ASSOCIATION OF *EGF*, *IGFBP-3* AND *TP53* GENE POLYMORPHISMS WITH MAJOR DEPRESSIVE DISORDER IN SLOVAK POPULATION

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SUMMARY

Background: Major depressive disorder (MDD) is a main public health concern worldwide. Despite extensive investigations, the exact mechanisms responsible for MDD have not been identified. Epidermal growth factor (EGF) and insulin growth factor binding protein-3 (IGFBP-3) are involved in brain function. Tumour suppressor protein p53 is widely involved in neuronal death in response to different forms of acute insults and neurological disorders. The present study focuses on the possible associations of the single-nucleotide polymorphisms (SNP) of *EGF* A61G (rs4444903), *IGFBP-3* C32G (rs2854746) and *TP53* G72C (rs1042522) genes with MDD risk in the Slovak population.

Methods: The present case-control association study was carried out in 111 confirmed MDD patients and 207 healthy subjects. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism methods.

Results: Logistic regression analysis showed no association between SNPs of selected genes and MDD risk in the Slovak population. However, the stratification of individuals by gender revealed that males carrying *IGFBP-3* G alleles (G32G or GG) had marginally increased risk for developing MDD as compared to CC homozygous males ($p = 0.09$). In women, inverse association was observed between SNP rs1042522 and MDD risk ($p = 0.04$ for recessive model).

Conclusion: Our results suggest the protective effect of minor allele 72C of *TP53* gene towards MDD. The disruption of mechanisms involved in cell survival and death regulation may be involved in pathophysiology of MDD.

Key words: major depressive disorder, EGF, IGFBP-3, p53, SNP, Slovak population

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INTRODUCTION

Neuropsychiatric disorders are the second cause of disability-adjusted life years (DALYs) in Europe (19%), with only 4% after cardiovascular disorders. It is estimated that 27% of the adult (18–65 years of age) EU population (including Iceland, Norway and Switzerland) suffer from at least one mental disorder (1) and almost every second person in the EU is or has been affected by mental disorders at some point in the lifetime. There is a significant gender gap concerning the depression as it is approximately twice as prevalent in women as it is in men in all EU countries. Even so, some of the Eastern and Northern European countries (e.g. Ireland, Slovakia and some Nordic countries) show bigger gender differences (2). Moreover, depression is substantially more common in patients with medical illness than it is in general population. For example, approximately 10% to 20% of patients with acute cardiac disease, diabetes, renal failure, or cancer also suffer from major depressive disorder (MDD) (3). However, assessing whether a medication has in fact caused depression or whether the relationship is coincidental can be challenging.

MDD is a subtype of unipolar depressive disorders that are the third cause of DALYs (5.6%). Genetic factors are one of the most important in the development of MDD, as indicated by family, twin and adoption studies (4). Since for the clinicians is difficult to collect samples from the central nervous system (CNS) environment, developing readily available biomarkers in peripheral tissues such as blood, serum or plasma provide a potential road of research. Accordingly, it is plausible that identifying susceptibility genes may eventually lead to targeted "cure therapeutics" (5), giving impulse to identifying the underlying susceptibility genes and genetic factors associated with MDD.

Role of Growth Factors in Development of MDD

One of the pathophysiological hypotheses states that abnormal neurogenesis may result in a malfunctioning hippocampus and decrease in the production of newborn dentate granule cells contributing to the deficits of cognitive, emotional and behavioural functioning observed in depressed patients and patients with neurodegenerative disorders such as Alzheimer's disease. Conversely, enhanced neurogenesis is beneficial and viewed necessary for the antidepressant treatment in rodent models (6). Several neurotrophic/growth factors affect the neurogenesis, including brain-derived neurotrophic factor (BDNF), fibroblast growth factor-2 (FGF-2), epidermal growth factor (EGF), insulin-like growth factor 1 (IGF-1), and neuropeptide vascular endothelial growth factor (7). Functional studies demonstrate that stress-induced changes in levels of these neurotrophic/growth factors result in consequences in behavioural models of depression (8, 9).

