Association of affective disorders and *MTHFR*, *MTR*, and *MTRR* gene polymorphisms: preliminary results of a family study

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Genetic polymorphisms associated with impaired one-carbon metabolism (1-CM) can be a risk factor not only for somatic and neurological diseases, but also for affective disorders (AD).

Objective: *to compare the frequency of genetic polymorphisms MTHFR, MTR, MTRR associated with 1-CM disorders among patients with AD, their blood relatives and healthy individuals.*

Patients and methods. *This cross-sectional study of the frequency of genetic polymorphisms (MTHFR, MTR, MTRR) associated with 1-CM included patients with AD (n=24), their blood relatives (n=40), as well as a group of healthy individuals (n=35). All study participants underwent a structured diagnostic interview, as well as genetic analysis using real-time polymerase chain reaction.*

Results and discussion. *Patients with AD were statistically more likely to carry the minor allele C of the 1298A>C polymorphism of the MTHFR gene and the minor allele G of the 2756A>G polymorphism of the MTR gene compared to the group of healthy individuals. The minor allele T of the 677C>T polymorphism of the MTHFR gene was associated with longer depressive episodes, as well as with the presence of concomitant cardiovascular diseases in blood relatives of patients with AD.*

Conclusion. *Genetic polymorphisms associated with 1-CM may contribute to familial aggregation of AD and somatic diseases. Further high-quality family studies using molecular genetic methods are needed.*

Keywords: *depression; bipolar disorder; family research; one-carbon metabolism; MTHFR.*

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Affective disorders (ADs) – recurrent depressive disorder (RDD) and bipolar disorder (BD) – are multifactorial polygenic pathological conditions, which implies the involvement of many molecular mechanisms in the formation of their phenotype [1]. These disorders are often registered in different generations of the same family, which makes it possible to make an assumption about the genetic nature of the family aggregation of RDD and BD [2, 3].

The association of AD with various metabolic and immune disorders can be traced at the genetic and molecular level [4–6], which largely explains their high clinical comorbidity with somatic diseases: patients with ADs are at increased risk of obesity, coronary heart disease, hypertension, stroke, as well as an increased risk of death from cardiovascular diseases (CVD) [7, 8]. The reverse trend is also true – patients with somatic diseases have increased risks of ADs [9].

These observations suggest that the pathophysiology of RDD and BD has a multisystem character and is not limited to the nervous system [10]. Moreover, common genetic risk loci or common biological pathways may underlie pleiotropy between ADs and somatic diseases [5, 11, 12].

Based on data on the multisystem and polygenic nature of ADs, their high prevalence in the population and the tendency to aggregate in families, it is advisable to consider widespread genetic variants as potential links in the pathogenesis of RDD and BD, which can also accumulate in families and have a pleiotropic effect on several physiological systems, and not only on the nervous system [6]. Recently, increased attention has been given to various inherited metabolic disorders as potential links in the pathogenesis of ADs [13]. In comparison with more promising genome-wide studies, this approach may be more clinically relevant, since a large number of metabolic disorders can be compensated for with the use of certain nutrients [6]. Among the metabolic factors involved in the pathogenesis of ADs, disorders of single-carbon metabolism (1-CM) have been the most well-studied, including folate deficiency and elevated homocysteine levels which play an important role [14].

Single-carbon metabolism (1-CM) consists of several interconnected biochemical cycles, the main task of which is the transfer of methyl groups from one molecule to another. Due to such transmethylation reactions, nucleic acids, individual amino acids, phospholipids, neurotransmitters, and other biologically active compounds are synthesized [15]. Individual markers of 1-CM disorders (hyperhomocysteinemia, folate deficiency) have previously demonstrated an association with schizophrenia, BD, RDD, attention deficit and/or hyperactivity disorder and autism [16, 17]. In addition, a number of genetic variants associated with 1-CM disorders are also associated with ADs, which makes it possible to speak about their participation in the pathogenesis of RDD and BD and their role in the family aggregation of these dis-

orders [16]. At the same time, a targeted study of the indicators of 1-CM and their role in the development of ADs in families has not yet been conducted.

