Original Article

Polymorphisms of Genes for Brain-Derived Neurotrophic Factor, Methylenetetrahydrofolate Reductase, Tyrosine Hydroxylase, and Endothelial Nitric Oxide Synthase in Depression and Metabolic Syndrome

(depression / metabolic syndrome / BDNF / gene polymorphism)

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Abstract. The prevalence of metabolic syndrome as well as the occurrence of depressive disorder, which are both connected with increased risk of diabetes mellitus type 2 and cardiovascular diseases, is continually increasing worldwide. These disorders are interconnected at various levels; the genetic one seems to be promising. Contribution of genetic factors to the aetiopathogenesis of depressive disorder weighs within the range 40–50 %, whereas the genetic background for the manifestation of metabolic syndrome is more complicated. In this pilot study, we investigated the incidence of polymorphisms in several genes supposed to play a role in the development of both depressive disorder and metabolic syndrome such as brain-derived neurotrophic factor, methylenetetrahydrofolate reductase, tyrosine hydroxylase, and endothelial nitric oxide synthase. The entire group consisted of 42 patients with depressive disorder, 57 probands with metabolic syndrome and 41 control individuals. We found that genotype Met/Met of the Val66Met polymorphism of the brain-derived neurotrophic factor gene was positively associated with depressive disorder ($P < 0.05$), but we were not able to find any significant associations of both the depressive disorder and metabolic syndrome with the remaining polymorphisms studied (methylenetetrahydrofolate reductase 677CT, methylenetetrahydrofolate reductase 1298AC, endothelial nitric oxide synthase Glu298Asp, and tyrosine hydroxylase).

Introduction

The modern world is faced with the growing prevalence of metabolic syndrome (MetS), which is a risk factor for the onset of diabetes mellitus type 2 (DM2) and cardiovascular diseases. Concomitantly, the rise in the occurrence of depressive disorder (DD) has been observed. At least one-fourth of adults carry the metabolic syndrome in the Americas, in Europe, and in India (Grundy, 2008). In the USA, approximately 10–20 % women and 5–12 % men experience one episode of main depression at least; half of these cases are recurrent (Nestler, 2002). One hundred sixty-eight new cases of affective disorders in 100,000 inhabitants were noticed in the Czech Republic in 2006; the incidence was two times higher in women than in men (Czech Health Statistics Yearbook, 2007). The key role in the aetiopathogenesis of both MetS and DM2 is inscribed to insulin resistance (IR) (Reaven, 2002), which (according to some authors) is also connected with the pathogenesis of DD (Okamura et al., 2000), associated with an in-

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Abbreviations: BDNF – brain-derived neurotrophic factor, BMI – body mass index, CAD – cardiovascular disease, CON – control group, DD – depressive disorder, DM – diabetes mellitus, DOPA – dihydroxyphenylalanine, eNOS – endothelial nitric oxide synthase, HDL-C – high-density lipoprotein cholesterol, HWE – Hardy-Weinberg equilibrium, IR – insulin resistance, MetS – metabolic syndrome, MTHFR – methylenetetrahydrofolate reductase, NOS – nitric oxide synthase, TCAT – tetranucleotide, TH – tyrosine hydroxylase, TG – triacylglycerols.

creased risk of type 2 diabetes mellitus (Musselman et al., 2003) and both cardiovascular and all-cause mortality (Muselman et al., 1998, Wulsin et al., 1999).

Despite increasing occurrence of depressive disorder we still know little about its underlying mechanisms. The susceptibility of given individuals to the manifestation of DD is significantly influenced by genetic factors – their contribution to the aetiopathogenesis of DD weighs within the range 40–50 % (Levinson, 2006). The age-adjusted risk of first-grade relatives of the patients with unipolar depression is approx. 5–25 % (McGuffin et al., 1996). According to Levinson (2006), the relative risk of these relatives reaches 2–3, and this risk is further increased in case of depressions manifested before the age of 30 with a tendency for recurrence.