Insulin-like Growth Factor Binding Protein-3

Insulin-like growth factors are a large family of insulin related peptides including IGF-1 and IGF-2 as well as their cell surface receptors (IGF-1R and IGF-2R), insulin like growth factors binding proteins (IGFBP-1-6), IGFBP proteases and several other IGFBP-interacting molecules which all regulate cell proliferation, differentiation and apoptosis (10). IGF-1 may increase the synthesis and activity of BDNF, and both factors are required to enhance neuronal survival and plasticity in the brain. IGF-1 exhibits multiple neurotrophic, neurogenic and neuroprotective actions. IGF-1 is mainly produced in the CNS and peripheral tissues such as liver. Moreover, it can penetrate through blood-brain barrier (BBB), which is crucial for the development of biomarkers for MDD or bipolar disorder (BD). In addition, IGF-1 can mediate antidepressant effect in MDD patients and its levels in the cerebrospinal fluid have been found to vary with antidepressant treatment (11). Currently, several studies have provided evidence about higher serum IGF-1 levels in MDD or bipolar disorder patients than in controls (11–14). More than 90% of the circulating IGF-1 is bound to IGFBP-3, which regulates the biological activity and availability of IGF-1. IGFBP-3 (OMIM #146732) is a multifunctional protein that is found to play a variety of roles in regulating cell proliferation and apoptosis (15). Mature deglycosylated human IGFBP-3 has a molecular weight of 28.7 kDa and comprises 264 amino acids. Using *in vitro* culture system of rat neural progenitor cells and animal models, the potential regulation role of IGFBP-3 in neurogenesis was demonstrated (16, 17). IGFBP-3 expression was increased after brain insults and upregulated in the brains of patients with Alzheimer's disease (18). Meanwhile, two genetic variants were mainly reported to link to IGFBP-3 with effect on the circulating level of IGFBP-3. One is a promoter SNP located at position -202 (rs2854744) resulting in a reduced promotor activity and decreased IGFBP-3 levels (19, 20). The other one is non-synonymous substitution of Gly to Ala at codon 32; Gly32Ala (rs2854746) (21), located in the region responsible for IGF-1 binding (22). Because of the biological significance of the *IGFBP-3* gene and a lack of studies of the effect of polymorphisms in this gene on MDD risk, we aimed to determine whether the *IGFBP-3* Gly32Ala polymorphism is associated with MDD risk.

Epidermal Growth Factor

The epidermal growth factor (OMIM #121530) is a small protein (6045 Da) and consists of 53 amino acid residues (23). The *EGF* gene, located at 4q25-27, encodes a ligand EGF for the epidermal growth factor receptor (EGFR) on the cell membrane that triggers the intrinsic protein tyrosine kinase activity and activates a series of intracellular signaling networks (24). On the basis of the neurotrophin hypothesis, EGF signal regulates the development of dopaminergic neurons and monoamine metabolism (25, 26). Previous studies indicated an association of MDD as well as schizophrenia with EGF abnormal or dysfunctional expression (27, 28). Schizophrenia has been shown to be associated with the functional SNP in the 5´ untranslated region of the *EGF* A61G (rs4444903) promoting *EGF* transcription (29, 30). Besides, the substitution of guanine (G) for adenine (A) at position 61 increased the serum level of EGF in cultured peripheral blood mononuclear cells (31) and thus *EGF* genotypes may be involved in the modulation of midbrain dopaminergic neurons development. Due to the essential role of EGF in the development of the brain and especially in the development of the dopaminergic neurotransmission, we tested the hypothesis that functional *EGF* A61G polymorphism might contribute to the development of MDD.

