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2 RESULTS OF A STUDY OF BIOCHEMICAL PROFILE INDICATORS IN CHILDREN WITH AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

INTRODUCTION

Since pathogenic polymorphic nucleotide substitutions in the genes of the folate and methionine cycles, as shown by the results of a number of recent meta-analyses and systematic reviews of randomized controlled clinical trials [1–5], are an associated factor with the ASD phenotype in children, a natural question arises regarding the diagnostic significance of conducting appropriate genetic tests in the routine practice of child clinical psychiatry specialists, as well as which part of the wide spectrum of known biochemical disorders identified in children with ASD is directly and/or indirectly related to genetically determined disorders in the functionally interconnected metabolic cycles of folic acid and methionine. The efforts of a number of research groups are currently focused on solving these key problems, as there is an opportunity to obtain informative and clinically applicable folate-associated laboratory biomarkers for rational prediction, risk assessment, and determination of the severity of the condition of patients with ASD, as well as to provide effective and convenient tools for optimizing family planning processes, preparation for pregnancy and childbirth, prenatal diagnosis, postnatal secondary prevention, and therapy of ASD and a number of related comorbid pathological conditions.

N. S. Mohammad et al., using the ANN (artificial neural network) model in a controlled clinical study involving 138 children with autism spectrum disorders and 138 healthy children, showed that the determination of pathogenic polymorphic variants of the GCPII C1561T, SHMT1 C1420T, MTHFR C677T, MTR A2756G, and MTRR A66G genes for diagnostic purposes allows determining the risk of developing autism spectrum disorders in a carrier with an accuracy of 63.8 % [2].

Various pathogenic polymorphic variants of folate cycle genes can act synergistically, significantly increasing the risk of autism spectrum disorders in children. Synergism between MTHFR C677T and MTRR A66G was discussed in the results of a meta-analysis by N. S. Mohammad et al. [2]. In contrast, A. H. Arab et al. in a controlled clinical study involving 112 children with autism spectrum disorders and 104 healthy children established a synergistic effect of MTHFR C677T and MTHFR A1298C in shaping the risk of autism spectrum disorders in children (**Fig. 1.1**, Chapter 1) [6]. The greater the number of pathogenic polymorphic variants of folate cycle genes in the carrier's genome, the higher the risk of autism spectrum disorders in him.

The results of clinical studies demonstrate that pathogenic polymorphic variants of folate cycle genes can lead to the development of encephalopathy with a clinical picture of autism spectrum disorders in at least three ways:

a) metabolic, closely related to the phenomenon of hyperhomocysteinemia and the induction of oxidative stress in the CNS tissue [7–9];

b) immune-dependent, caused by the development of neurotropic opportunistic infections, antineuronal autoimmunity and persistent systemic/intracerebral inflammation [10–12];

c) gene regulatory, mediated by derepression of other pathogenic mutations/polymorphisms in the carrier's genome due to disruption of DNA methylation processes as a universal mechanism of gene censorship [13].

There is an assumption that both direct and immune-dependent mechanisms of encephalopathy development are associated with metabolic disorders, therefore it seems important to study the profile of biochemical disorders in children with genetic deficiency of the folate cycle associated with autism spectrum disorders.

The aim of the work: study of biochemical disorders in children with genetic deficiency of the folate cycle associated with autism spectrum disorders, to understand the mechanism of encephalopathy and immunodeficiency formation, as well as the search for biomarkers – monitoring the condition and targets of further therapeutic interventions to prevent and/or reduce neurotoxicity and immunosuppression.

