

Research Paper

Genetic Analysis of *BDNF*, *GNB3*, *MTHFR*, *ACE* and *APOE* Variants in Major and Recurrent Depressive Disorders in Russia

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Abstract

This study was conducted to explore the possibility of association between the single-nucleotide polymorphisms rs6264 of *BDNF*, rs5443 of *GNB3*, and rs1801133 of *MTHFR*; the *In/Del* polymorphism of *ACE*; and the $\epsilon 2$ allele of *APOE* and major depressive disorder (MDD) and recurrent depressive disorder (RDD) in an East Slavic population. Generalized multifactor dimensionality reduction (GMDR) method was applied to detect gene–gene interactions. One hundred fifty patients with RDD (101 females and 49 males) and 208 patients with MDD (115 females and 93 males) were included in the study. The comparison group consisted of 200 unrelated individuals. There was no significant difference in genotype distributions or allele frequencies between the controls and any of the diagnostic groups. Nevertheless, the frequency of the G allele of rs1801133 of *MTHFR* was higher in the RDD group and the frequency of the C allele of rs6264 of *BDNF* was higher in the MDD group. The difference between the controls and specific disease groups almost reached statistical significance ($P = 0.08$). A GMDR did not reveal optimal two- and three-dimensional models with significant prediction accuracies ($P > 0.05$) for the MDD or RDD groups.

Key words: *BDNF*, *GNB3*, *MTHFR*, *ACE* and *APOE*

1. Introduction

Major depressive disorder (MDD) is a mental disorder with a high incidence. Its most severe presentations can lead to the death of the patient as a result of suicide. The World Health Organization (WHO) predicts that MDD will be the second leading cause of disability by 2020 [1, 2]. Clinically, MDD is a highly heterogeneous disorder, both in its psychopathological manifestations and in the current of illness and responses to therapeutic effects. According to one of the classifications, ICD-10, two large clinical groups were identified based on the severity of the depressive symptoms: a depressive episode (F.32) and recurrent depressive disorder (F.33).

Numerous data stemming from clinical, physiological, and genetic studies indicate that MDD is a complex, multifactorial disorder, and that the predisposition to it is determined by the action of many genes and their interaction with each other and a variety of environmental factors. Twin studies and, albeit to a lesser extent, studies of adopted children suggest that genetic factors play an important role in the development of depression. Thus, according to the large meta-analysis of family and twin studies of MDD conducted by Sullivan et al. (2000), the heritability of MDD was estimated to be 37% (95% confidence interval 31%–42%) [3], although certain subsets of MDD may be more heritable (e.g., recurrent

and an early-onset depression) [4].

Since the publication of the first study of candidate genes for MDD published in 1978 [5], a significant number of case-control studies have been carried out worldwide to identify genes that are involved in the development of this disease. However, despite these efforts, no specific loci that predispose to the disease have been identified, and the precise mechanism underlying its pathogenesis remains unknown. Lo'pez-Leo'n et al. performed a large review of MDD case-control genetic association studies and a meta-analysis for polymorphisms that had been investigated in at least three studies. The final analysis included 20 polymorphisms in 18 genes, for which pooled odds ratios (ORs) with 95% confidence intervals (CIs) have been calculated. Statistically significant associations were found for *APOE e2* (OR, 0.51), *GNB3* 825T (OR, 1.38) (rs5443), *MTHFR* 677T (OR, 1.20) (rs1801133), *SLC6A4* 44 bp Ins/Del S (OR, 1.11) alleles and for the *SLC6A3* 40 bpVNTR 9/10 genotype (OR, 2.06) [6].

Because association studies of ethnic components will play an important role in the determination of whether the loci mentioned above contribute to the pathogenesis of the disease in the Slavic population, it is necessary to conduct a study of replication samples of specific ethnic backgrounds. This study was conducted to search for associations of polymorphisms that showed a high degree of association with MDD in the study of Lo'pez-Leo'n et al. (2008): rs5443 (*GNB3*), rs1801133 (*MTHFR*), rs429358 and rs7412 (*APOE*) and recurrent depression and MDD in the Slavic population. Moreover, the analysis included rs6264 of the *BDNF* gene and insertion/deletion (In/Del) polymorphism in the *ACE* gene.