Role of Tumour Suppressor Gene *TP53*

p53 (OMIM #1911170) is a nuclear phosphoprotein involved in DNA repair, cell cycle progression, and apoptosis (32). It has been reported that p53 is involved in an early stage of brain development and might constitute a candidate susceptibility gene for mental as well as neurodegenerative disorders and its neurocognitive deficits (33). Additionally, increased apoptosis in frontal cortex and smaller brain tissue volumes, decreased neuropil and fewer neuronal progenitor cells observed in patients suffering from MDD (34) include apoptosis in the aetiology of disease. Several polymorphisms have been identified within *TP53* gene, both in noncoding and coding regions (35). The best studied human *TP53* apoptosis-associated polymorphism G72C (rs1042522) arises from a single-base-pair substitution at codon 72, where either CCC encodes proline instead CGC encodes arginine (hereinafter designated as Arg72Pro) (36). Codon 72 is in exon 4 in the segment of *TP53* that encodes the polyproline domain which has been shown to be important for p53 function, especially for its ability to induce apoptosis (37). The both variants exhibit different abilities to activate gene expression, since Arg72 variant induces apoptosis and suppresses transformation more efficiently than the Pro72 variant (36). It has been recently established that this polymorphism impacts the apoptotic function of p53 in a tissue- and age-specific manner along with ethnicity-specific genetic background and environmental exposure (24, 38).

In the present study we investigate the possible association between the independent *TP53* Arg72Pro, *EGF* A61G and *IGFBP-3* G32C gene polymorphisms and MDD risk in the Slovak population.

MATERIALS AND METHODS

Study Population

The case group comprised 111 patients (from 19–82 years, median 51), 61.3% were women with average age 50.9 ± 11.6

(from 19–82 years, median 53) and 38.7% were men with average age 47.3 ± 10.9 (from 21–71 years, median 49), suffering from MDD with single or recurrent episode. Diagnoses were established according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) and the ICD-10 Classification of Mental and Behavioural Disorders (ICD-10) criteria. Two independent expert psychiatrists interviewed all of the patients. Diagnosis of bipolar disorder led to exclusion from the study. For the complexity of diagnosis a rating scale (MADRS score) was used too. Patients with primary depression were hospitalized from four to seven weeks at the Psychiatric Clinic of University Hospital Martin (Slovakia) after the failure of ambulance treatment. The control group comprised 207 healthy volunteers (median 56 years) who visited the general health check-up or medical and paramedical staff. The composition of the control group was comparable to the cases in terms of ethnicity (Caucasian – Central European only), age and gender (61.4% of women with average age 46.5 ± 13.7, median 48.5; and 38.6% of men with average age 55.6 ± 14.1, median 56). Exclusion criteria for controls: blood relatives who had been diagnosed with MDD. Inclusion criteria was age > 18. The present study was approved by the Ethical Board of Jessenius Faculty of Medicine, Comenius University and the written informed consent was obtained from all individuals prior to the study.

Genotype Analysis

Genomic DNA was isolated from peripheral leukocytes by proteinase K digestion (Applichem, Germany), phenol/chloroform extraction and ethanol precipitation, dissolved in TE buffer (pH = 7.5) and stored at −20°C until genotype analysis.

