The interaction of folate cycle enzyme genes and the risk of extrapyramidal side effects of antipsychotics

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Personalized medicine means the selection of therapy for patients, taking into account the assessment of genetic risk factors for side effects. A number of studies show that folate metabolism disorders, including single nucleotide polymorphisms (SNPs) in the genes of folate-metabolizing enzymes, are more frequently detected in schizophrenic patients than in the general population. The role of SNPs of the key folate cycle enzymes in developing the extrapyramidal side effects of antipsychotics has not yet been studied, although there is evidence of their association with other movement disorders.

Objective: *to analyze the association between the carriage of SNP alleles of MTHFR 677C>T, MTR 2756A>G, and MTRR 66A>G and the severity of extrapyramidal side effects of antipsychotics in patients with schizophrenia.*

Patients and methods. *The investigation included 61 patients with schizophrenia (according to the criteria for ICD-10 Code F20). All the patients took antipsychotics for at least 7 hospital days were examined using real-time polymerase chain reaction (PCR) with allele-specific primers, followed by detection for the carriage of SNP alleles of MTHFR 677C>T, MTR 2756A>G, and MTRR 66A>G. The standardized Simpson–Angus scale (SAS) was used to evaluate the severity of extrapyramidal symptoms; the PCR test results were unknown during their examination.*

Results and discussion. *In the patients carrying a low-functional 677 T allele in the gene of the key folate cycle enzyme MTHFR, the severity of extrapyramidal side effects of antipsychotics was statistically significantly higher than in the carriers of the wild-type genotype: 13.27±5.10 versus 9.84±6.03 SAS scores, respectively (t=-2.40; p=0.020). In addition, the carriage of the wild allele A of SNP in the MTRR 66A>G gene (F=3.83; p=0.0283; $p_{corr.}$=0.043) is associated with the severity of extrapyramidal symptoms. There was a direct moderate correlation of the number of risk alleles at two loci with the total SAS score (r=0.51; p=0.00017).*

Conclusion. *The polymorphic allele of MTHFR 677T and the wild allele of MTRR 66A can be regarded as risk alleles for the development of extrapyramidal side effects of antipsychotics.*

Keywords: *schizophrenia; antipsychotics; extrapyramidal side effects; folate metabolism disorders; tetrahydrobiopterin.*

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Currently, personalized medical treatment implies individualized selection of therapy for patients, considering the assessment of genetic risk factors related to the development of adverse effects [1].

According to the results of a number of studies, disorders of folate metabolism are detected more often in people with schizophrenia than in the general population: such disorders also include single nucleotide polymorphisms (SNP) in folate (one-carbon) metabolism genes. It has been demonstrated, in particular, that in patients with schizophrenia the minor T-allele of SNP methylenetetrahydrofolate reductase (MTHFR) 677C> T is present more often than in general population [2]. According to the results of a meta-analysis, the presence of this allele in its homozygous form increases the probability of schizophrenia development by 36%, compared with the wild gene pattern carrier state [3]. It has been demonstrated in several studies that carriage of the defective T-allele MTHFR 677C> T is associated with the severity of negative symptoms in schizophrenia [4]. In the case of this allele carriage, functional activity of the MTHFR enzyme decreases, whereas biochemical disorders develop (hyperhomocysteinemia, deficiency of tetrahydrobiopterin resynthesis and dopamine synthesis, methylation deficiency, etc.), which have a multimodal pathogenetic effect on various tissues and organs [5, 6].

Extrapyramidal neurological adverse effects are recorded to a greater extent with the use of traditional antipsychotics, but these can also be seen during treatment with atypical antipsychotics [7]. There have been no studies of the influence of one-carbon metabolism (deficiency of folate or genetic polymorphisms of the folate cycle enzymes) disorders on the development of neurolepsy symptoms during treatment with antipsychotics. However, the association of the carrier state of the defective allele of T-polymorphism MTHFR 677C> T with the catatonic [8] and negative schizophrenia symptoms [4] was previously identified. Besides, one cannot exclude the influence of extrapyramidal adverse effects of antipsychotic treatment on the assessment of negative and catatonic symptoms. The same structures, neuronal networks, and neurotransmitter systems of the brain which are involved in the development of catatonic symptoms (basal ganglia, cerebral cortex; dopamine, glutamate and GABA-ergic sys-

