the British population which included 1222 depressed patients and 835 control subjects. The study was not decisive and authors found no linkage between the C677T polymorphism and major depression in British Caucasians (OR = 1.05). Results suggest for more equivocal role of the *MTHFR* C677T polymorphism in conferring susceptibility to recurrent unipolar depression. Later studies on Caucasian population showed also various results (Hernandez et al. 2009; Huang et al. 2010). While Huang et al. (2010) in the group of 178 patients did not find any positive association of the *MTHFR* C677T with unipolar depression, Hernandez et al. (2009) in a smaller cohort (21 patients) found significant statistical association of MTHFR and depression. Recent meta-analysis published by Peerbooms et al. (2011) have examined the association between *MTHFR* and multiple psychiatric disorders (such as schizophrenia, bipolar disorder, and depression together) using a cross-disorder design, not separately for particular disorders and found no decisive results. However, till now, no study was done in the Slovak population of depressed patients in association with *MTHFR C677T* polymorphism. Present paper is the first to prove that in Slovak population does not exist higher prevalence of the risk CT or TT genotypes of the MTHFR C677T polymorphism among the control and depressed group. Likewise, genotype distribution in men and women showed similar trend and we found no statistically significant differences. Thus, we can conclude that results of our study fits well with two other meta-analyses performed on Caucasian cohort of depressed patients (Zintzaras 2006, Gaysina et al. 2008).

Only one genetic study which shows the *MTHFR* A1298C polymorphism in connection with MDD was reported till now (Reif et al. (2005). Authors have reported for a positive association with *MTHFR* A1298C and MDD in Caucasians and found an odds ratio 3.25, 95% CI = 1.19–8.88, when CC variants were compared with the AA homozygote wild type genotype. In accordance with this study, we found a statistically significant association of the AC + CC and, the CC genotype and the C allele of the *MTHFR* A1298C polymorphism with MDD in our Slovak cohort. Statistically significant increases were shown in those patients who were carriers of C1298C (14.9% in patients compared with 8.4% in healthy volunteers), and those who were carriers of C1298C and A1298C (63.4% in patients and 51.1% in healthy volunteers). In men we found statistically significant increased risk of depression for those who were CC carriers ($p < 0.05$), in women we observed higher frequency of the CC genotype in depressed group compared to controls, but not statistically significant.

This study also provides for the first time a double association analysis of the C677T and the A1298C *MTHFR*

polymorphic sites in association with MDD. Combined heterozygous genotypes for both *MTHFR* mutations resulted in features similar to those observed in TT homozygotes for the C677T mutation (van der Put et al. 1998). Even though, we found a 5.5 times higher prevalence of the variant TT combination and the heterozygous AC genotype in MDD group of Slovak Caucasian patients, result was not statistically significant. Thus, the final prove of positive association with statistical significance should be approved by further study with larger group of patients.

Major depressive disorder is a complex neuropsychiatric disorder, with heritability up to 40%, but pathophysiology is still not fully understood. Analysis of genes polymorphisms which could be involved in the etiopathology of MDD could thus lead to discovery of novel markers for identification of patients with higher risk of development of MDD. In addition, findings detecting particular combinations of polymorphisms of different genes may confer to the exploration of earlier onset and /or presence of the severe forms of MDD.

Limitation: The main limitation of this study is relatively small sample size.

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