MATERIALS AND METHODS OF THE RESEARCH

The medical data of 225 children aged 3 to 8 years with genetic deficiency of the folate cycle, who had autism spectrum disorders, were analyzed. All of them were patients of the specialized neuroimmunological clinic Vivere (registration file dated 12/22/2018 No. 10/2212–M). Obtaining data for the study and processing the material were carried out in accordance with contract No. 150221 dated February 15, 2021, and the conclusion of the bioethical examination commission (protocol No. 140 dated December 21, 2020, Bogomolets NMU). The diagnosis of autism spectrum disorders was made by child psychiatrists according to the criteria of DSM–IV–TR (Diagnostic and Statistical Manual of mental disorders) and ICD–10 (The International Statistical Classification of Diseases and Related Health Problems). Pathogenic polymorphic variants of folate cycle genes were determined by PCR based on the detection of the MTHFR C677T nucleotide substitution in monoform (27 patients), as well as – in combination with other nucleotide substitutions – MTHFR A1298C, MTRR A66G and/or MTR A2756G (111 people). These individuals constituted the study group (SG). The control group (CG) consisted of 51 children (37 boys and 14 girls) of similar age distribution who did not suffer from genetic deficiency of the folate cycle. We analyzed biochemical profile indicators that, according to recent studies, are considered informative biomarkers of genetic deficiency of the folate cycle, in particular, serum concentrations of homocysteine (*N* = <5.2 μmol/l), vitamins B6 (*N* = 8.7–27.2 μg/l), B12 (*N* = 197–771 pg/ml), D3 (*N* = 30–60 ng/ml), folic acid, or vitamin B9 (*N* = 3.89–26.8 ng/ml), creatinine (1–3 years *N* = 21–36 μmol/l; 3–5 years *N* = 27–42 μmol/l; 5–8 years *N* = 28–52 μmol/l), creatine phosphokinase (total) (*N* = 39–308 U/l) and lactate dehydrogenase (*N* = 135–225 U/l).

Statistical processing of the material was carried out by comparative and structural analyses. To determine the probability of differences between indicators in the observation groups, the parametric Student's T-test with the confidence probability indicator p and the nonparametric criterion – the number of signs Z according to Yu. Urbach were used. To study the associations between pathogenic polymorphic variants of folate cycle genes and parameters of the biochemical profile, the odds ratio (OR) and 95 % confidence interval (95 % CI) were used. Microsoft Excel was used to perform statistical calculations.

RESULTS AND DISCUSSION OF THE RESEARCH

As a result of structural and comparative analyses, it was found that the following pattern of biochemical disorders was characteristic of SG patients: hyperhomocysteinemia, decreased serum concentrations of vitamins B6, B12, D3 and folic acid, hypercreatininemia, increased serum concentrations of CPK and LDH, which significantly distinguished them from CG individuals (**Fig. 2.1**). In particular, an increase in the concentration of homocysteine beyond the reference values in the serum of SG children at the time of examination occurred in 88 %, a decrease in the serum concentration of vitamin B6 – in 76 %, vitamin B12 – in 79 %, vitamin D3 – in 72 %, folic acid – in 69 %, hypercreatininemia – in 65 %, increased serum concentrations of CPK – in 57 % and LDH – in 79 % of cases.

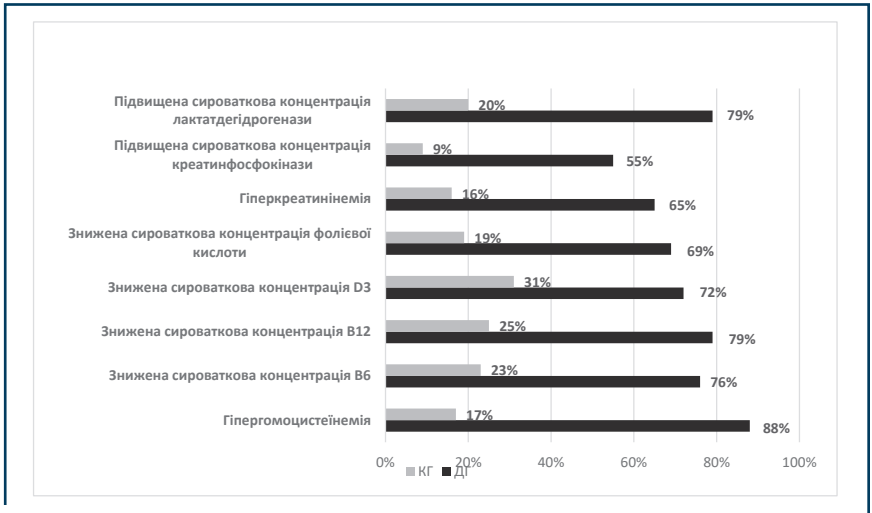

Fig 2.1 Results of structural analyses of biochemical profile indicators among patients SG (n = 225) and CG (n = 51)