Genomic DNA (100 ng) was amplified in a total volume of 25 μl reaction mixture containing 25 pmol of the either exon 1 *IGFBP-3* gene sequence primers, (forward 5'-TTCCTGCCTGGATTCCACAGCTT-3' and reverse 5'-GGCACTAGCGTTGACGCAGA-3') or exon 4 TP53 (forward 5'-TTGCCGTCCCAAGCAATGGATGA-3' and reverse 5'-TCTGGGAAGGGACAGAAGATGAC-3') or *EGF* gene sequence primers (forward 5'-TGTCACTAAAGGAAAGGAGGT-3' and reverse 5'-TTCACAGAGTTTAACAGCCC-3', VBC-Biotech, Austria), and RedTaq ReadyMix PCR reaction mix (20 mM Tris-HCl, pH 8.3, 100 mM KCl, 3 mM $MgCl_2$, 0.002% gelatin, 0.4 mM dNTP mix, stabilizers, and 0.06 unit/μl of Taq DNA Polymerase, Sigma-Aldrich, USA). In case of *IGFBP-3*, samples were initially denaturated for 5 min at 96°C, then 35 cycles were performed for 30 sec at 96°C (denaturation), for 45 sec at 60°C (annealing) and for 60 sec at 72°C (extension), followed by a final step for 5 min at 72°C. In case of *TP53* reaction mixtures were preincubated for 5 min at 94°C, 35 cycles were performed for 40 sec at 94°C, for 30 sec at 68°C and extension for 40 sec at 72°C, followed by a final step of 10 min at 72°C. The PCR products were digested with 2 units of *Ava1* in case of *IGFBP-3* or 5 units of *BstUI* in case of *TP53* (New England, Biolabs) at 37°C for 2 hours. After digestion, the fragments were electrophoresed on 2% agarose gel and visualized by UV light after ethidium bromide staining. Thus, the G allele of *IGFBP-3* was identified by the presence of a 187 bp and 263 bp fragments and C allele by single 450 bp fragment (22). *IGFBP-3* was evaluated with individuals being categorized as having a *C32C*, *G32C*, or *G32G* genotype. In case of *TP53*, the proline allele was identified by the presence of a single fragment of 199 bp and the arginine allele by two fragments of 113 bp and 86 bp, respectively. Heterozygous (Arg72Pro) samples displayed all three fragments of 199 bp, 113 bp and 86 bp. Genotyping of *EGF* was done by PCR-RFLP as described previously (31). Briefly, the reaction mixtures were preincubated at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 57°C for 1 min, 72°C for 1 min and a final extension step at 72°C for 10 min. The *EGF* amplification product of the size 242 bp was digested with 5 units of *Alu1* (New England, Biolabs) at 37°C for 16 hours. Restriction enzyme digestion products G61G (193 bp, 34 bp, and 15 bp), A61A (102 bp, 91 bp, 34 bp, and 15 bp), and A61G (193 bp, 102bp, 91 bp, 34 bp, and 15 bp) were analyzed using the Shimadzu MCE-202 MultiNA microchip technology and MultiNA Viewer software (Shimadzu Corporation, Kyoto, Japan). As a quality control, 10–20% of all samples were repeated as blinded duplicates.

Statistical Analysis

The Mann Whitney test was used to assess the differences between the frequency distribution of variables in cases and controls. Hardy-Weinberg equilibrium was tested using a goodness-of-fit χ^2 test with one degree of freedom to compare observed genotype frequencies with expected genotype frequencies among the subjects. Odds ratios (OR), 95% confidence intervals (CI) for OR and χ^2 test were used to test frequencies of genotypes/allele in MDD patients and controls. ORs were calculated for all genetic models: codominant, dominant (A/G + A/A vs. G/G), recessive (G/G + A/G vs. A/A), additive (A/G vs. G/G + AA). Logistic regression was used that treats disease status as the outcome variable and genotype according to genetic model as an explanatory variable. Logistic models were adjusted for age and gender, if it was possible. The statistical programs Microsoft Excel and GraphPad Instat version 3.00 for Windows 95, GraphPad Software, San Diego California USA were used. Logistic regression was done using SNP and Variation Suite v8 (39). All tests were two-sided and considered significant if $p < 0.05$.

RESULTS

Study Population

In total, 111 cases and 207 controls of Slovak Europeans were included in this analysis. Mann Whitney statistics tested no significant differences between age distributions in case and control study groups ($p > 0.05$). Statistical analysis did not reveal difference related to gender distribution between the groups investigated as well ($x^2 = 0.0003$, $p = 0.99$).