2. Materials and methods

2.1. Subjects

This study was performed using two groups of patients. The first group consisted of 208 unrelated patients with depressive disorder who met ICD-10 classification system criteria for major depressive disorder (93 men (44.7%) and 115 women (55.3%); mean age, 32.7 ± 7.9 years). Henceforth, this group will be called "patients with major depressive disorder" (MDD). The inclusion criteria were as follows: age of onset between 18 and 45 years; East Slavic origin; and an established diagnosis of "moderate depressive episode" (F 32.1) and "mixed anxiety and depressive disorder" (F 41.2) within the ICD-10. The exclusion criteria were as follows: schizophrenia; alcoholism and drug addiction in the patient's life history; serious neurological diseases

(stroke, Parkinson's disease, dementia, epilepsy, etc.); and severe somatic diseases (oncology).

The second group consisted of 150 unrelated patients with "recurrent depressive disorder" (49 men (32.7%) and 101 women (67.3%); mean age, 34.2 ± 7.3 years). Henceforth, this group will be called "patients with recurrent depressive disorder" (RDD). The criteria used for the inclusion of patients in this study were: age of onset between 18 and 45 years; East Slavic origin; and an established diagnosis of "recurrent depressive disorder, current episode moderate" (F 33.1). The exclusion criteria were the same as those used for the first group. A survey of patients and blood samples was carried out at the Moscow Research and Clinical Center for Neuropsychiatry of the Healthcare Department of Moscow. The control group (N=200) was a sample of the East Slavic population of the city of Moscow and the regions of Central Russia, with a comparable sex and age structure to that of the sample of patients (99 men (49.5%) and 101 women (50.5%); mean age, 36 ± 8.2 years). Informed consent was obtained from all patients. All blood samples were collected with the informed consent of the investigated persons. The Ethics Committee of the Institute of Molecular Genetics approved the study.

2.2. DNA isolation and genotyping

Genomic DNA was obtained from 250 μ L of EDTA- anticoagulated venous blood using innuPREP Blood DNA Mini Kit (Analytik Jena AG, Germany), according to the manufacturer's recommendations. For SNP genotyping, we used a set of reagents from TaqMan® SNP Genotyping Assay (Applied Biosystems, USA): C__11592758_10 (rs6264), C__2184734_10 (rs5443), C__1202883_20 (rs1801133), C__3084793_20 (rs429358), and C__904973_10 (rs7412). The In/Del *ACE* gene polymorphism was determined as described previously [7, 8].

2.3. Statistical analysis

The frequencies of genotypes and alleles were calculated using GraphPad InStat3 (<http://www.graphpad.com/>), STATISTICA (data analysis software system, version 7, <http://www.statsoft.com>). The strength of associations between the studied polymorphisms and MDD and RDD was estimated using odds ratios (ORs), with the corresponding 95% confidence intervals (95% CIs). Hardy-Weinberg equilibrium calculator software (<http://www.oege.org/software/hardy-weinberg.html>) was used to calculate the correspondence of the genotype distribution in the population sample to the Hardy-Weinberg equilibrium. A χ^2 test was used to

determine the difference between the frequencies of the genotypes and alleles in the control group and patients with different types of depressive disorder. Differences were considered significant at $P < 0.05$. The assessment of associations of *APOE* genotypes were performed using by 5-degree-of-freedom tests.

The SNP-SNP interactions was conducted using the GMDR software (Generalized multifactor dimensionality reduction), (<http://www.ssg.uab.edu/gmdr/>) [9]. This method provides a number of output parameters, including cross-validation consistency, the testing balanced accuracy, and the sign test, for all the selected attributes. The cross-validation consistency score is a measure of the degree of consistency with which the selected interaction is identified as the best model among all the possibilities. To assess interactions between genes using this method, the GMDR comprehensive search algorithm (exhaustive search algorithm) was applied, which evaluated all the possible combinations of DNA markers with respect to the risk of MDD and RDD. The testing balanced accuracy is a measure of the degree to which the interaction accurately predicts case-control status with scores between 0.50 and 1.00 and a sign test used to measure the significance of an identified model.