The pleiotropic effect of genetic variants associated with 1-CM disorders may help explain the high comorbidity of mental and somatic disorders. Thus, in a meta-analysis of genome-wide studies, the most studied genetic variant of 1-CM – *MTHFR*, associated with impaired folate metabolism and hyperhomocysteinemia – showed a genetic "overlap" with ADs, CVDs, and metabolic disorders [5]. It has previously been demonstrated that genetic variants associated with 1-CM disorders due to common biological pathways are also risk factors for stroke [18], essential hypertension [19], heart failure [20], aneurysm and dissection of the aorta, calcification of the coronary arteries [21], neurodegenerative diseases, vascular dementia [22], as well as pathologies of pregnancy and birth [23]. These data once again point to the biological determinacy of high compatibility of mental and somatic disorders.

The aim of the study was to compare the frequency of genetic polymorphisms (*MTHFR*, *MTR*, *MTRR* genes) associated with 1-CM among patients with ADs, their relatives and a group of healthy individuals, as well as to analyze their impact on the clinical characteristics of the disease.

Patients and methods. This cross-sectional study was aimed at studying the frequency of occurrence of polymorphic variants of the *MTHFR*, *MTR*, *MTRR* genes associated with 1-CM disorders among patients with ADs (n=24), their relatives (n=40) in comparison with a group of healthy individuals (n=35). Thus, the study groups included: patients over 18 years old who met the diagnostic criteria of the International Classification of Diseases of the 10th revision (ICD-10) for a depressive episode (DE) or RDD (F32 or F33), as well as BD (F31); relatives of probands with DE/RDD or BD of the first and second degrees of kinship over the age of 18; healthy persons over the age of 18 without a history of mental disorders.

Non-inclusion criteria for all study participants were: concomitant psychiatric diagnosis that met the criteria of ICD-10 (headings F00–09, F20–29, and F60–99); episodes of seizures in the anamnesis, except for cases of single simple febrile seizures at the age from 6 months to 5 years; the history of severe brain injury.

The exclusion criteria for all participants in the study were: the participantsX desire to stop participating in the study; participantsX inability to continue participating due to their aggressive behavior, as well as threats to their own lives or the lives of other people.

The scientific study was approved by the independent Ethics Committee of V.M. Bekhterev National Research Medical Center for Psychiatry and Nerology. The collection of material for the study was carried out on the basis of the Department of Translational Psychiatry in the period from 2016 to 2020. The data analysis was carried out in 2020–2021.

Each of the participants underwent a comprehensive examination according to a specially developed study map, which included standard sociodemographic and anthropometric data, information about the family history of mental disorders and somatic diseases, as well as various clinical characteristics of AD. At the stage of inclusion in the study, all participants (patients and their blood relatives) underwent a diagnostic examination using a semi-structured interview – MINI (Mini-International Neuropsychiatric Interview).

The sample size was calculated post factum for χ^2 criterion with the following criteria: effect size (ES) – 0.3, degrees of freedom (dF) – 1, significance level (p) – 0.05. Thus, in order to reach the threshold value of statistical power of 80%, it was enough to include 87 people in the comparison groups. In order to reduce the probability of making a type II error in cases of statistical power <80%, the size of the effects was additionally calculated according to Cramer's V criteria.

Table 1. *Description of genetic polymorphisms considered in this study (according to www.snpedia.com)*

Gene	Name of polymorphism	Encoded enzyme and main function
MTHFR	C677T, Ala222Val (rs1801133) – replacement of the cytosine nucleotide with thymine in the coding region of the gene, leading to the replacement of the amino acid alanine with valine in the protein A1298C, Glu429Ala (rs1801131) – replacement of the adenine nucleotide with cytosine in the coding region of the gene, leading to the replacement of glutamic acid with alanine in the protein	Methylenetetrahydrofolate reductase – conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate
MTR	A2756G, Asp919Gly (rs1805087) – replacement of the adenine nucleotide with guanine in the coding region of the gene, leading to the replacement of aspartic acid with glycine in the protein	Methionine synthase – remethylation of homocysteine to methionine
MTRR	A66G, Ile22Met (rs1801394) – replacement of the adenine nucleotide with guanine in the coding region of the gene, leading to the replacement of isoleucine with methionine in the protein	Methionine synthase reductase – methylation of methionine synthase

Genetic analysis. For long-term storage of biomaterial – blood – modern methods of its stabilization were applied. Deoxyribonucleic acid (DNA) was isolated from the venous blood by phenol-chloroform extraction. Molecular diagnostics of alleles of genetic polymorphisms (Table 1) was carried out by polymerase chain reaction (PCR) with allele-specific primers ("SNP-express-RV") and subsequent detection in real time. The test system "DNA-Express-blood" was used to isolate DNA from leukocytes. To determine the alleles of single-nucleotide polymorphisms, kits manufactured by NPF Litech (Moscow, Russia) were used. PCR analysis was performed using programmable amplifiers with a "real-time" fluorescence signal detection system – Rotor-Gene3000/6000 (CorbettResearch, Australia), Rotor-GeneQ (Qiagen, Germany).