As can be seen from **Fig. 2.1**, there were significant differences in the structural distribution of patients with the studied biochemical status disorders in SG and CG. As expected, hyperhomocysteinemia was the most typical sign of SG individuals and was 5 times more common than in CG. Reduced serum concentrations of vitamins B6 and B12 occurred three times more often, and low vitamin D3 concentrations were twice as common in SG than in controls. Increased serum CPK concentrations in SG were less common than changes in other studied parameters, but were five times more common than in CG.

The results of the analysis of variance in the observation groups indicate that the studied serum biochemical disorders are typical disorders specifically for children with GDFC associated with ASD, and are not characteristic of healthy children. **Table 2.1** compares the mean values $\left(\overline{X} \pm m\right)$ of the studied indicators of the biochemical profile of blood serum in SG and CG.

Table 2.1 Mean values $\left(\overline{X} \pm m\right)$ of the studied biochemical profile parameters in patients SG (n = 225) and CG (n = 51)

Indicator	SG $\left(\overline{X} \pm m\right)$	CG $\left(\overline{X} \pm m\right)$	T-test	Z
Homocysteine, μmol/L	9.63 ± 0.36	3.42 ± 0.11	$p < 0.05$	$Z < Z0.05$
Vitamin B12, pg/mL	112.64 ± 25.12	337.78 ± 38.47	$p < 0.05$	$Z < Z0.05$
Vitamin B6, μg/L	6.32 ± 0.24	18.11 ± 1.72	$p < 0.05$	$Z < Z0.05$
Vitamin D3, ng/mL	13.98 ± 1.37	35.65 ± 2.94	$p < 0.05$	$Z < Z0.05$
Folic acid, pg/mL	2.97 ± 0.46	17.19 ± 2.99	$p < 0.05$	$Z < Z0.05$
Creatine, μmol/L	69.13 ± 4.31	32.36 ± 2.12	$p < 0.05$	$Z < Z0.05$
Creatine phosphokinase, U/L	314.2 ± 29.76	52.17 ± 19.53	$p < 0.05$	$Z < Z0.05$
Lactate dehydrogenase, U/L	378.47 ± 29.78	178.24 ± 17.83	$p < 0.05$	$Z < Z0.05$

Thus, serum concentrations of homocysteine, creatinine, CPK and LDH are significantly higher, and serum concentrations of vitamins B6, B12, D3 and folic acid are significantly lower in patients with GDFC associated with ASD than in healthy children of similar age and sex.

Similar data on most of the studied biochemical parameters have already been demonstrated in other clinical studies published in PubMed, but so far no comprehensive analysis of the biochemical profile in such cases has been carried out. The conducted scientific works concerned the study of only individual metabolic parameters without correlation with other indicators, often without specifying the diagnosis of GDFC and only taking into account the clinical manifestations of ASD, which may have a heterogeneous origin. In this work, a comprehensive analysis of key indicators of biochemical status in children with ASD associated with GDFC is performed for the first time, which allows us to recreate a holistic picture of the metabolic profile in such cases.

The question of the pathogenetic significance of the identified biochemical abnormalities in children with ASD associated with GDFC is important. Currently, direct neurotoxic effects are being discussed, such as in homocysteine and creatinine [7]. Vitamin deficiency can disrupt metabolism in the CNS, in particular, affecting the metabolism of key neurotransmitters. As noted by F. Gevi et al., vitamin B6 deficiency slows down the transformation of the excitatory amino acid glutamate into the inhibitory neurotransmitter gamma-aminobutyric acid, which may play an important role in the induction of symptoms of hyperactivity and hyperexcitability in children with ASD [14]. Also, biochemical disorders can negatively affect the development and functioning of the child's immune system, contributing to the formation of a state of immunodeficiency and associated immune dysregulation [56], which can mediate a number of severe immune-dependent complications involved in the pathogenesis of encephalopathy in children with ASD [13, 15].