The genetic background is also significant for the manifestation of MetS. An important role is probably played by genes in various *loci*, which act *via* gene-gene interactions or together with exogenous (diet, physical activity, stress, smoking, infection) and metabolic factors. Many candidate genes are known to be associated with MetS in various ethnical groups (López-Miranda et al., 2007): genes for insulin receptor substrate 1*,* peroxisomal proliferator-activated receptor gamma, adiponectin, leptin, leptin receptor, and many others.

In depression, several candidate gene polymorphisms were investigated, such as the serotonin transporter, serotonin 2A receptor, tyrosine hydroxylase (TH) (the limiting enzyme for dopamine synthesis), tryptophan hydroxylase 1 (serotonin synthesis), catechol-O-methyltransferase (dopamine catabolism) (Lesch, 2004; Levinson, 2006), and methylenetetrahydrofolate reductase (MTHFR) (metabolism of homocysteine, which is toxic to neurons and is also a risk factor for cardiovascular disease) (Arinami et al., 1997, Lewis et al., 2006, López-León et al., 2008). The studies conducted in last years support the hypothesis on the importance of stress connected with a decreased volume of hippocampus (McEwen, 2005). Brain-derived neurotrophic factor (BDNF) significantly influences hippocampal neurogenesis and plays an important role in the pathogenesis of the depression (Altar, 1999, Duman 2004). Several studies revealed lower plasma concentrations of BDNF in depressive patients (Karege et al., 2002), and a decreased content of BDNF was proved in the brain cortex and hippocampus of deceased depressive patients (Dwivedi et al., 2003). The association of *BDNF* gene polymorphisms with depression was reported by several authors (Sklar et al., 2002, Sen et al., 2003, 2008; Chen et al., 2006, Kaufman et al., 2006).

The above-mentioned disorders (cardiovascular diseases, type 2 diabetes mellitus MetS, and DD) are interconnected at various levels; the genetic one seems to be promising. The genes in question also involve those taking part in various neuronal processes. We therefore decided to compare the occurrence of polymorphisms of genes for BDNF, MTHFR, TH, and endothelial nitric oxide synthase (eNOS, metabolism of NO influencing intersynaptic signal mediation and vessel tonus) in patients with depression, metabolic syndrome and in healthy controls.

Material and Methods

Patients and controls

A group of 140 Caucasian subjects (55 M/85 F) was recruited from outpatients, who were subsequently examined (from May 2006 until May 2008) at the 4th Department of Medicine and Psychiatric Department of Charles University in Prague. The entire group consisted of 41 (14 M/27 F) healthy control subjects (medical staff of the 1st Faculty of Medicine), 57 patients with MetS, diagnosed according to the International Diabetes Federation (Alberti et al., 2006) (35 M/22 F), and 42 patients (6 M/36 F) with depressive disorder, diagnosed according to the criteria of American Psychiatric Association (1994). The basic clinical characteristics of the studied groups are shown in Table 1. The study protocol was approved by the Joint Ethical Committee of the 1st Faculty of Medicine and General University Hospital of Charles University in Prague.

Laboratory procedures

Blood samples were collected after a 12-h fasting. The concentrations of triacylglycerols (TG) and glucose

Table 1. Basic clinical characteristic of the studied groups

	Depressive disorder (42)	Metabolic syndrome (57)	Controls (41)	Statistical significance[c]
Age (years)	61.4 ± 15.8[1]	56.4 ± 9.1	58.1 ± 13.5	NS[c]
Number (men/women)	6/36	35/22	14/27	0.0001*
Weight (kg)	70.9 ± 13.9	88.5 ± 14.4	71.1 ± 9.6	0.00001
BMI (kg.m^{-2})	26.3 ± 4.9	29.7 ± 3.9	25.0 ± 3.4	0.00001
Systolic blood pressure (mm Hg)	126 ± 16	137 ± 17	130 ± 12	0.00075
Diastolic blood pressure (mm Hg)	78 ± 9	89 ± 8	81 ± 6	0.00001
Glucose (mmol/l)	5.24 ± 1.07	5.72 ± 1.39	4.80 ± 0.43	0.00059
Insulin (mIU/l)	12.47 ± 10.77	13.44 ± 8.26	7.66 ± 3.79	0.00341
Triacylglycerols (mmol/l)	1.68 ± 1.11	3.74 ± 4.10	1.19 ± 0.59	0.00001
HDL-cholesterol (mmol/l)	1.51 ± 0.50	1.22 ± 0.23	1.67 ± 0.37	0.00001