3. Results

In the control samples, polymorphisms rs6264 (*BDNF*), rs5443 (*GNB3*), and rs1801133 (*MTHFR*), and In/Del polymorphism of *ACE* were in the Hardy-Weinberg equilibrium (HWE) ($p=0.84$, $p=0.7$, $p=0.67$, and $p=0.51$ by χ^2 test, respectively). Table. 1 shows the results of the genotyping of polymorphic loci rs6264 (*BDNF*), rs5443 (*GNB3*), rs1801133 (*MTHFR*), and In/Del polymorphism of the *ACE* gene in patients with MDD and RDD and in the control group. The genotype and allele distributions of *GNB3* rs5443 were not significantly different between MDD cases and the control group, or between RDD cases and the control group. No significant associations between MDD and *BDNF* rs6264, *MTHFR* rs1801133, and *ACE* In/Del genotypes were discovered. However, the C allele frequency of *BDNF* rs6264 showed a tendency to be increased in patients with MDD (88.2%) compared with the control group (83.8%). In the group of patients with RDD, the frequency of the G allele polymorphic variant of *MTHFR* (rs1801133) was higher than it was in the control group ($p = 0.08$). Thus, despite the results of the abovementioned studies of the effect of polymorphisms in the *BDNF*, *GNB3*, and *MTHFR* genes on the risk of MDD (and of RDD in particular) we did not find statistically significant allelic associations between *BDNF* rs6264 and MDD and between the G allele polymorphic variant of *MTHFR* (rs1801133) and RDD ($P = 0.08$).

Table 1. Genotype and allele frequency of *BDNF* (rs6264), *GNB3* (rs5443), *MTHFR* (rs1801133), and *ACE* In/Del polymorphisms in major and recurrent depression.

Genotype/ Allele	Control, (Genotype/allelic frequency, %)	RDD cases, (Genotype/allelic frequency, %)	<i>P</i> value	MDD cases, (Genotype/allelic frequency, %)	<i>P</i> value
<i>BDNF</i> rs6264					
C/C	141 (70.5)	104 (69.3)	$\chi^2 = 1.3; P = 0.52$	161 (77.4)	$\chi^2 = 3.8; P = 0.15$
C/T	53 (26.5)	44 (29.3)		45 (21.6)	
T/T	6 (3)	2 (1.4)	$\chi^2 = 0.008; P = 0.93$	2 (1)	$\chi^2 = 3.03; P = 0.08$
C	335 (83.8)	252 (84)		367 (88.2)	
T	65 (16.2)	48 (16)		49 (11.8)	
<i>MTHFR</i> (rs1801133)					
G/G	90 (45)	80 (53.3)	$\chi^2 = 3.37; P = 0.18$	91 (43.7)	$\chi^2 = 0.38; P = 0.83$
G/A	86 (43)	59 (39.3)		95 (45.7)	
A/A	24 (12)	11 (7.4)	$\chi^2 = 3.1; P = 0.08$	22 (10.6)	$\chi^2 = 0.0007; P = 0.98$
G	266 (66.5)	219 (73)		277 (66.6)	
A	134 (33.5)	81 (27)		139 (33.4)	
<i>GNB3</i> (rs5443)					
C/C	95 (47.5)	71 (47.3)	$\chi^2 = 0.33; P = 0.85$	99 (47.6)	$\chi^2 = 1.7; P = 0.43$
C/T	90 (45)	70 (46.7)		86 (41.3)	
T/T	15 (7.5)	9 (6)	$\chi^2 = 0.00; P = 1.0$	23 (11.1)	$\chi^2 = 0.21; P = 0.65$
C	280 (70)	212 (70.7)		284 (68.3)	
T	120 (30)	88 (29.3)		132 (31.7)	
<i>In/Del</i> polymorphism <i>ACE</i>					
I/I	52 (26.1)	49 (32.7)	$\chi^2 = 3.5; P = 0.17$	49 (23.6)	$\chi^2 = 2.2; P = 0.33$
I/D	98 (49.3)	59 (39.3)		94 (45.1)	
D/D	49 (24.6)	42 (28)	$\chi^2 = 0.11; P = 0.74$	65 (31.3)	$\chi^2 = 1.54; P = 0.21$
I	202 (50.8)	157 (52.3)		192 (46.2)	
D	196 (49.2)	143 (47.7)		224 (53.8)	