tems) may partly participate in the development of extrapyramidal neuroleptic disorders. Folate deficiency can lead to a deficiency in tetrahydrobiopterin resynthesis and dopamine synthesis, which may form the basis for motor disorders [9]. Hyperhomocysteinemia, which develops in folate deficiency, affects the glutamatergic system of the brain being also involved in the pathogenesis of movement disorders; moreover, homocysteine may demonstrate excitotoxicity and participate in oxidative stress, which is a risk factor for extrapyramidal disorders development [9]. It is known that pyridoxine, which decreases the level of homocysteine by turning the latter into other sulfur-containing amino acids, is used in treatment regimens for movement disorders associated with antipsychotics [10, 11], despite the fact that its efficacy was confirmed only in tardive dyskinesia in a single randomized placebo-controlled study [12]. Other group B vitamins (B_9, B_{12}), participating in one-carbon metabolism and utilization of homocysteine, have not yet been studied from the point of view of extrapyramidal neuroleptic disorders management.

Taking into account the above mentioned facts, one can make an assumption about the influence of disorders of one-carbon metabolism – carriage of the minor low-function alleles of genetic polymorphisms of folate cycle enzymes MTHFR 677C> T, methionine synthase (MTR) 2756A> G and methionine synthase reductase (MTRR) 66A>G on the risk of development of extrapyramidal adverse effects of antipsychotic treatment for schizophrenia.

The purpose of the study is to analyze the association between the carriage of SNP alleles of single-nucleotide genetic polymorphism MTHFR 677C> T, MTR 2756A> G, and MTRR 66A> G and the severity of extrapyramidal adverse effects of antipsychotics in patients with schizophrenia.

Patients and methods. The study enrolled patients (n = 61) complying with the following criteria: diagnosis of schizophrenia (F20) established in accordance with the 10th revision of the International Classification of Diseases (ICD-10); antipsychotics administration; inpatient treatment for at least 7 days; signing of a voluntary informed consent to participate in the study by the patient. The study protocol was approved by the local Ethics Committee No. 1 of the Federal State Budgetary Educational Institution of Higher Education «Privolzhsky Research Medical University» of the Ministry of Health of the Russian Federation.

All patients were examined for the carrier status of SNP alleles MTHFR 677C> T, MTR 2756A> G, and MTRR 66A> G using polymerase chain reaction (PCR) with allele-specific primers followed by real-time detection of these alleles.

During the pilot study, which enrolled 36 patients, the Simpson-Angus Scale (SAS) [13] and the Abnormal Involuntary Movement Scale (AIMS) designed to detect tardive dyskinesia [14]

Characteristics of the studied sample and indicators of severity of extrapyramidal adverse effects of antipsychotics in the groups of carriers of various allelic variants of the single nucleotide polymorphism MTHFR 677C> T

Indicator	Carriers of the T-allele (gene patterns: TT, CT; n = 30)	Carriers of the CC gene pattern (n = 31)	Significance level of differences
Gender: Female Male	 17 13	 19 12	χ^2=0,01; p=0,92
Patient's age, years, Me [25th; 75th percentile]	46 [31; 56]	50 [35; 58]	Z=0.46; p=0.64
Average equivalent aminazin dose of antipsychotics, mg, Me [25th; 75th percentile]	350 [170; 580]	325 [140; 660]	Z=0.12; p=0.91
Number of patients taking first-generation antipsychotics, n (%)	25 (83)	28 (90)	χ2=0.18; p=0.67
Number of patients taking correctors for extrapyramidal disorders, n (%)	14 (47)	12 (39)	χ2=0.14; p=0.71
SAS average score, M ± SD	13.27±5.10	9.84±6.03	t=-2.40; p=0.020

Note. The significance level of differences was assessed as follows: for qualitative characteristics – by using cross tables (χ^2 criterion with Yates' correction); for quantitative characteristics – by using the Mann – Whitney test (Z) or Two-sample t-test with different variances (two-tailed test).