[1]Average ± SD; [c]-(+)ANOVA, * Pearson χ^2-test; DD vs. CON: P = 0.0631 (Yates' correction),
DD vs MetS: P = 0.0001 (Yates' correction), CON vs. MetS: P = 0.0078

Table 2. Conditions for detection of allelic variants

genes	polymorphisms	forward primers (5′→ 3′) reverse primers (5′→ 3′)	annealing (°C)	restriction enzymes	product sizes (bp)
BDNF rs 6265	V66M (G→A)	AAACATCCGAGGACAAGGTG CCTCATGGACATGTTTGCAG	60	Eco72I	G(W) 124, 180 A(M) 304
MTHFR rs 1801133	A222V, 677CT (C→T)	TCCCTGTGGTCTCTTCATCC CAAAGCGGAAGAATGTGTCA	59.9	HinfI	C(W) 206 T(M) 146, 60
MTHFR rs 1801131	E429A, 1298AC	AGGAGGAGCTGCTGAAGATG ACAGGATGGGGAAGTCACAG	59.8	MboII	A(W) 150, 54 C (M) 204
eNOS rs 1799983	E298D, 894GT (G→T)	GAGATGAAGGCAGGAGACAGT TCCATCCCACCCAGTCAAT	59	MboI	G(W) 263 T(M) 164, 99
TH microsatellite repeat	(TCAT)	GGCAAATAGGGGGCAAAA GGCTTCCGAGTGCAGGTC	56	–	A(146), B(148), C(152), D(156), E(160)

Product sizes – W means wild-type allele, M variant-type allele.

were assessed by enzymatic-colorimetric methods, HDL-C in supernatant after precipitation of lipoproteins-B with PTA/Mg^{2+}, immunoreactive insulin by the radioimmunoassay method.

Genetic analyses

The isolation of DNA was performed according to standard desalting procedures (Miller et al., 1988). In all study subjects we examined five genetic variants (four single-nucleotide polymorphisms and one microsatellite repeat) using the PCR-RFLP method, direct sequencing and fragment analysis. Table 2 shows the studied genes, primers, temperature °C of annealing, restriction enzymes, and the length of product sizes. The typing of TCAT repeat of the *TH* gene and polymorphism E298D of the *eNOS* gene were published in more detail previously (Jindra et al., 2000, Jachymova et al., 2001).

Statistics

All data were processed and statistical analyses performed in the statistical environment STATISTICA CZ ver 7.1 (StatSoft Inc., Tulsa, OK) and GenAlEx V6 (Peakall, 2005). Categorical data are summarized in absolute and relative frequencies; continuous data are summarized as a mean and standard deviation. The variables without normal distributions were log-transformed. Pearson χ^2-test was employed in testing for differences in the distribution of genotype frequencies in respective groups (Yates' correction for small numbers in 2 × 2 tables or Monte Carlo simulation in larger tables). The statistical significance was defined as $P < 0.05$; Bonferroni correction was used when multiple comparisons were carried out.

Results

Basic clinical and demographic data of the studied groups are shown in Table 1. The MetS group included significantly more men in comparison with both the control group (CON) and DD groups. Differences between CON and DD groups in the men vs. women representation did not reach statistical significance. Expected significant differences in anthropometric and metabolic variables (BMI, systolic and diastolic blood pressure, fasting blood glucose, triglycerides and HDL-cholesterol) were seen between the MetS on the one hand and both CON and DD groups on the other, except for insulin, where the difference between MetS and DD groups was not statistically significant and moreover, serum insulin in DD was significantly higher than in the CON group. Interestingly, serum TG and BMI were also higher in the DD in comparison with the CON group, which indicates that some features of the MetS were presented in depressive patients.

Tables 3a and 3b show the prevalence of genotypes and allele frequencies of the investigated genetic variants. The validity of the Hardy-Weinberg equilibrium (HWE) was tested in each group separately and the distributions of genotypes were in HWE except for the *BDNF* Val66Met genotype in the DD group. In the case of *BDNF* gene Val66Met polymorphism we found a higher frequency of genotype AA in the DD group, as compared to the control and MetS group; these differences were significant after Bonferroni correction at the 5% level. Other polymorphisms did not reveal statistically significant changes between genotypes or allele frequencies.