Statistical analysis. Statistical analysis and data visualization were carried out in the R programming language

(version 4.0.2) in RStudio v1.4.1717. The arithmetic mean and standard deviation – M (σ), as well as median and interquartile range – Me [25th; 75th percentile] were used as measures of the central trend. Categorical variables were described as absolute values with percentages – n (%). The deviation of the genotype frequency from the Hardy–Weinberg equilibrium was evaluated using the criterion χ^2. The distribution of the quantitative variables was determined according to the Kolmogorov–Smirnov criterion. In order to identify statistical differences in the distribution of genotypes and alleles of the polymorphic locus in the studied subgroups, as well as comparisons by demographic and clinical characteristics, Pearson's criterion χ^2 was used, as well as Fisher's two-sided exact criterion in the case of analyzing the tables with a small number of observations. When conducting multiple hypothesis testing, the Bonferroni correction was applied. Additionally, the size of the effects was calculated according to Cramer's V criteria, which were interpreted as follows: ES I0.2 – weak result, 0.2< ES I0.6 – moderate result, ES >0.6 – strong result. Binary logistic regression with various dependent and independent variables was used to determine risk factors. The value 0.05 was chosen as the critical significance level (p) at which the null hypothesis was rejected. The value 0.05< p <0.09 was taken as a 3trend towards significanceИ.

Results. Sixty-four study participants underwent genotyping, including 24 patients (probands) with diagnoses of RDD – 11 (45.8%) and BD – 13 (54.2%) and 40 their blood relatives, of whom: healthy – 20 (50%), with RDD – 11 (27.5%) and with BD – 9 (22.5%). The comparison group included 35 people with no history of mental disorders. Thus, the full final sample included 99 people. As can be seen from the descriptive characteristics in Table 2, patients and their relatives significantly differed in current age (p=0.02), but did not differ in sex ratio, and education status (p>0.05). In addition, when comparing patients with relatives with RDD or BD diagnoses, it was revealed that the first manifestation of AD occurred at an earlier age (p=0.03). No other statistically significant differences in the proportions of diagnoses or the duration of the longest DE in the groups of patients and relatives with ADs were revealed (p>0.05). There were also no statistically significant differences in the characteristics of the probands and the comparison group (p>0.05).

The frequency of occurrence of genotypes and allelic variants of polymorphisms 677C>T and 1298A>C of the methylenetetrahydrofolate reductase *(MTHFR)* gene, as well as polymorphism 2756A>G of the methionine synthase *(MTR)* gene and polymorphism 66A>G of the methionine synthase reductase *(MTRR)* gene in the studied groups is presented in Table. 3. The frequency distribution of these genetic polymorphisms in the studied groups corresponded to the Hardy–Weinberg equation (p>0.05).

Patients with ADs were statistically more likely to carry the minor allele C (genotypes AC and CC) of polymorphism 1298A>C of the *MTHFR* gene (χ^2=4.1, p=0.04; Cramer's V=0.3), as well as the minor allele G (genotypes AG and GG) of polymorphism 2756A>G of the *MTR* gene (χ^2=9.4, p=0.002; Cramer's V=0.4) compared with the group of healthy individuals. There were no differences between the minor allele T of polymorphism 677C>T of the *MTHFR* gene (χ^2=1.7; p=0.2; Cramer's V=0.2) and the minor allele G of polymorphism 66A>G of the *MTRR* gene (χ^2=0.4; p=0.5; Cramer's V=0.08) in these groups.