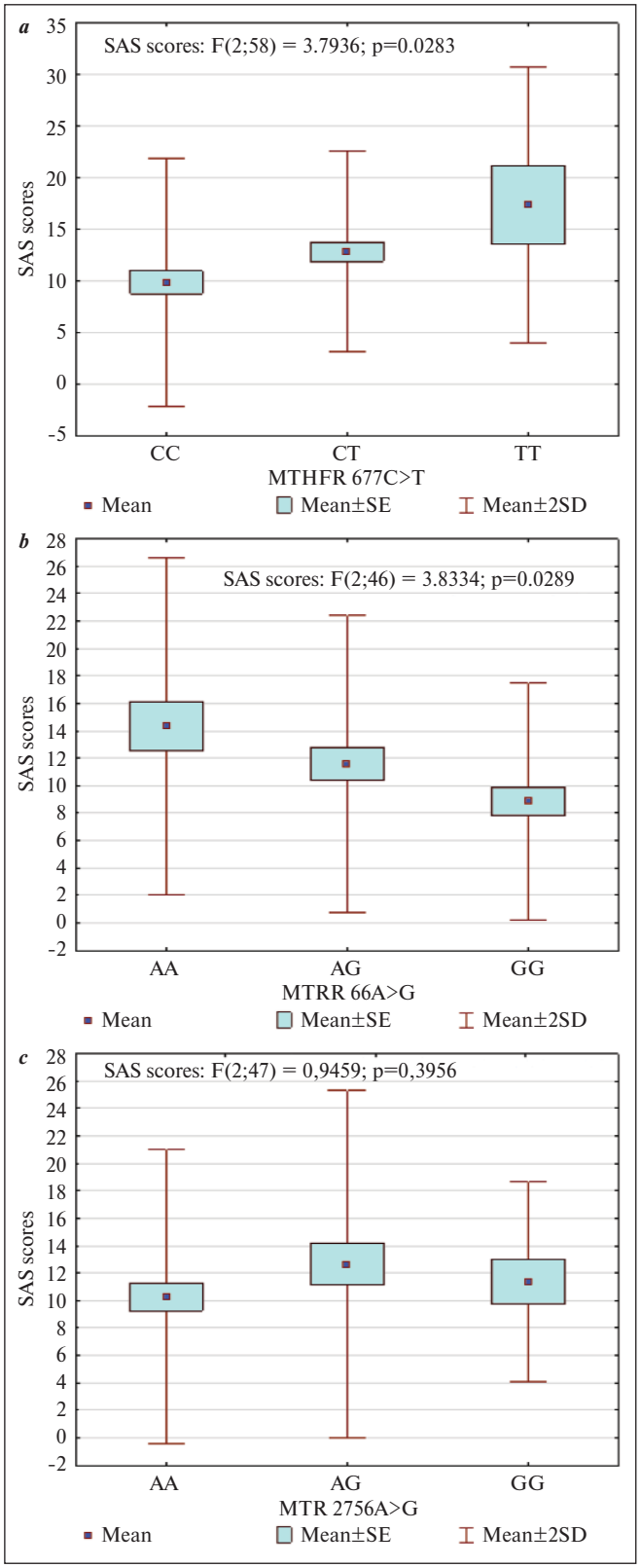

Fig. 1. *Results of examining patients with different genotypes of MTHFR 677C>T (a), MTRR 66A>G (b) and MTR 2756A>G (c), by using the Simpson-Angus Scale (SAS)*

were used to assess extrapyramidal symptoms. Considering that the trend in the differences between subgroups of carriers of the minor