Discussion

BDNF polymorphism

In this pilot study we found a significant association of the MetMet genotype of Val66Met *BDNF* polymorphism with DD. To our knowledge, this is the first report of the investigation of the *BDNF* gene polymorphism in Czech population.

BDNF belongs to a superfamily of neurotrophins, proteins playing roles in neuronal development, survival and plasticity (Maisonpiere et al., 1991). Recently it was found that BDNF also takes part in the regulation of energy homeostasis and influences blood glucose levels and insulin sensitivity (Levin, 2007). Decreased levels of the serum BDNF were found in the patients with

Table 3a. Selected polymorphisms, genotypes in the studied groups

	genotype				allele			HWE	χ^2 test
***BDNF* Val66Met**	**AA**	**AG**	**GG**	**total**	**A**	**G**	**total**		**P**
depression	5	7	30	42	17	67	84	0.002	0.003 genotype*
MetS	0	19	38	57	19	95	114	0.131	0.788 alleles
control	0	16	25	41	19	16	66	0.121	
***MTHFR* 677CT**	**CC**	**CT**	**TT**	**total**	**C**	**T**	**total**		
depression	15	18	9	42	48	36	84	0.418	0.495 genotype
MetS	30	19	8	57	79	35	114	0.102	0.170 alleles
control	16	17	8	41	49	33	82	0.377	
***MTHFR* 1298AC**	**AA**	**AC**	**CC**	**total**	**A**	**C**	**total**		
depression	22	17	3	42	61	23	84	0.908	0.965 genotype
MetS	28	23	6	57	79	35	114	0.696	0.879 alleles
control	20	18	3	41	58	24	82	0.699	
***eNOS* E298D**	**GG**	**GT**	**TT**	**total**	**G**	**T**	**total**		
depression	21	19	2	42	61	23	84	0.373	0.682 genotype
MetS	31	20	6	57	82	32	114	0.322	0.896 alleles
control	19	19	3	41	27	25	82	0.550	

*Pearson χ^2-test (Yates' correction for low numbers, Bonferroni correction for multiple comparison); DD vs. CON: P = 0.034, DD vs MetS: P = 0.027, CON vs. MetS: NS

Table 3b. Tyrosine hydroxylase microsatellite repeats in the studied groups

genotype	**AA**	**AB**	**AC**	**AD**	**AE**	**BB**	**BC**	**BD**	**BE**	**CC**	**CD**	**CE**	**DD**	**DE**	**EE**	**HWE**
depression	2	7	0	3	8	1	2	2	3	0	2	3	0	3	6	0.565
MetS	3	6	2	6	8	2	1	3	7	0	1	4	3	8	3	0.962
control	1	5	2	1	9	0	1	3	3	0	4	2	0	7	3	0.1854

allele	**A**	**B**	**C**	**D**	**E**	**total**	**χ^2 test**	
depression	22	16	7	10	29	84	0.727	genotype
MetS	28	21	8	24	33	114	0.813	alleles
control	19	12	9	15	27	82		

MetS and type 2 diabetes mellitus (Chaldakov et al., 2001; Krabbe at al., 2007). The human *BDNF* gene is mapped to chromosome 11p14, and three common polymorphisms (C270T, Val66Met, and dinucleotide-repeat $(GT)_N$ polymorphism located 1040 bp upstream from the transcription start site of the 1.6 kb BDNF mRNA) were described (Pröschel et al., 1992; Kunugi et al., 2003). The single-nucleotide polymorphism rs6265, which causes a valine to methionine substitution at codon 66 (Val66Met), is in the experiment connected with the disturbance of intracellular packaging and regulated secretion of BDNF by hippocampal neurons (Chen et al., 2004).