The results of the analysis of the genotype distribution at the polymorphic locus of the *APOE* gene are presented in Table 2. As in the control group and in patients with RDD, the $\epsilon 3$ allele was predominant (77.8% and 80.3%, respectively). The frequency of allele $\epsilon 2$, which showed an association with MDD in the meta-analysis of Lopez-Leon et al. [6], was only 6% in patients with MDD and 8% in the control group, whereas the frequency of allele $\epsilon 4$ was higher in both groups (13.7% in patients with MDD and 14.2% in the control group). However, the differences in the frequencies of occurrence observed between the two groups were not statistically significant for either a 5-degree-of-freedom test for *APOE* genotype ($P = 0.33$), or for the 2-degree-of-freedom test for alleles ($P = 0.56$).

In the group of patients with RDD, the $\epsilon 3$ allele was also predominant (80.2%) and its frequency coincided with that observed in patients with MDD. The frequency of the $\epsilon 2$ allele in this group was 1.1% higher than that recorded in the control group, and 3.1% higher than that detected in patients with MDD. The frequency of allele $\epsilon 4$, in contrast, was lower than that observed in the other groups (10.7%). Further analysis revealed an absence of association with the polymorphic locus in the *APOE* gene, based on a

5-degree-of-freedom test ($P = 0.17$), or a 2-degree-of-freedom test ($P = 0.26$).

A generalized multifactor dimensionality reduction (GMDR) method [9] was used to analyze the interaction between the five SNPs (rs6264, rs5443, rs1801133, rs429358, rs7412) and In/Del in the *ACE* gene. The following parameters were calculated: the cross-validation consistency, the testing balanced accuracy, the training balanced accuracy, and the sign test to assess the reliability of each model regarding intergenic interaction. In the process of simulation, a 10-fold cross-validation (CV) was established to search for the configuration model. Models with the lowest prediction error (the greatest testing balanced accuracy) and the highest CV consistency were selected as the best n-locus models. Significant data were considered a model of intergenic interaction ($P < 0.05$ in the sign test). The analysis identified the best two- and three-locus model intergenic interaction in a group of patients with MDD, as well as for patients with RDD (Table 3).

However, for all four models identified, the intergenic interaction prediction accuracy (testing balanced accuracy) does not exceed 60%, and the sign test P was >0.05 ; thus, the resulting model was not statistically significant.

Table 2. *APOE* genotype and allele distribution in controls, patients with MDD, and patients with RDD.

Genotype/ Allele	Control, (Genotype/allelic frequency, %)	RDD cases, (Genotype/allelic frequency, %)	P value	MDD cases, (Genotype/allelic frequency, %)	P value
$\epsilon 2/\epsilon 4$	6 (3)	5 (3.3)	$\chi^2 = 5.8; P = 0.33^*$	2 (1)	$\chi^2 = 7.7; P = 0.17^*$
$\epsilon 2/\epsilon 2$	1 (0.5)	1 (0.7)		5 (2.4)	
$\epsilon 2/\epsilon 3$	24 (12)	11 (7.3)		26 (12.5)	
$\epsilon 3/\epsilon 3$	125 (62.5)	98 (65.3)		135 (64.9)	
$\epsilon 3/\epsilon 4$	37 (18.5)	34 (22.7)		38 (18.2)	
$\epsilon 4/\epsilon 4$	7 (3.5)	1 (0.7)		2 (1)	
$\epsilon 2$	32 (8)	18 (6)	$\chi^2 = 1.15; P = 0.56^{**}$	38 (9.1)	$\chi^2 = 2.69; P = 0.26^{**}$
$\epsilon 3$	311 (77.8)	241 (80.3)		334 (80.2)	
$\epsilon 4$	57 (14.2)	41 (13.7)		44 (10.7)	

* – 5-degree-of-freedom test across all genotypes, ** – 2-degree-of-freedom test across all alleles.

Table 3. Best gene–gene interaction models, as identified by GMDR for RD and MDD.