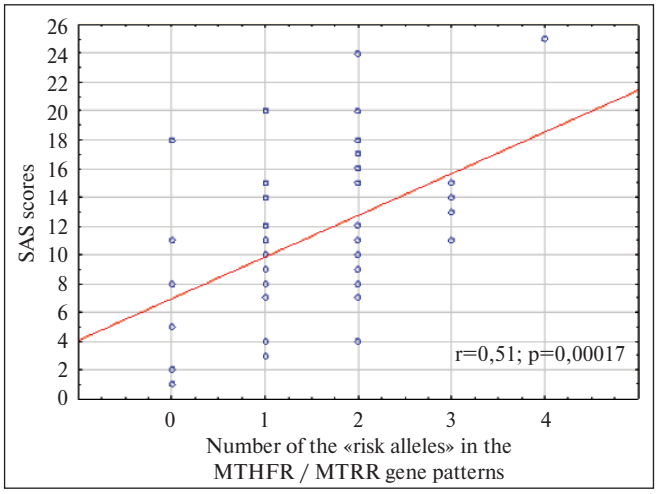

Fig. 2. *Patient survey scores (by the SAS) and the number of risk alleles (MTHFR 677T/MTRR 66A)*

T-allele of the key SNP of the folate cycle MTHFR 677C> T was identified only based on the results of the assessment with the SAS scale, whereas the results of the AIMS scale assessment in the subgroups were identical, and the severity of the symptom scores was minimal (0.9 points in patients with the wild MTHFR 677CC gene pattern and 0.92 points in patients with the minor MTHFR 677T allele), further use of the AIMS and the assessment of tardive dyskinesia in this study were considered unreasonable.

The genetic test results were not known to the researchers assessing movement disorders («blind research»). The inpatient medical records provided data on the medications received by the patients at the time of the examination (taking into account the prolonged injectable antipsychotics that were used within a month before the examination). For the convenience of medication doses comparison, the dose conversion was made in terms of the chlorpromazine equivalent.

Social and demographic characteristics of the studied sample are shown in the table below.

The normality of distribution was checked using Shapiro-Wilk W test. Two-sample t-test was used to process the obtained data (to compare two groups), one-way ANOVA test (to compare three groups) – in case of normal data distribution; Mann P Whitney test (to compare two groups) – in case of abnormal distribution of data (patient age: W = 0.95, p = 0.011; doses of antipsychotics in aminazin equivalent: W = 0.88, p = 0.00002). Cross tables were analyzed using χ^2 criterion with Yates' correction for continuity (Statistica 6.0, StatSoft Inc., USA). Benjamini P Hochberg correction was introduced for multiple comparisons. Spearman rank correlation coefficient was used to analyze correlations.

Results. As one can see from the table, the groups of carriers of the minor T-allele and the wild CC gene pattern of MTHFR 677C> T polymorphism were comparable in terms of gender and age; the patients in these groups received comparable doses of antipsychotics in aminazin equivalent, as well as "correctors" of extrapyramidal disorders and first generation antipsychotics in similar proportions. However, the average SAS score in the group of T-allele carriers was statistically significantly higher than in the comparison group.

As one can see in Figure 1 (a P c), the differences in the severity of extrapyramidal symptoms in carriers of different SNP gene patterns of MTHFR 677C> T and MTRR 66A> G are sta-

tistically significant (one-way ANOVA test). Moreover, it is shown that the carriage of the minor T-allele MTHFR 677C> T is associated not only with a greater severity of extrapyramidal disorders, but also with the wild (standard) A-allele MTRR 66A> G polymorphism. SNP MTR 2756A> G allele carriage is not associated with the severity of extrapyramidal symptoms. With the Benjamini-Hochberg correction for multiple testing (taking into account that three different polymorphism types were assessed), $p < 0.05$ was demonstrated only by the p-value for SNP MTRR 66A> G ($p = 0.085$ for MTHFR 677C> T; $p = 0.043$ for MTRR 66A> G; $p = 0.40$ for MTR 2756A> G).

Based on the results obtained and considering the MTHFR 677T and MTRR 66A alleles as "risk alleles" from the point of view of extrapyramidal adverse effects of antipsychotics development, the authors conducted post hoc analysis of the association of the total indicator of the carriage of these alleles in the patient's gene pattern (Figure 2); thus, the authors identified a direct moderate, statistically significant Spearman rank correlation between the number of the "risk alleles" at two loci with the severity of extrapyramidal symptoms (total SAS score): $r = 0.51$; $p = 0.00017$.