In the presented study the analysis of allele frequencies revealed 20 % of the Met allele in the DD group, 18 % in the MetS group and 19 % in the CON group. These differences did not reach statistical significance. The frequency of genotypes has shown a higher occurrence (P = 0.044) of the MetMet genotype in the DD group (12 %) in comparison with the other groups (1.8 % for the MetS and 2.0 % for the CON group). The frequency of the Met allele in our non-depressive probands (CON and MetS groups) is similar to those described in the Caucasian population by other authors. Shimizu et al. (2004) found frequency of the Met allele 17–27 % and prevalence of MetMet homozygosity 2–3 %. Montag et al. (2008) described the Met allele frequency 19.2 % and prevalence of MetMet genotype 2.7 % in German healthy non-smokers, and in healthy former + current smokers 18 % and 3.4 %, respectively. Quite different findings were observed in Asians. The prevalence of the MetMet genotype in Asians was 17.3 % (Kynugi et al., 2004), 20.1 % (Iga et al., 2007), 21.1 % (Choi et al., 2006) or 23.3% (Hong et al., 2003) in the control subjects. Interestingly, in these populations there were no associations of Val66Met *BDNF* polymorphism with major depressive disorder (Choi et al., 2006; Iga et al., 2007), with bipolar disorder (Kynugi et al., 2004) or with Parkinson's disease (Hong et al., 2003). In the study using a sample of probands participating in the EPIC study, no association between the *BDNF* Val66Met polymorphism and mood status in a non-clinical community sample was shown (Surtees et al., 2007).

Recently it was published that *BDNF* SNP rs6265 (Val66Met) was associated with major DD in Mexican-Americans, but the associated allele was Val (major allele) and ValVal homozygous was 70 % more likely to be found in the depressed group than in controls (Ribeiro et al., 2007). It seems that ethnic differences play a role in both distribution of alleles and in their association with DD.

Polymorphism of MTHFR gene

Decreased activity of the MTHFR enzyme with concomitant folate deficiency is connected with elevated plasma total homocysteine levels (Folstein et al., 2007), which are considered to be a risk factor for cardiovascular diseases (Žák and Zeman, 2004; Herrmann 2006). Clinical and epidemiological studies have found the association of hyperhomocysteinaemia with DD (Bottiglieri et al., 2000). Homocysteine is directly toxic to neurons and endothelial cells and can induce DNA strand breakage, oxidative stress, and apoptosis (Folstein et al., 2007). The most prevalent genetic cause of hyperhomocysteinaemia seems to be the SNP 677C>T polymorphism of the *MTHFR* gene. Another described mutation of the *MTHFR* gene (1298A>C) also reduces MTHFR activity (although to a lesser extent than the 677C>T) (Castro et al., 2003).

Recently it was published that among the patients with schizophrenia, TT genotype subjects were at greater risk for insulin resistance with increasing central adiposity, independently of age, gender or BMI (Ellingrod et al., 2008). The occurrence of the 677CT *MTHFR* polymorphism was investigated in the Czech population and it was reported that the C allele was associated with DM2 in women, while the T allele was associated with coronary artery disease only in normotensive subjects (Beneš et al., 2001).

The relationships of the *MTHFR* gene polymorphism 1298A>C with psychiatric diseases and MetS were investigated in several works. In one study, the 1298CC genotype was associated with the bipolar disorder (Kempisty et al., 2007). In the study of Teruzzi et al. (2007), *MTHFR* (AC) heterozygous genotype was statistically different in the obese compared to the control group ($P = 0.03$).

In this study we were not able to find any significant associations of both SNP 677C>T (677CT) and 1298A>C of *MTHFR* gene, either with depression or with MetS. In SNP 677CT polymorphism the frequency of the T allele was 40 % in the CON group, 31 % in MetS and 43 % in the DD group, while the prevalence of the TT genotype in the groups studied was 20 %, 14 % and 21 %, respectively. In the case of the 1298AC polymorphism, the allele C frequencies in the CON, MetS and DD groups were: 29 %, 31 % and 27 %, while the prevalence of genotype CC was 8 %, 11 %, and 7 %, respectively. There are significant differences in *MTHFR* gene polymorphisms between different populations. In the Czech population, Veselá et al. (2005) found the frequencies of the T allele of SNP 677CT polymorphism to be about 34.0 %, genotype TT 10 % and in SNP 1298A>C of *MTHFR*, gene frequencies of C alleles were about 32 % and CC genotypes 10 %. Among the healthy controls of the study of Beneš et al. (2001), there were 33.5 % T-allele carriers and 8.1 % TT homozygotes.