Prevalence of *IGFBP-3* G32C Genotypes in MDD Patients

No significant deviation from expected genotype frequencies under Hardy-Weinberg equilibrium was observed in the control group. However, *IGFBP-3* genotype distribution of the men control group was not in agreement with Hardy-Weinberg equilibrium ($\chi^2 = 4.77$, $p < 0.05$). Table 1 shows the frequencies of *IGFBP-3* genotypes in MDD patients and control group. Fisher test and χ^2

Table 1. *Frequency distribution of IGFBP-3 C32G, EGF A61G or TP53 Arg72Pro polymorphisms adjusted for gender and age*

Genotypes	Cases (N = 111)		Controls (N = 207)		OR (95% CI)	p value
	n	%	n	%		
IGFBP-3 C32G						
CC	40	36.04	76	36.71	1.00	
GC	53	47.75	105	50.72	0.95 (0.58–1.60)	0.97
GG	18	16.22	26	12.56	1.30 (0.65–2.70)	0.50
GC + GG vs CC	71	63.96	131	63.29	0.99 (0.61–1.61)	0.97
GC vs CC + GG	58	52.25	102	49.28	1.10 (0.78–1.57)	0.57
CC + GC vs GG	93	83,78	181	87.44	1.48 (0.76–2.88)	0.25
EGF A61G						
AA	41	36.94	85	41.06	1.00	
AG	48	43.24	90	43.48	1.10 (0.70–1.80)	0.70
GG	22	19.82	32	15.46	1.40 (0.70–2.75)	0.30
AG + GG vs AA	70	63.06	122	58.94	1.17 (0.72–1.91)	0.52
AG vs AA + GG	63	56.76	117	56.52	1.12 (0.81–1.55)	0.50
AA + AG vs GG	89	80.18	175	84.54	1.16 (0.62–2.15)	0.65
p53 Arg72Pro (C→G)						
CC	59	53.15	111	53.62	1.00	
CG	41	36.94	80	38.65	0.96 (0.60–1.60)	0.90
GG	6	5.41	16	7.73	0.70 (0.26–1.90)	0.50
CG + GG vs CC	47	42.34	96	46.38	0.87 (0.54–1.40)	0.57
CG vs CC + GG	65	58.56	127	61.35	0.86 (0.58–1.26)	0.43
CC + GC vs GG	100	90.09	191	92.27	0.67 (0.25–1.79)	0.42

test showed no significant association between *IGFBP-3* G32C gene polymorphism and MDD risk. However, subgroup analysis by gender has revealed marginally significant increased risk of MDD in men patients in a dominant model (p = 0.09) (Table 2) suggesting that *IGFBP-3* GG genotype might increase MDD risk in men whereas this genotype may be a potential protective factor in women with diagnosed MDD (Table 3).

Prevalence of *EGF* A61G Genotypes in MDD Patients

Genotype frequencies of A61G polymorphism of *EGF* gene are listed in Table 1. Subgroup analysis by gender has shown that the genotype *EGF* G61G as well as allele G was associated with non significantly increased risk of MDD in women (Table 3). In contrast, G allele and *EGF* G61G genotype has shown to be protective regarding the MDD risk in men (Table 2).

Prevalence of *TP53* Arg72Pro Genotypes in MDD Patients

Genotype frequences of *TP53* Arg72Pro polymorphism (Table 1) indicate that individuals homozygous for the Arg allele have higher frequency than other alleles and that MDD may be related to Arg allele frequency. Logistic regression analysis showed no significant association of *TP53* Arg72Pro genotypes with MDD after adjustment for gender and age. However, an inverse association between Pro72 (C) variant allele of *TP53* gene and MDD risk (p = 0.04) was revealed by using recessive model in women (Table 3).

DISCUSSION

Since neutrophins and neurodegeneration appear to play critical role in the pathophysiology of MDD, we investigated the contribution of the *IGFBP-3* G32C, *EGF* A61G and *TP53* Arg72Pro polymorphisms to MDD risk in the Slovak population. This is the first study showing a possible association of the above mentioned genetic polymorphic variants with MDD risk.