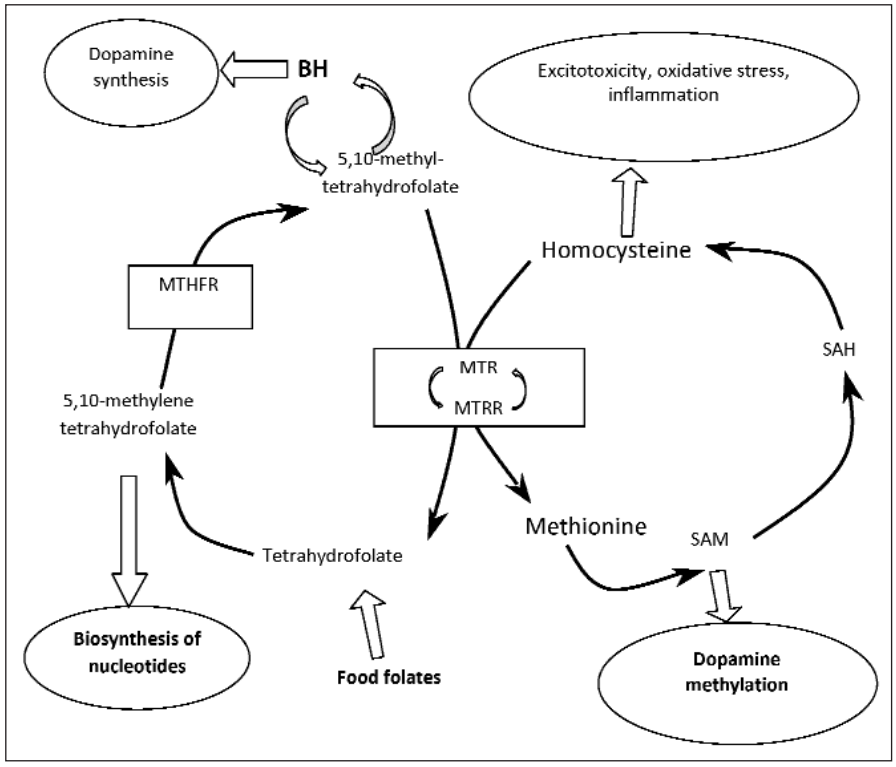

Fig. 3. *Basic processes of one-carbon exchange. BH4 – tetrahydrobiopterin; SAH – S-adenosyl homocysteine; SAM – S-adenosylmethionine*

Discussion. The obtained evidence of the association of the MTHFR 677C> T T-allele carriage with the total SAS score confirms the working assumption that folate metabolism disorders are associated with the severity of extrapyramidal adverse effects of antipsychotics. Extensive meta-analyses demonstrated that the carriage of the minor T-allele SNP MTHFR 6677C> T is associated with biochemical disorders in the folate metabolism cycle, such as 5-methyltetrahydrofolate deficiency and hyperhomocysteinemia [15]. This adds up to the earlier obtained data that the carriage of T-allele MTHFR 677C> T is associated with the development of secondary pharmacogenic negative symptoms of schizophrenia [16], and confirms that patients with folate metabolism disorders have more adverse effects (in particular, extrapyramidal symptoms).

Currently, meta-analyses provide convincing evidence that the MTHFR 677C> T polymorphism is associated with an increased risk of Parkinson's disease [17]. Apart from that, miscellaneous studies of the association of other SNP folate cycle enzymes with the development of Parkinson's disease are underway; moreover, molecular mechanisms have not been confirmed yet [18]. Taking into account common phenotypic manifestations, the mechanisms of the development of extrapyramidal symptoms in Parkinson's disease and medication-induced parkinsonism may be partly common. Hypothetically, folate metabolism disorders (deficiency in the synthesis of 5-methyltetrahydrofolate from its precursor using the MTHFR enzyme in carriers of the minor T-allele SNP MTHFR 677C> T) can affect the severity of extrapyramidal symptoms by reducing the resynthesis of tetrahydrobiopterin, the key cofactor in dopamine synthesis reactions (Figure 3). In addition, due to a decrease in the MTHFR function and a decrease in the level of 5-methyltetrahydrofolate, the transformation of homocysteine into methionine decelerates followed by development of hyperhomocysteinemia, which is involved in the development of oxidative stress; also, homocysteine and its derivatives are characterized by NMDA receptor activity, including excitotoxicity (see Figure 3).