There is also an important question regarding the origin of the identified biochemical disorders. It has now been established that the mechanism of biochemical imbalance in children with ASD is complex and multicomponent. Thus, some disorders are a direct consequence of the presence of pathogenic polymorphic

nucleotide substitutions in the genes of folic acid cycle enzymes, that is, they are directly related to the dysfunction of the folate cycle. In particular, we are talking about the phenomenon of hyperhomocysteinemia [7]. Other disorders may have an indirect mechanism of development. For example, the deficiency of a number of vitamins, in addition to GDFC, is explained both by impaired absorption of nutrients in the small intestine in connection with the development of persistent enterocolitis in children with ASD, and by behavioral disorders that involve dietary restrictions due to pathological selectivity in food in ASD [53].

Signs of mitochondrial dysfunction, including increased serum creatinine, LDH, and CPK, are a consequence of oxidative stress, which develops both through the direct effect of homocysteine on enzymes of the antioxidant system and the indirect effect of immune dysregulation caused by biochemical disorders, which is associated with abnormally increased production of prooxidant compounds in the process of persistent systemic inflammation [16].

Thus, with GDFC, children with ASD have a specific pattern of biochemical disorders that differs from the biochemical profile of healthy children and may have significant practical significance, in particular, becoming a cornerstone of the diagnostic algorithm for ASD associated with GDFC.

Important issues are the association of biochemical profile abnormalities with pathogenic polymorphic nucleotide substitutions in genes encoding folic acid cycle enzymes and the differences in associations between different folate cycle deficiency genotypes and certain biochemical disorders. Data on the association of biochemical profile abnormalities (OR; 95 % CI) with genetic testing results among SG patients are presented in **Table 2.2**.

● **Table 2.2** Results of the study of the association of disorders of the studied biochemical parameters (OR; 95 % CI) with the combination of identified polymorphisms of folate cycle genes SG (n = 225)

Genotype	Homo	B12	B6	D3	B9	Creatinin	CPK	LDH
1	2	3	4	5	6	7	8	9
MTHFR C677T	4.094; 1.851–9.051	2.841; 1.235–6.533	2.506; 1,110–5.660	2.402–1.068–5.401	2.367; 1.056–5.304	2.882; 1.257–6.609	3.267; 1.399–7.626	2.917; 1.268–6.707
MTHFR C677T + + MTHFR A1298C	5.444; 2.314–12.807	5.464; 2.320–12.872	4.992; 2.132–11.685	4.958–2.124–11.577	4.516–1.949–10.463	5.464; 2.320–12.872	3.857; 1.669–8.911	6.612; 2.762–15.831
MTHFR C677T + + MTRR A66G	4.737; 2.080–10.788	3.111; 1.340–7.223	2.917; 1.268–6.707	3.182; 1.362–7.431	3,598; 1,530–8,462	3.857; 1.669–8.911	3.231; 1.384–7.541	3.947; 1.780–8.756
MTHFR C677T + + MTR A2756G	4.334; 1.945–9.660	3.947; 1.780–8.756	3.750; 1.701–8.265	4.737; 2.080–10.788	3,553; 1,623–7,775	3.947; 1.780–8.756	3.801; 1.708–8.461	3.725; 1.672–8.300
MTHFR C677T + + MTHFR A1298C + + MTRR A66G	6.629; 2.629–16.718	6.261; 2.500–15.682	5.392; 2.209–13.166	7.292; 2.831–18.782	5,367; 2,179–13,218	4.911; 1.944–12.403	5.612; 2.143–14.698	4.819; 1.917–12.114