Due to ability of IGF-1 to influence many processes such as adult neurogenesis, synaptic plasticity, neuromodulation, myelination and remyelination, it has been suggested that disturbances in the IGF-1 system are involved in the development of affective disorders (40). Besides, IGF-1 level was shown to be elevated in patients with MDD (11, 40) suggesting that peripheral IGF-1 levels may be a disease trait marker or indicator of cognition. Although, it has been estimated that up to 60% of the variance in IGF-1 serum level has a genetic basis (41), results of studies that evaluated the relationship between the IGF-1 polymorphisms and IGF-1 levels are contradictory (42). Recent results on healthy middle-aged Caucasian individuals confirm the association between IGF-1 polymorphisms and IGF-1 levels but found no association between IGF-1 polymorphisms and IGF-1 levels with cognitive functioning (n = 698) (42). IGFBP-3, an

Table 2. *Frequency distribution of selected polymorphisms between men cases and controls and its association with risk of MDD adjusted for age*

Genotypes	Cases (N = 43)		Controls (N = 80)		OR (95% CI)	p value
	n	%	n	%		
IGFBP-3 C32G						
CC	15	34.88	28	35.00	1.00	
GC	19	44.19	46	57.50	0.80 (0.34–1.76)	0.50
GG	9	20.93	6	7.50	2.80 (0.84–9.40)	0.10
GC + GG vs CC	28	65.12	52	65.00	0.74 (0.32–1.68)	0.47
CG vs CC + GG	24	55.81	34	42.50	1.10 (0.60–2.03)	0.76
CC+GC vs GG	34	79.07	74	92.50	2.76 (0.81–9.37)	0.09
EGF A61G						
AA	18	41.86	28	35.00	1.00	
AG	16	37.21	36	45.00	0.70 (0.30–1.60)	0.40
GG	9	20.93	16	20.00	0.88 (0.32–2.40)	0.80
AG + GG vs AA	25	58.14	52	65.00	0.87 (0.39–1.95)	0.74
AG vs AA + GG	27	62.79	44	55.00	0.87 (0.52–1.47)	0.61
AA + AG vs GG	34	79.07	64	80.00	0.75 (0.28–2.03)	0.57
p53 Arg72Pro (C→G)						
CC	21	48.84	43	53.75	1.00	
CG	15	34.88	32	40.00	0.95 (0.40–2.20)	0.90
GG	5	11.63	5	6.25	2.05 (0.50–7.80)	0.30
CG + GG vs CC	20	46.51	37	46.25	0.97 (0.44–2.14)	0.94
CG vs CC + GG	26	60.47	48	60.00	1.06 (0.58–1.95)	0.85
CC + CG vs GG	36	83.72	75	93.75	1.48 (0.38–5.83)	0.58