However, according to literature data, the minor allele MTRR 66G also contributes to the development of hyperhomocysteinemia, its carriage results in a decrease in function of MTRR enzyme, which activates MTR, and MTR, in turn, converts (methylates) homocysteine into methionine [19]. According to this study data, the carriage of the wild A-allele (and not the low-function G-allele), is associated with the severity of extrapyramidal symptoms, which contradicts the assumption that hyperhomocysteinemia (as well ass the associated disorders) is involved in the development of extrapyramidal adverse effects of antipsychotics. Thus, the data obtained demonstrate a higher probability of 5-methyltetrahydrofolate (its deficiency) involvement in this process and BH4 resynthesis defects, as in the case of a biochemical loop in the folate cycle («methyl-trap») at the level of the MTHFR enzyme (see Figure 3), a decrease in the enzyme function at the next stage enables compensation of the 5-methyltetrahydrofolate level by slowing down its consumption for methylation within the homocysteine P methionine cycle. The normal functioning of the MTR and MTRR enzymes supports a faster consumption of 5-methyltetrahydrofolate for homocysteine remethylation, which results in a depletion of its pool for BH4 resynthesis. This assumption requires confirmation in a similar study with the assessment of biochemical parameters -such as the levels of BH4 and plasma homocysteine in carriers of different MTHFR 677C> T and MTRR 66A> G gene patterns. Furthermore, a sufficient synthesis of methionine enables better

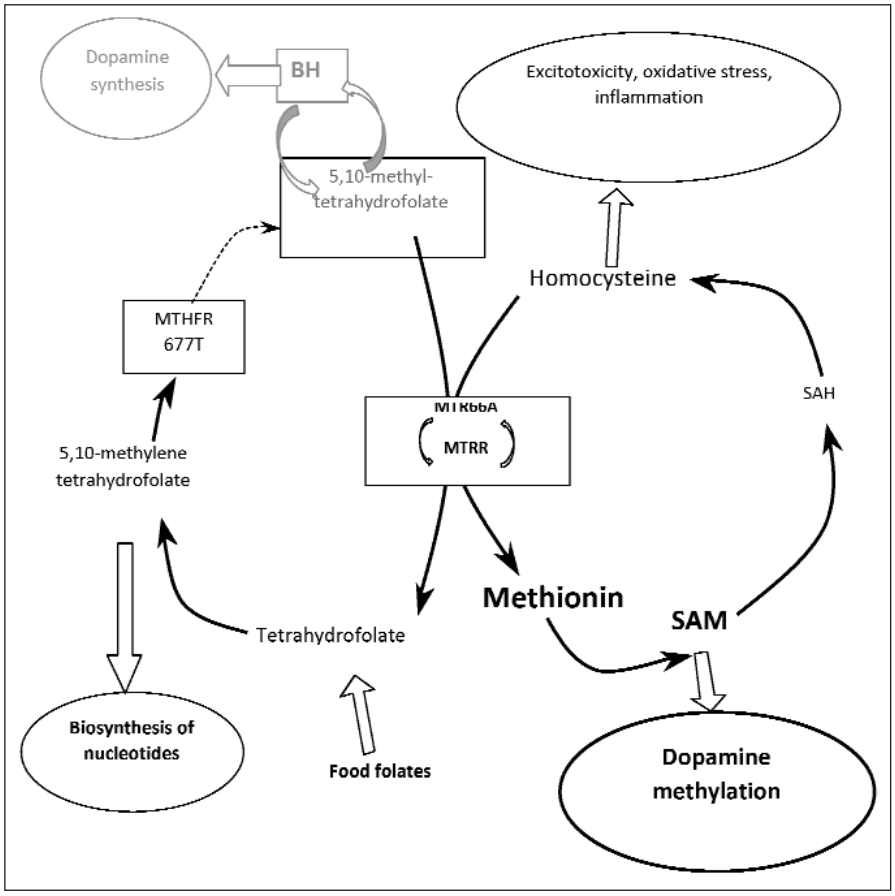

Fig. 4. *A model of biochemical disorders during carriage of MTHFR 677T and MTRR 66A. Deficiency of the metabolic pathway in the carriers of the indicated genetic variants are shown in gray, and its redundancy is shown in bold*

utilization of dopamine at the synapse by methylation with participation of the catechol-O-methyltransferase enzyme, which can also contribute to an increase in extrapyramidal symptoms.