Continuation of Table 2.2

1	2	3	4	5	6	7	8	9
MTHFR C677T + + MTHFR A1298C + + MTR A2756G	6.432; 2.606–15.870	5.404; 2.380–12.272	5.765; 2.514–13.217	6.125; 2.649–14.163	5,921; 2,554–13,727	5.224; 2.295–11.895	4.644; 2.073–10.401	5.573; 2.424–12.811
MTHFR C677T + + MTRR A66G + + MTR A2756G	6.176; 2.570–14.842	4.871; 2.100–11.297	5.412; 2.315–12.651	5.828; 2.462–13.793	5,701; 2,406–13,508	5.701; 2.406–13.508	5.729; 2.341–14.023	3.947; 1.780–8.756
MTHFR C677T + + MTHFR A1298C + + MTR A2756G + + MTRR A66G	7.206; 3.026–17.157	7.212; 2.861–18.177	6.657; 2.677–16.555	5.641; 2.316–13.740	6,044; 2,456–14,873	4.583; 1.942–10.816	6.509; 2.614–16.205	6.111; 2.475–15.091

As shown in **Table 2.2**, all genotypes studied are associated with a characteristic pattern of biochemical changes, which was discussed above, and not only with deviations in the levels of individual biochemical indicators. The expected frequency of certain biochemical disorders in the presence of a certain genotype increases from 2 to 7 times depending on the biochemical indicator and the pathogenic polymorphic variant of nucleotide substitution in the gene of the folic acid cycle enzyme. This indicates a complex and interconnected nature of the detected biochemical disorders in SG patients. For each genotype studied, mostly conjugate deviations in the levels of various biochemical indicators were characteristic, which allows us to speak about characteristic differences in biochemical status depending on the genotype (genotype-associated biochemical profiles). Thus, for the MTHFR C677T genotype, the frequency of biochemical status abnormalities increases at least 2–4 times, while for the MTHFR C677T + MTHFR A1298C genotype, it increases 3–6 times. The unequal influence of different pathogenic polymorphic variants of nucleotide substitutions in the genes of folate cycle enzymes on the severity of the studied biochemical disorders in blood serum was demonstrated. Thus, MTHFR A1298C was characterized by a closer association with deviations in the levels of the studied biochemical parameters compared to MTRR A66G and MTR A2756G. It was also shown that the accumulation of pathogenic polymorphic variants of nucleotide substitutions in the genes of folic acid cycle enzymes in the patient's body is associated with more pronounced changes in the biochemical profile, which indicates a cumulative effect of the identified genotype-associated biochemical profiles in SG. Accordingly, the most severe in terms of violations of the biochemical status of blood serum is the broadest genotype MTHFR C677T + MTHFR A1298C + MTR A2756G + MTRR A66G, which includes all the main pathogenic nucleotide substitutions, and the lightest is the mono-form MTHFR C677T. Homocysteine, among other studied biochemical indicators, was more closely associated with GDFC, and changes in its serum concentration better correspond to differences in the patient's genetic status. Thus, homocysteine is the most representative serum biochemical indicator for GDFC, which should be taken into account in the algorithms of laboratory screening of GDFC among children with ASD. This feature can be attributed to the direct relationship of the phenomenon of hyperhomocysteinemia with the specific metabolic block formed in GDFC, while, for example, serum vitamin concentrations are expected to be influenced by some other factors, such as the quality of absorption in the small intestine and the patient's dietary habits.

Hyperhomocysteinemia is known to be the main biochemical abnormality in genetic deficiency of the folate cycle in humans [7, 17]. A meta-analysis of randomized controlled clinical trials by B. Q. Guo et al. of 31 trials involving 3304 children, including 1641 patients with autism spectrum disorders, demonstrated that hyperhomocysteinemia is associated with autism spectrum disorders (Hedges's $g = 0.56$; 95 % CI = 0.36–0.76, $p < 0.001$) [17]. The neurotoxic effects of homocysteine are now well understood [7], and direct dysmetabolic mechanisms of encephalopathy are associated with them, which usually lead to certain clinical outcomes [17]. According to our observations, the immunotoxic effects of homocysteine and oxidative stress induced by this agent are of greater pathogenetic importance, leading to impaired development of the child's immune system with the formation of a specific form of immunodeficiency [18–20]. This immunodeficiency is responsible for the development of immune dysregulation and the implementation of immune-mediated and immunoinflammatory pathways of neuronal damage with the formation of encephalopathy, which is manifested by a delay in psycho-speech development in a child with manifestations of autism spectrum disorders [10, 12]. This is confirmed by the clinical effectiveness of some immunotherapeutic interventions [11].