Polymorphism of eNOS

Nitric oxide synthase (NOS) catalyzes the oxidative conversion of L-arginine to citrulline and nitric oxide. The enzyme is expressed in three isoforms: endothelial (eNOS), neuronal, and inducible form (Chrapko et al., 2004). In the central nervous system the enzyme exerts a mediatory role within synapses (Pacher et al., 2007). A polymorphism (894G to T) in exon 7 of the *eNOS* gene causes conversion of Glu to Asp in position 298, connected with a poorer functional accessibility of the enzyme (Jáchymová et al., 2001). The changes in enzyme function play a role in the pathophysiology of hypertension (Jáchymová et al., 2001) and are probably involved in the development of DD (Le Mellédo et al., 2004) due to lower neurogenesis (Kempermann and Kronenberg, 2003).

In a case-control study performed in Italy in non-diabetic subjects with cardiovascular disease (CAD) and stent implantation, the presence of the T allele was associated with both increased insulinaemia and endothelial dysfunction (Galluccio et al., 2008).

In the presented study we could not find any associations of E298D polymorphism with the presence of either DD or MetS. Genotype TT was found in 2.4 % patients of DD, 7 % of MetS and 6.1 % of CON groups and the frequency of the T allele was also similar in the studied groups (25 %; 26 %; 27 %).

Polymorphisms of TH

TH catalyzes hydroxylation of L-tyrosine to L-DOPA (dihydroxyphenylalanine), which is a rate-limiting step in the biosynthesis of catecholamines; these substances are important for physiology of the sympathetic neuronal system and they also serve as neurotransmitters in the central nervous system (Serreti et al., 1998). The *TH* gene is localized on the locus 11p15.5. The functional activity of the enzyme can be modified by at least three SNPs: Val81Met, Leu205Pro, Val468Met, and microsatellite polymorphism of tetranucleotide (TCAT) repeats in the first intron (Rao et al., 2007). Several studies found the relationship of depression and gene variants, e.g. in the case of TCAT repeats and bipolar affective disorder (Leboyer et al., 1990). Contrary to that, other studies indicate low depressive symptomatology in this polymorphism (Serretti et al., 1998), or the association was not found (Furlong et al., 1999).

Essential hypertension, a component of MetS, is associated with microsatellite polymorphism in the 1st intron of the *TH* gene ("long allele" 9.3 and 10, Klintschar et al., 2005). The $(TCAT)_6$ and $(TCAT)_{10}$ polymorphisms are cardioprotective, because the carriers have a lower haemodynamic response to the stress stimulus, contrary

to $(TCAT)_7$ (Zhang et al., 2004). However, the stratification of the probands in our study for $(TCAT)_6 + (TCAT)_{10}$ vs. $(TCAT)_7$ genotypes did not reveal any differences between the groups, nor were we able to find any association of the microsatellite polymorphism of *TH* with the presence of either DD or MetS.

Conclusion

In conclusion, we found that the genotype MetMet of the Val66Met polymorphism of the *BDNF* gene was positively associated with depressive disorder. The finding is in concordance with the present hypothesis that *BDNF* genotyping could significantly contribute to the possibility of prediction of many neurologic and psychiatric diseases. The association may probably be caused by the fact that *BDNF* gene Val66Met polymorphism influences the BDNF expression and/or activity. The limitation of this pilot study lies in the small size of samples. No associations were found between MetS and Val66Met polymorphism of the *BDNF* gene.

In the study we were not able to find any significant associations of both depressive disorder and metabolic syndrome with the remaining polymorphisms studied (*MTHFR* 677CT, *MTHFR* 1298AC, *eNOS* Glu298Asp and *TH*). It is evident from the literature that the individual gene polymorphisms have quite different distribution among different ethnic populations and can also have different associations with the studied disturbances.

We suppose that large-scale studies on the association of the individual polymorphisms with depressive disorder and/or metabolic syndrome in specific populations are needed to elucidate the hypothetical links.

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