When comparing relatives of patients with the group of healthy individuals, there were no statistically significant differences in the carriage of minor alleles of the studied polymorphisms (p>0.05; see Table 3). The absence of statistical significance was also noted when comparing groups of patients and their relatives (p>0.05). At the same time, when

Table 2. *Characteristics of patients (probands), their blood relatives, including when stratified by the presence of AD, and comparison groups*

Characteristics	Patients (n=24)	Relatives (n=40)			Comparison Group (n=35)
		all	with AD (n=20)	without AD (n=20)	
Sex, n (%):					
female	17 (70,8)	23 (57,5)	14 (70)	9 (45)	21 (60)
male	7 (29,2)	17 (42,5)	6 (30)	11 (55)	14 (40)
Age, years, M (σ)	27,5 (10,3)	47,6 (16,6)*	44,7 (170)*	50,5 (16,1)*	26,2 (4,3)
Duration of education, years, M (σ)	14,2 (2,9)	11,2 (5,1)	10,1 (4,6)	12,3 (3,2)	14,4 (3,8)
Clinical characteristics					
Diagnosis, n (%):					
RDD	11 (45,8)	–	11 (55)	–	–
BD	13 (54,2)		9 (45)		
Age at onset, years, Me [25th; 75th percentile]	20 [16; 23]	–	23 [21,3; 27,5]*	–	–
Duration of the longest DE, month, Me [25th; 75th percentile]	8 [5; 12]	–	8,5 [6; 12]	–	–
Duration of the longest hypomania/mania episode, month, Me [25th; 75th percentile]	1 [0,5; 1,6]	–	1 [0,5; 1,4]	–	–

Note. The significance of the differences from the proband group: * – p<0.05; ** – p<0.01 (here and in Tables 3, 4).

comparing the genotypes of patients and their relatives, the former showed a tendency to statistical significance (χ^2=6; p=0.05; Cramer's V=0.3) with respect to more frequent carriage of the TT genotype of polymorphism 677C>T of the *MTHFR* gene.

When stratifying relatives of patients by the presence (n=20) or absence (n=20) of ADs (Table. 4) it was found that probands, in comparison with healthy relatives, are statistically more often carriers of the minor allele C of polymorphism 1298A>C of the *MTHFR* gene (χ^2=4.1; p=0.04; Cramer's V=0.3) and the minor allele G of polymorphism 2756A>G of the *MTR* gene (χ^2=4.1; p=0.04; Cramer's V=0.3). There was also a tendency to statistical significance in relation to more frequent carriage of the minor allele T of polymorphism 677C>T of the *MTHFR* gene (χ^2=3.8; p=0.05; Cramer's V=0.3).

At the same time, when comparing probands and their relatives with ADs, no statistically significant differences were revealed (p>0.05), as well as when comparing healthy relatives and persons in the comparison group (p>0.05). At the same time, relatives with ADs showed a tendency to significance (χ^2=3; p=0.08; Cramer's V=0.1) in terms of greater occurrence of the minor allele G of polymorphism 66A>G of the *MTRR* gene, as well as the minor allele G of polymorphism 2756 A>G of the *MTR* gene (χ^2=3.4; p=0.06; Cramer's V=0.2) compared with the group of healthy individuals.

A logistic regression model was used to assess the effect of the carriage of the minor allele T of polymorphism 677C>T of the *MTHFR* gene on the clinical course of AD. According to the results obtained, the carriage of the minor allele T of polymorphism 677C>T of the *MTHFR* gene is associated with longer DEs [p=0.01; odds ratio (OR) 1.3; 95% confidence interval (CI) 1.06–1.53], which may indirectly indicate a higher severity of the course of AD (see Figure). At the same time, there was no statistically significant association of polymorphisms linked with 1-CM with the age of AD manifestation and with the duration of hypomanic/manic episodes (p>0.05).

The carriage of the minor allele T of polymorphism 677C>T of the *MTHFR* gene in the relatives of patients with ADs (n=40) was associated with the presence of concomi-

Table 3. *The frequency of occurrence of genotypes and allelic variants of polymorphisms of the MTHFR, MTR and MTRR genes among patients with AD and their blood relatives, and the comparison group*