The results of a meta-analysis of controlled clinical trials prepared by Z. Wang et al. in 2020, which included the results of 34 trials involving 20,580 children, indicate that reduced serum vitamin D concentrations are a characteristic feature of children with autism spectrum disorders (mean difference (MD): –7.46 ng/mL, 95 % CI: –10.26; –4.66 ng/mL, $p < 0.0001$, $I2 = 98$ %) [14]. Accordingly, the data of a systematic review and meta-analysis of controlled clinical trials by B. Li et al. in 2020, which includes the results of 5 trials involving 349 people, indicate that vitamin D supplementation for serum vitamin D deficiency significantly reduces the severity of hyperactivity (pooled MD: –3.20; 95 % CI: [–6.06; –0.34]) with low heterogeneity ($I2 = 10$ %, $p = 0.33$) in children with autism spectrum disorders [21].

The results of a controlled clinical trial by Ç. Yektaş et al. with the participation of 118 children demonstrated a significant increase in serum homocysteine concentrations and a decrease in vitamin B12, but not folic acid, in children with autism spectrum disorders and attention deficit hyperactivity disorder compared with healthy individuals [22]. Data from a controlled clinical study by A. Belardo et al. with the participation of 120 patients indicate a significant decrease in serum concentrations of vitamins B6 and B12, as well as folic acid in children with autism spectrum disorders compared with healthy children [23].

The results of controlled clinical studies by M. Lv et al. [24] and O. A. Al-Mosalem et al. [25], conducted independently of each other, indicate a probable increase in serum concentration and activity of creatine phosphokinase in children with autism spectrum disorders compared with healthy individuals. The data of a controlled clinical study by A. El-Ansary et al. indicate that increased serum concentrations of lactate dehydrogenase and creatine phosphokinase are biomarkers of autism spectrum disorders in children along with some other indicators of the metabolic profile [26].

In 2018, Y. J. Li et al. conducted a systematic review of the results of randomized controlled clinical trials on micronutrient deficiencies observed in children with autism spectrum disorders. The results of 7 such trials indicate that vitamin B6 supplementation is ineffective in correcting mental status disorders in children with autism. Data from two other trials showed that the use of methyl form of vitamin B12 leads to some improvement in mental status indicators in children with autism spectrum disorders. The results of three studies using vitamin D3 preparations indicate insufficient effectiveness of this approach

in correcting mental disorders in children with autism. Data from another trial showed the benefit of prescribing folic acid in autism spectrum disorders in children [27].

The question of the pathogenetic significance of the identified biochemical disorders in children with autism spectrum disorders associated with genetic deficiency of the folate cycle is important. Currently, there is talk of direct neurotoxic effects, such as homocysteine [7]. Vitamin deficiency can disrupt metabolism in the CNS, in particular, affect the metabolism of neurotransmitters. As noted by F. Gevi et al., vitamin B6 deficiency slows down the transformation of the excitatory amino acid glutamate into the inhibitory neurotransmitter gamma-aminobutyric acid, which may play an important role in the induction of symptoms of hyperactivity and hyperexcitability in children with autism spectrum disorders [15]. Also, biochemical disorders can negatively affect the development of the immune system, contributing to the formation of an immunodeficiency state, which can mediate a number of immune-dependent complications involved in the pathogenesis of encephalopathy in children with autism spectrum disorders [10, 12].