The study data demonstrating that the wild (standard) MTRR 66A> G allele promotes the risk of extrapyramidal symptoms development contradicts the results of studies of the folate cycle polymorphism association with Parkinson's disease, according to which «risk alleles» are low-function alleles that contribute to hyperhomocysteinemia development [20]. This can be explained by the fact that for Parkinson's disease the primary role is vested with the mechanisms of neurodegeneration contributing to the loss of dopaminergic neurons of the substantia nigra, and these mechanisms are hypothetically associated to a greater extent with hyperhomocysteinemia (excitotoxicity and cell apoptosis, oxidative stress, neuroinflammation). In the situation of medication induced parkinsonism development, of major importance is a reversible deficiency of dopaminergic neurotransmission in the nigrostriatal pathways, which can be explained by a balance shift due to a decrease in dopamine synthesis (in case of deficiency of BH4 resynthesis using 5-methyltetrahydrofolate) and its normal utilization with catechol-O- methyltransferase (Figure 4).

Probably, the mechanisms of tardive dyskinesia development, different from the mechanisms of medication-induced parkinsonism development (the assumed compensatory increase in the density and hypersensitivity of Type 1 dopamine receptors due to their prolonged blockade by antipsychotics), explain the fact that there were no differences found during the pilot study in the severity of abnormal involuntary movements (using the AIMS scale) between subgroups of carriers of the minor T-allele MTHFR 677C> T and the wild gene pattern MTHFR 677CC. However, the limitation of this study is that the symptoms of tardive dyskinesia were minimal and were demonstrated by a relatively small part of the patient population examined in the pilot study. Hypothetically, assessment of the contribution of the folate cycle SNP to the risk of tardive dyskinesia requires examination of patients with a longer duration of the disease and a longer exposure to antipsychotics in their medical history.

Taking into account the possibility of correction of the folate metabolism disorders in the carriers of the minor alleles of the studied SNP with vitamins (folates and B_{12}), the authors believe it relevant to conduct double-blind, randomized, placebo-controlled prospective intervention studies with a standardized assessment of the severity of adverse effects in dynamics while taking folates in carriers of the MTHFR 677T allele, as well as with assessment of biochemical markers (levels of homocysteine, tetrahydrobiopterin, folates and plasma cobalamin) over time. The up-to-date recommendations typically provide for a combined use of folates and cobalamin, but in this case, given the results obtained, this does not seem to be justified and requires further confirmation.

Conduction of a larger pharmacogenetic study of the risk alleles of MTHFR 677T and MTRR 66A association with antipsychotics tolerance is highly demanded, as confirmation of this study results will allow to select medications for patients, taking into account the probable contribution of the alleles of the studied SNPs, or to prescribe highly potent antipsychotics in combination with 5-methyltetrahydrofolate.

The assumption of the possible influence of one-carbon metabolic disorders on the risk of motor adverse effects of antipsychotics is evidenced by a number of clinical observations of patients taking folates in addition to antipsychotic therapy (within the framework of another study [21, 22]), in whom a decrease in extrapyramidal symptoms was registered; however, the assessment of extrapyramidal symptoms was not a part of that study and was based only on general clinical impression.

Conclusion. As can be seen from the above, schizophrenic patients with disorders of one-carbon metabolism (in particular, carriers of the polymorphic T-allele MTHFR 677C> T) face an increased risk of extrapyramidal adverse effects of antipsychotics. In addition, the wild A-allele at the MTRR 66A> G locus can be considered a «risk allele» for development of extrapyramidal adverse effects. Thus, a possibility of correction of folate metabolism disorders with vitamin augmentation opens up perspectives for a personalized reduction in these adverse effect risks, which is a relevant objective for further research.

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Conflict of Interest Statement

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