Gene	Polymorphism	Genotypes and alleles	Probands (n=24), n (%)	Relatives (n=40), n (%)	χ^2/ p-value	Cramer's V	Comparison Group (n=35), n (%)	vs probands χ^2/ p-value	vs probands Cramer's V	vs relatives χ^2/ p-value	vs relatives Cramer's V
MTHFR	677C>T	CC CT TT	11 (45,8) 8 (33,3) 5 (20,8)*	24 (60) 15 (37,5) 1 (2,5)*	**6/0,05***	0,3	22 (62,9) 12 (34,3) 1 (2,9)	3,9/0,1	0,2	0,09/0,9	0,3
		T in homo- and heterozygous forms C in homozygous form	13 (54,2) 11 (45,8)	16 (40) 24 (60)	1,2/0,3	0,1	13 (37,1) 22 (62,9)	1,7/0,2	0,2	0,06/0,8	0,02
	1298A>C	AA AC CC	6 (25) 15 (62,5) 3 (12,5)	19 (47,5) 18 (45) 3 (7,5)	3,2/0,2	0,2	18 (51,4) 12 (34,3) 14 (14,3)	4,9/0,084	0,3	1,4/0,5	0,1
		C in homo- and heterozygous forms A in homozygous form	18 (75) 6 (25)	21 (52,5) 19 (47,5)	3,2/0,07	0,2	17 (48,6) 18 (51,4)	**4,1/0,04**	**0,3**	0,1/0,7	0,04
MTR	2756A>G	AA AG GG	6 (25)* 15 (62,5)* 3 (12,5)*	19 (47,5) 19 (47,5) 2 (5)	3,7/0,2	0,2	23 (65,7)* 12 (34,3)* 0*	**11,6/0,003***	**0,4**	3,6/0,2	0,2
		G in homo- and heterozygous forms A in homozygous form	18 (75) 6 (25)	21 (52,5) 19 (47,5)	3,2/0,07	0,2	23 (65,7) 12 (34,3)	**9,4/0,002**	**0,4**	2,5/0,1	0,2
MTRR	66A>G	AA AG GG	7 (29,2) 7 (29,2) 10 (41,7)	10 (25) 20 (50) 10 (25)	2,9/0,3	0,2	13 (37,1) 18 (51,4) 4 (11,4)	5,7/0,057	0,3	2,7/0,3	0,2
		G in homo- and heterozygous forms A in homozygous form	17 (70,8) 7 (29,2)	30 (75) 10 (25)	0,1/0,7	0,04	22 (62,9) 13 (37,1)	0,4/0,5	0,08	1,2/0,3	0,1

Note. Here and in table 4 – significant differences are highlighted in bold.

tant CVD (p=0.007; OR 5.2; 95% CI 1.6–17.2). No such association was found for the patients with ADs (p>0.05), which can be explained, among other things, by a younger age and a complex CVD phenotype (hypertension, coronary heart disease, chronic heart failure and myocardial infarction).

Discussion. The first important observation of our study was that patients with ADs and a burdened family history were statistically significantly more likely to be carriers of the minor allele C of polymorphism 1298A>C of the *MTHFR* gene and the minor allele G of polymorphism 2756A>G of the *MTR* gene in comparison with the group of healthy individuals. At the same time, despite the fact that the minor allele T of polymorphism 677C>T of the *MTHFR* gene did not occur statistically significantly more often in patients, in comparison with the group of healthy individuals, it was associated with longer DEs, reflecting a more severe course of the underlying disorder.

To date, a lot of works have been accumulated that have demonstrated the important role of 1-CM disorders in the development of ADs such as RDD or BD [16]. However, most foreign studies have focused specifically on the study of polymorphisms of the *MTHFR* gene, for which about 14 common and rare variants have been discovered [24]. In general, the contribution to the development of RDD and BD was confirmed for polymorphism 1298A>C of the *MTHFR* gene [16. 25. 26-28], as well as polymorphism 677C>T of the *MTHFR* gene [16. 26. 28-30] both in large original studies in different countries and in several meta-analyses. However, there were no studies in the Russian population. For the polymorphism 2756A>G of the *MTR* gene, there are only a few studies in patients with ADs in samples consisting of elderly individuals and premenopausal women [31-33]. Another B12-vitamin-associated polymorphism of the *MTRR* – 66A>G gene was considered a predictor of the response to antidepressants of the class of selective serotonin reuptake inhibitors in DE/RDD at an older age in a sample of the white population of the USA [34]. At the same time, in a study on the Polish population, this polymorphism was not associated with BD (as were polymorphisms 1298A>C and 677C>T of the *MTHFR* gene) [35]. No other studies of 66A>G polymorphism of the *MTRR* gene in a sample of patients with ADs have been published in open sources.