There is also an important question regarding the origin of the identified biochemical disorders. It has now been established that the mechanism of biochemical imbalance in children with autism spectrum disorders is complex and multicomponent. Thus, some disorders are a direct consequence of the presence of pathogenic polymorphic variants of the folic acid cycle genes, that is, they are directly related to the dysfunction of folate cycle enzymes. In particular, we are talking about the phenomenon of hyperhomocysteinemia [17]. Other disorders may have an indirect mechanism of development. For example, the deficiency of a number of vitamins is explained both by impaired absorption of nutrients in the small intestine in connection with the development of persistent enterocolitis in children with autism spectrum disorders, and by behavioral disorders that involve dietary restrictions due to food selectivity in autism spectrum disorders [2]. Signs of mitochondrial dysfunction, including increased serum concentrations of creatinine, lactate dehydrogenase, and creatine phosphokinase, are a consequence of oxidative stress, which develops both through the direct effect of homocysteine on enzymes of the antioxidant system and the indirect effect of immune dysregulation caused by biochemical disorders, which is associated with increased production of prooxidant compounds [8, 12].

Thus, for children with GDFC associated with ASD, a specific pattern of pathological biochemical changes in the blood serum, determined by GDFC, is characteristic, which is not typical for healthy children, and may be an important component of the pathogenesis of immunodeficiency and encephalopathy, which usually occur in such cases. Hyperhomocysteinemia, deficiency of certain vitamins and signs of mitochondrial dysfunction are noted. The mechanism of development of these serum biochemical disorders can be complex and multicomponent, however, all the pathological biochemical abnormalities studied are closely associated with pathogenic polymorphic variants of nucleotide substitutions in the genes of folate cycle enzymes, and their severity depends both on the type of pathogenic polymorphic variant of nucleotide substitution in the gene of the folic acid cycle, and on the number and composition of these pathogenic nucleotide substitutions in the patient's genome. The most favorable in biochemical terms is the MTHFR C677T genotype, which includes only one pathogenic nucleotide substitution, while the most severe is the broadest genotype with a combination of all major polymorphisms of the folate cycle enzyme genes MTHFR C677T + MTHFR A1298C + MTR A2756G + MTRR A66G.

The obtained data allow us to better understand the pathogenesis of the disease in children with ASD associated with GDFC. The identified pattern of laboratory biochemical abnormalities in serum can be used in the diagnostic process both in laboratory screening of GDFC among children with m, and in assessing the severity of the patient's current condition, predicting the further course of the disease and conducting clinical monitoring of children with ASD associated with GDFC. In addition, these GDFC-associated serum biochemical abnormalities can be the object of targeted therapeutic interventions to correct the patient's metabolic status, reduce the manifestations of GDFC-induced immunosuppression and reduce the severity of associated mental disorders in a child with ASD.

CONCLUSIONS TO THE SECTION 2

Children with genetic deficiency of the folate cycle associated with autism spectrum disorders are characterized by a specific pattern of biochemical changes that is not typical for healthy children and may explain the pathogenesis of immunodeficiency and encephalopathy. Hyperhomocysteinemia, deficiency of some B vitamins and signs of mitochondrial dysfunction are noted. The mechanism of development of these biochemical disorders can be complex and multicomponent, however, all studied biochemical abnormalities are closely associated with pathogenic polymorphic variants of the genes of folate cycle enzymes, and their severity depends on both the type of pathogenic polymorphic variant of the gene and the number and composition of pathogenic nucleotide substitutions in the patient's genotype. The most favorable in biochemical terms is the MTHFR C677T genotype, while the most severe is the genotype with the combination of polymorphisms MTHFR C677T + MTHFR A1298C + MTR A2756G + MTRR A66G. The obtained data allow us to better understand the pathogenesis of the disease. The identified pattern of biochemical disorders can be used in the diagnostic process both in screening for genetic deficiency of the folate cycle, and in assessing the severity of the condition and conducting clinical monitoring of children with autism spectrum disorders. In addition, these disorders can be the object of therapeutic interventions in order to correct the biochemical status of the patient and reduce the manifestations of immunosuppression and mental disorders.

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