Locus number	Best combination	Training Balanced Accuracy	Testing Balanced Accuracy	Sign Test (P)	Cross-validation Consistency
RDD patients					
2	rs5443 In/Del <i>ACE</i>	0.5748	0.4632	5 (0.6230)	4/10
3	rs6264 rs1801133 rs429358	0.6103	0.5016	6 (0.3770)	6/10
MDD patients					
2	rs1801133 In/Del <i>ACE</i>	0.5713	0.5347	6 (0.3770)	9/10
3	rs1801133 rs5443 In/Del <i>ACE</i>	0.6072	0.5256	6 (0.3770)	10/10

4. Discussion

To confirm the associations identified in the meta-analysis reported by Lopez-Leon et al. [6], we analyzed the possible association between the *BDNF* rs6264, *GNB3* rs5443, *MTHFR* rs1801133, and *ACE* In/Del polymorphisms and major depressive

disorder and recurrent depressive disorder. The study was conducted in two clinically well-defined samples: MDD and RDD. In our study, we found that the *BDNF* rs6264, *MTHFR* rs1801133, and *ACE* In/Del polymorphisms did not influence the development of MDD or RDD. In both samples, we found no statistical differences in allele and genotype

frequencies between patients and controls. However, it should be noted that there was a tendency toward an association between allele C of *BDNF* rs6264 and MDD and between the G allele polymorphic variant of *MTHFR* rs1801133 and RDD ($P = 0.08$).

Brain-derived neurotrophic factor (BDNF) is involved in the regulation of neuronal plasticity and in the survival of neurons. It is widely expressed in the adult mammalian brain [10]. There is increasing evidence that BDNF may play an important role in the pathogenesis of MDD and participate in the mechanism of antidepressant action. For example, a human postmortem study showed that BDNF immunoreactivity in the hippocampus is higher at death in patients with MDD treated with antidepressants than it is in those who did not receive such treatment [11]. Moreover, BDNF is involved in a wide range of neuropsychiatric and neurodegenerative disorders [12]. A number of studies have demonstrated decreased BDNF levels in major depression [13, 14] and bipolar disorder [15]. The *BDNF* rs6264 (Val66Met) polymorphism has been associated with various psychiatric illnesses, including bipolar disorder [16] and schizophrenia [17]. Despite the role of BDNF in the pathogenesis of confirmed clinical depression and the results of pharmacological studies in animal models, association studies are contradictory and mostly do not confirm the association between polymorphisms in this gene and the pathogenesis of MDD. Moreover, a meta-analysis [6] did not confirm this association. Further studies in larger samples of the same ethnic group, as well as the inclusion of additional polymorphisms in the analyses, are necessary to rule out definitively the potential role of BDNF in the pathogenesis of MDD, including RDD.

Methylenetetrahydrofolate reductase (*MTHFR*) is a crucial enzyme in one-carbon metabolism (OCM; a pathway that is essential for purine and thymidylate biosynthesis and DNA methylation and is necessary for reactions that generate neurotransmitters) [16]. *MTHFR* catalyzes the conversion of 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-MTHF, the prevailing circulating form of folate [18]. 5,10-MTHF is involved in DNA synthesis as a main donor molecule for the synthesis of purines and as a substrate for thymidine synthase [18, 19]. In the methylation cycle, the methyl group of 5-MTHF is used for the remethylation of homocysteine to methionine and the conversion of methionine to S-adenosylmethionine (SAM), which is the main donor of methyl groups to DNA, proteins, neurotransmitters, hormones, and phospholipids [18-20]. Abnormalities in the OCM cycle have been associated with neural tube defects [21] and with the

pathogenesis of various diseases, including cardiovascular diseases [22] and congenital abnormalities [23].

Folate is a cofactor in the metabolic pathway that leads to the synthesis of serotonin and other neurotransmitters. It decreases homocysteine levels, which seems to have an excitotoxic effect via Ca^{2+} glutamate brain receptors. Folate is also required for the biosynthesis of SAM, which, in its turn, has antidepressant properties [24-26].