Only one study with a family design was found, aimed at studying the role of genetic polymorphisms associated with 1-CM disorders in the development of AD (there are no studies

Table 4. *Prevalence of genetic polymorphisms (MTHFR, MTR, MTRR) associated with 1-CM among patients with AD and their blood relatives depending on the presence or absence of AD*

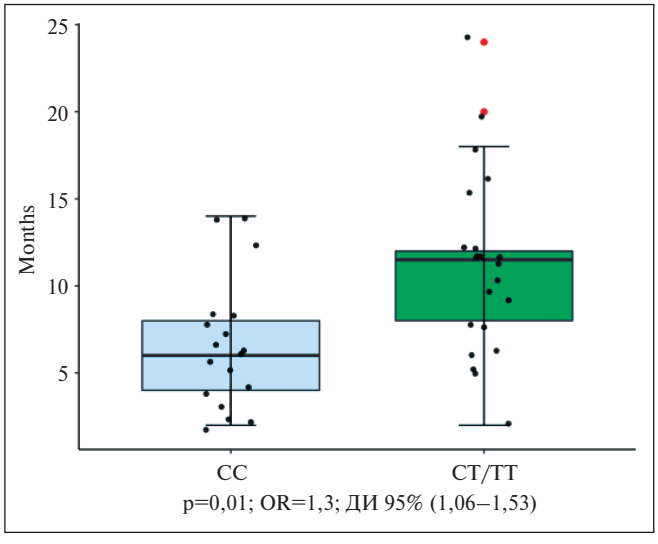
Gene	Polymorphism	Genotypes and alleles	Probands (n=24), n (%)	Healthy relatives (n=20), n (%)	χ2/ p-value	Cramer's V	Relatives with AD c AP (n=20), n (%)	χ2/ p-value	Cramer's V
MTHFR	677C>T	CC	11 (45,8)	15 (75)	4,3/0,1	0,3	9 (45)	5,4/0,07	0,4
		CT	8 (33,3)	4 (20)			11 (55)		
		TT	5 (20,8)	1 (5)			0		
		T in homo- and heterozygous forms	11 (45,8)	5 (25)	**3,8/0,05**	**0,3**	11 (55)	0,003/0,9	0,008
		C in homozygous form	13 (54,2)	15 (75)			9 (45)		
	1298A>C	AA	6 (25)	11 (55)	4,3/0,1	0,3	8 (40)	1,6/0,4	0,2
		AC	15 (62,5)	7 (35)			11 (55)		
		CC	3 (12,5)	2 (10)			1 (5)		
		C in homo- and heterozygous forms	18 (75)	9 (45)	**4,1/0,04**	**0,3**	12 (60)	1,1/0,3	0,3
		A in homozygous form	6 (25)	11 (55)			8 (40)		
MTR	2756A>G	AA	6 (25)	11 (55)	4,3/0,1	0,3	8 (40)	1,5/0,4	0,2
		AG	15 (62,5)	8 (40)			11 (55)		
		GG	3 (12,5)	1 (5)			1 (5)		
		G in homo- and heterozygous forms	18 (75)	9 (45)	**4,1/0,04**	**0,3**	12 (60)	1,1/0,3	0,1
		A in homozygous form	6 (25)	11 (55)			8 (40)		
MTRR	66A>G	AA	7 (29,2)	7 (35)	0,6/0,7	0,1	3 (15)	5,6/0,06	0,4
		AG	7 (29,2)	7 (35)			13 (65)		
		GG	10 (41,7)	6 (30)			4 (20)		
		G in homo- and heterozygous forms	17 (70,8)	13 (65)	0,1/0,6	0,06	17 (85)	1,3/0,3	0,2
		A in homozygous form	7 (29,2)	7 (35)			3 (15)		

for polymorphisms of the *MTR* and *MTRR* genes). In the work of Z. Ozbek et al. [36] there were no significant differences in the carriage of polymorphisms 1298A>C and 677C>T of the *MTHFR* gene among patients (probands) with BD, their blood relatives, and healthy controls. At the same time, in patients and their relatives who were carriers of the minor alleles of these *MTHFR* polymorphisms, the level of homocysteine was increased and the levels of folate and vitamin B_{12} were reduced, which, in turn, is a risk factor for the development of BD.