important regulator of cell responsiveness to IGF-1, is the most abundant IGFBP protein in the bloodstream and is expressed at a low level in the CNS, mainly in nonneuronal structures including epithelial cells. Associations with circulating IGFBP-3 have been previously evaluated with cardiovascular disease, diabetes and cancer (43). Contradictory results were published regarding the association between the IGFBP-3 level and cognitive changes. Duron et al. (44) found that low IGF-1 and IGFBP-3 serum levels were significantly associated with age-related cognitive changes and development of dementia in men with Alzheimer's disease (n = 694). In another study of men (n = 746), similar results were found regarding the relation between IGFBP-3 and IGF-2 but not IGF-1 and cognitive aging and development of dementia (45). On the contrary, Johansson et al. (46) in a small cross-sectional study of patients found increased serum but not cerebrospinal fluid levels of IGF-1 and IGFBP-3 in patients with AD (n = 60). In German epidemiologic KORA-Age study (47), IGFBP-3 was positively associated with well-being in women and less so in men whereas IGF-1 was positively correlated with depression and negatively with well-being (n = 985). Here, opposite and independent associations of IGF-1 and IGFBP-3 on well-being particularly in women suggest for the neuroprotective effects of IGFBP-3 in cognitive aging. Previous studies have shown that genetic factors are important determinants of circulating IGFBP-3 levels. Five *IGFBP-3* SNPs (rs3110697, rs2854747, rs2854746, rs2854744, rs2132570) were highly correlated with IGFBP-3 level in the multiethnic cohort study including white men and women (n = 826) (48). They observed consistently lower levels of IGFBP-3 in the presence of the minor allele across five racial/ethnic groups. Because IGFBP-3 is the principal binding protein of circulating IGF-1, lower levels of IGFBP-3 due to genetic variation may increase the bioavialability of IGF-1 and bioactivity of IGF-1 in circulation and tissues. In the study of African American and Caucasian women, the *IGFBP-3* SNP rs2854746 (Ala32Gly) was shown to be associated with plasma IGFBP-3 (49). In the present study we investigated the correlation between rs2854746 polymorphism of *IGFBP-3* gene and MDD risk. We did find no association between the *IGFBP-3* SNP rs2584746 and MDD risk. However, some gender differences were noted when the variant genotype *IGFBP-3* G32G was associated with a marginally significant increased risk of MDD in men (p = 0.1) but not in women. The question remains whether this gender difference may be the effect of sex hormones. Although sex hormones cannot change DNA sequence, it is known they can be potent modifiers of epigenetic status and gene expression (50). However, due to the nature of hospital-based case control study design and deviations from Hardy-Weinberg equilibrium in men, a potential selection bias should be taken into consideration when interpreting this result. Here, using hospital-based controls as well as medical and paramedical staff could generate Berkson bias which might influence the frequencies of I*GFBP-3* genotypes and the susceptibility to MDD risk. Therefore, this result requires validation in a further

Table 3. *Frequency distribution of selected polymorphisms between women cases and controls and its association with risk of MDD adjusted for age*

Genotypes	Cases (N = 68)		Controls (N = 127)		OR (95% CI)	p value
	n	%	n	%		
IGFBP-3 C32G						
CC	25	36.76	48	37.80	1.00	
GC	34	50.00	59	46.46	1.10 (0.58–2.10)	0.80
GG	9	13.24	20	15.75	0.86 (0.34–2.17)	0.80
GC + GG vs CC	43	63.24	79	62.20	1.03 (0.55–1.94)	0.92
CG vs CC + GG	34	50.00	68	53.54	0.97 (0.62–1.51)	0.88
CC+GC vs GG	59	86.76	107	84.25	0.83 (0.34–1.98)	0.67
EGF A61G						
AA	23	33.82	57	44.88	1.00	
AG	32	47.06	54	42.52	1.50 (0.80–2.80)	0.30
GG	13	19.12	16	12.60	2.00 (0.82–4.80)	0.10
AG + GG vs AA	45	66.18	70	55.12	1.54 (0.80–2.84)	0.21
AG vs AA + GG	36	52.94	73	57.48	1.31 (0.84–2.02)	0.23
AA + AG vs GG	55	80.88	111	87.40	1.30 (0.56–3.01)	0.54
p53 Arg72Pro (C→G)						
CC	38	55.88	68	53.54	1.00	
CG	26	38.24	48	37.80	0.97 (0.50–1.80)	0.90
GG	1	1.47	11	8.66	0.16 (0.02–1.31)	0.10
CG + GG vs CC	27	39.71	59	46.46	0.79 (0.42–1.46)	0.45
CG vs CC + GG	39	57.35	79	62.20	0.70 (0.42–1.18)	0.18
CC + CG vs GG	64	94.12	116	91.34	0.18 (0.02–1.42)	0.04

cohort. Furthemore, it remains to be determined whether this genetic variant may influence circulating *IGFBP-3* level in MDD patients particularly with regard to different gender. Taking into account our results and previous findings we suggest that defect in *IGFBP-3* may be involved in the development of MDD and we presume that gender specific expression of *IGFBP-3* may enhance the risk for MDD among male patient with the G allele of rs2854746 but not for female with the G allele.