A common polymorphism localized in 4 exon of the *MTHFR* gene – a substitution of C to T at position 677 (rs1801133) leads to the substitution of alanine to valine (Ala222Val), a catalytic protein domain, which in turn results in a thermolabile variant of this enzyme, which has approximately 30% of the wild-type enzyme's activity. *MTHFR* rs1801133 T/T homozygotes have been shown to have higher levels of homocysteine compared with C/C homozygotes; the levels in heterozygotes appear to be intermediate between the two [27]. Although we were unable to confirm the results of a meta-analysis [6], the lack of association between *MTHFR* polymorphism and MDD and RDD does not exclude the possibility that MDD or RDD is associated with alternative forms of a gene.

The APOE plasma protein plays a central role in the transport of cholesterol and other lipids between the liver and peripheral tissues [28]. The gene that encodes apolipoprotein E (*APOE*) is located on chromosome 19. There are three major isoforms of APOE, which are denoted as E2 (Cys 112, Cys 158), E3 (Cys 112, Arg 158), and E4 (Arg 112, Arg 158) [29]. These variants are encoded by alleles $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, respectively. In different populations, the frequency of the alleles of APOE differ significantly between populations. Thus, the frequency of the $\epsilon 4$ allele in Japanese and Chinese populations are much lower than that observed in Europeans. This fact must be considered when carrying out research based on a case-control approach, as the ethnic component can affect the final result. The study reported by Feng et al. (2015) [30] showed a significant association between ApoE gene polymorphism and susceptibility to depression in the Caucasian population ($\epsilon 2$ allele versus $\epsilon 3$: OR 0.75, 95% CI 0.58–0.97). In our study, there was no statistically significant difference between the control samples and patients with MDD ($\chi^2 = 7.7$, $df = 5$, $P = 0.17$) or patients with RDD ($\chi^2 = 5.8$, $df = 5$, $P = 0.33$) regarding the genotypes of *APOE*.

To analyze the interaction between the five SNPs (rs6264, rs5443, rs1801133, rs429358, rs7412) and the *ACE* In/Del polymorphisms), a GMDR method (Generalized multifactor dimensionality reduction) was used. Among patients with RDD the best of two-

and three-locus models of interaction of the studied genes were obtained. Thus, the best two-locus model was a model of the interaction between *GNB3* and *ACE*, although this model did not reach statistical significance ($P = 0.62$). It was shown previously that a combination of the risk allele rs5443 of *GNB3* and the D allele of *ACE* increases the risk of MDD several times [1, 31]. Perhaps an analysis of a larger sample of patients with RDD is needed to identify a more accurate model of the interaction of genes.

Thus, our results demonstrated again the need for replication studies of previously identified associations using samples of patients with a corresponding ethnicity. For analysis, polymorphisms in five genes (rs6264, rs5443, rs1801133, rs429358, rs7412, and In/Del in the *ACE* gene) were selected for which a high degree of association with MDD was identified previously. However, the study of patients of East Slavic origin from Russia regarding any of the analyzed polymorphisms showed no significant association with the development of the disease in patients in both clinical groups. One of the reasons why the present study could not confirm the results by Lopez-Leon et al. (2008) is the fact that depression is a heterogeneous phenotype. It is possible to meet different diagnostic criteria for a major depressive disorder in distinct studies (DSM-IV or DSM-5). These subtypes of MDD could reflect different genetic contributions. Another possible reason is ethnic diversity among the sample population. It is known that the frequency of genetic polymorphisms tends to vary between different ethnic groups. Disease associations with polymorphisms arise either because the polymorphisms themselves play a causal role or because they are in linkage disequilibrium with another causative variant. In addition, the degree of linkage disequilibrium between neighboring SNPs is also dependent on different factors including allelic frequency, therefore ethnic differences in linkage disequilibrium may arise. Finally, our study has investigated only one SNP per gene. Although we were unable to find genes that were significantly associated to MDD, there may still be other variants in these genes that are associated to MDD or RDD. In the future, the analysis of other genes and polymorphisms in patients with MDD from Russia, as well as the study of a larger number of different clinical forms of the disease, will help identify the genetic variants that influence both the risk of MDD and the current of illness.

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Conflicts of Interest

The authors indicated no potential conflicts of interest.

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