In addition, in our study the presence of the minor allele T of polymorphism 677C>T of the *MTHFR* gene in the relatives of patients was associated with the presence of concomitant CVDs, which also correlates with the results of recent meta-analyses on the role of polymorphism 677C>T of the *MTHFR* gene in the development of these diseases [16. 24].

The familial nature of ADs and 1-CM disorders associated with polymorphisms of the *MTHFR*, *MTRR* and *MTR* genes suggests the search for endophenotypes – measurable, quantitative specific biomarkers correlating with the disease due to common or maximally similar genetic mechanisms. Thus, the level of genetic influence on endophenotypes is higher and better amenable to statistical and logical analysis than heterogeneous clinical symptoms. From these positions, the following enzymes are the most genetically determined: methylenetetrahydrofolate reductase encoded by the *MTHFR* gene, methionine synthase reductase encoded by the *MTRR* gene, and methionine synthase encoded by the *MTR* gene. It is an decrease in the function of these enzymes due to polymorphisms of the genes described above that leads to a disorder of 1-CM and, as a consequence, an increase in homocysteine levels (which can only worsen with the exposure to exogenous factors).

A reasonable question is the possibility of using augmentation methods in patients with ADs and identified polymorphisms of the *MTHFR*, *MTR* and *MTRR* genes. At the same time, it should be borne in mind that such an approach should be used only after confirmation of biochemical deviations in the levels of homocysteine, folate, and vitamin B_{12} [36]. The choice of the form of folate for therapy is discussed in the scientific literature, but at the moment there are no recommendations based on evidence-based studies. Methylfolate (5-MTHF, 5-methyltetrahydrofolate, metafolin) is a biologically active form of folate, the closest to folate in natural foods, which easily penetrates the central nervous system (unlike synthetic folic acid and dihydrofolate in food products), does not require preliminary transformation in the liver using the enzyme dihydrofolate reductase, is immediately integrated into the folate exchange cycle, and, in addition, its further biochemical exchange (in particular, the supply of methyl groups to the homocysteine–methionine cycle) does not depend on the MTHFR enzyme, and therefore, allows to avoid transformation problems in carriers of polymorphism 677C>T of the *MTHFR* gene [37]. Therefore, methylfolate has been studied in a large number of studies of the effect of folates on mood.



The duration of the longest depressive episode depending on the carriage of the minor allele T of the 677C>T polymorphism of the MTHFR gene

This study has a number of limitations. Firstly, the study was not longitudinal, but had a cross-sectional design with a retrospective assessment of the course of the disease, which could also, to some extent, reduce the quality of data on the course of ADs in patients. Secondly, the study included patients from a large clinical center, which, in turn, could artificially overestimate the percentage of patients with a burdened family history due to their more severe course of AD and, consequently, more frequent medical treatment. Thirdly, insufficient statistical power could lead to a type II error (acceptance of the null hypothesis when it is false). Finally, the presence of a family history burdened by somatic diseases was assessed only on the basis of self-reports of patients and medical documentation of participants and was not confirmed clinically and instrumentally within the framework of this study.

Conclusion. When analyzing the frequency of polymorphic variants of the *MTHFR*, *MTR* and *MTRR* genes associated with 1-CM, it was revealed that patients with ADs and a burdened family history were statistically significantly more likely to be carriers of the minor allele C of polymorphism 1298A>C of the *MTHFR* gene and the minor allele G of polymorphism 2756A>G of the *MTR* gene compared with the group of healthy individuals. At the same time, despite the fact that the minor allele T of polymorphism 677C>T of the *MTHFR* gene did not occur statistically significantly more often in patients, in comparison with the group of healthy individuals, it was associated with longer DEs reflecting a more severe course of the underlying disorder. In addition, this allele was associated with the presence of concomitant CVD in relatives of probands with AD. Patients, in comparison with their healthy relatives, were statistically significantly more often carriers of the minor allele T of polymorphism 677C>T of the *MTHFR* gene, the minor allele C of polymorphism 128A>C of the *MTHFR* gene and the minor allele G of polymorphism 2756A>G of the *MTR* gene. At the same time, relatives with ADs showed a tendency to significance in terms of greater occurrence of the minor allele G of polymorphism 66A>G of the *MTRR* gene, as well as the minor allele G of polymorphism 2756A>G of the *MTR* gene in comparison with the group of healthy individuals.

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