Concerning the *EGF* A61G polymorphism, there were no significant differences in genotype or allele frequencies between the patient and control groups, suggesting that this polymorphism does not affect the development of MDD. Previous studies in patients with schizophrenia showed similar results (29, 51, 52). However, we observed some gender differences. The genotype *EGF* G61G was associated with marginally significant increased risk of MDD in women ($p = 0.10$) whereas negative association was found in men. Previously, Lee et al. (30), in Korean study revealed that male patients with early onset schizophrenia ($n = 190$ patients) were more likely to exhibit the common A61A homozygote than male patients with adulthood onset schizophrenia suggesting that AA genotype might play a disease-modifying role differentially according to gender. In contrary, Anttila et al. (53) ($n = 94$) and Hänninen et al. (29) ($n = 149$) in Finnish studies found *EGF* G61G genotype to be associated with schizophrenia in male patients. No association was found between treatment response and *EGF* polymorphism. According to the study of Tian et al. (27) ($n = 463$ patients) among a Chinese population, the EGF levels in plasma were significantly lower in the patients with MDD than in the control group ($p < 0.001$) showing EGF as a possible biomarker for the early diagnosis, treatment and prognosis of MDD. *EGF* promoter polymorphisms were previously observed to modulate EGF levels and thought to have effect on susceptibility to various carcinomas but the results are contradictory (24). Thus, it will be compulsive to investigate effect of *EGF* A61G polymorphism on modulating EGF level in MDD patients.

Apoptosis, which is mainly under the control of the nuclear phophoprotein p53, has long been considered one of the likely factors in the aetiology of dysregulation process in the CNS of patients suffering from mood disorders including MDD (54). Distributions of *TP53* Arg72Pro genotypes in our control group are almost identical to those reported for the European white, comparable in ethnicity and latitude (24, 55, 56). We found a preferential loss of the 72Pro allele in MDD patients. Inverse association of homozygous *TP53* -Pro72 genotype with decreased risk of MDD was shown in women (OR = 0.18; 95% CI: 0.02–1.42; $p = 0.04$) in a recessive model. The Arg72 variant of *TP53*, but not Pro72, has been described *in vitro* in primary cultured neurons to have a higher capacity to trigger neuronal apoptosis (57). Furthemore, the Arg72 but not Pro72 was shown to be localized in the mitochondria leading to cytochrome c release from the mitochondria to the cytosol and indicating the mechanism of apoptosis as mitochondrial (intrinsic) apoptotic pathway

(57). Recent literature data at least in cultured cell systems show long term effects of lithium on the apoptosis induced by various stimuli, Bcl-2 up regulation and p53 and Bax down regulation and suggest possible association between the action of lithium and mood stabilization (58). It should be interesting to investigate possible association between *TP53* Arg72Pro polymorphism and degree of prophylactic response to lithium in patients with MDD.

Major limitations of this study are that it has low power to detect significant associations and that the confounding factors such as smoking, alcohol consumption, dietary nutritional habits, and other environmental impacts have not been analyzed in this study, as detailed information could not be obtained from clinical records.

Moreover, a comprehensive epigenetic analysis of candidate SNPs is desirable as could be shed new light on the gender differences observed in our association study.

In conclusion, our study provides evidence that particularly *TP53* Arg72Pro polymorphism may contribute to the depression aetiology in the Slovak population. The potential future role of *EGF* A61G and *IGFBP-3* G32C may lie in their ability to identify patients at risk for developing MDD. However, novel correlations require verification in other large patient populations with MDD, particularly in other races/ethnics, since our population was overwhelmingly Caucasian.

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Conflict of Interests

None declared

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