Biochemical profile of children with autism spectrum disorders associated with genetic deficiency of the folate cycle

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ABSTRACT

Introduction: the results of 5 randomized controlled meta-analysis studies showed the association between genetic deficiency of folate cycle and autistic spectrum disorders in children. The purpose of the present study is to investigate the biochemical alterations in children with autism spectrum disorders associated with genetic deficiency of the folate cycle.
Methods: the experimental group involved 225 children diagnosed with autism spectrum disorders (Diagnostic and Statistical Manual of Mental Disorders IV-TR, International Statistical Classification of Diseases and Related Health Problems 10), who suffered from a genetic deficiency of the folate cycle (methylenetetrahydrofolate reductase C677T + methylenetetrahydrofolate reductase A1298C and/or methionine synthase A2756G and/or methionine synthase reductase A66G; polymerase chain reaction). The control group included 51 healthy children who followed the same age and gender distribution pattern of the patients group.
Results: the study revealed that the patients had a specific pattern of biochemical alterations in serum ($p<0.05$; $Z<Z0.05$). Mean value and (SD) are reported. Hyperhomocysteinemia: 9.63 (5.36) μmol/L, r.v. <5.2); deficiencies of vitamins: B6 [6.32 (3.58) μg/L, r.i. 8.7-27.2], folic acid [2.97 (6.85) pg/mL, r.i. 3.89-26.8], B12 [112.64 (374.2) pg/mL, r.i. 197-771], and D3 [13.98 (20.41) ng/mL, r.i. 30-60]; hypercreatininemia [69.13 (64.21) μmol/L, r.i. 3 years: 21-36, 3-5 years: 27-42, 5-8 years: 28-50], increased creatine kinase [314.12 (443.42) U/L, r.i. 39-308 U/L]; and lactate dehydrohenase [378.47 (443.72) U/L, r.i.135-225]. At the time of examination, an increase in the serum concentration of homocysteine was registered in 88% of the patients. The associations between different folate cycle genes polymorphisms with certain biochemical abnormalities were shown
Discussion: autism spectrum disorders associated with genetic deficiency of the folate cycle in children are characterized by a specific pattern of biochemical changes that is not found in healthy children and may be involved in the pathogenesis of immunodeficiency and encephalopathy. These data can be used in clinical practice for diagnostic purposes to identify a subgroup of children with autism spectrum disorders associated with genetic folate cycle deficiency and for the selection of biochemical correction during treatment.

Keywords: *homocysteine, B vitamins, folic acid*

INTRODUCTION

Solving the problem of childhood neuropsychiatric disorders is a priority task of modern medicine. The greatest attention among the various pathologies of the mental sphere is currently focused on the study of the etiology and pathogenesis of autism spectrum disorders (ASD) in children; its frequency is steadily increasing and now in the United States reaches 1 case per 50 children (1,2). ASD is a group of heterogeneous neuropsychiatric disorders that are variable in phenotype and clinically characterized by deficits in social interactions, impaired communication and narrowing of the circle of interests. Facing the problem of ASD in children is rather difficult without clarifying the mechanisms of the disease, which still remain poorly understood. One of the important recent advances is the elucidation of the association between genetic deficiency of the folate cycle (GDFC) and ASD (2,3). The folate cycle

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is functionally related to the methionine cycle and is a key mechanism for regulating DNA methylation: an universal form of gene censorship. The key enzymes of folate-methionine metabolism are methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), and methionine synthase reductase (MTRR). The results of 5 meta-analyses and systematic reviews of randomized clinical trials indicate an association of ASD phenotype and MTHFR C677T in children (2-6). Two other meta-analyses demonstrated an association of ASD with MTHFR A1298C (5-6), and one meta-analysis with MTRR A66G (3), while an association with MTR A2756G was found in only one controlled study (7).

Using an artificial neural network (ANN) model, Shaik et al. (3) conducted a controlled clinical trial involving 138 children with ASD and 138 healthy children. The researchers showed that diagnostic detection of pathogenic polymorphic variants of GCPII C1561T, SHMT1 C1420T, MTHFR C677T, MTR A2756G, and MTRR A66G allows assessing the risk of developing ASD in the carrier with an accuracy of 63.8% (3).

Different pathogenic polymorphic variants of folate cycle genes can act synergistically, significantly increasing the risk of developing ASD in children. The synergism between MTHFR C677T and MTRR A66G was discussed in the meta-analysis performed by Shaik et al. (3). Arab et al. (8) conducted a controlled clinical study involving 112 children with ASD and 104 healthy children, and demonstrated a synergistic effect of MTHFR C677T and MTHFR A1298C on the risk of ASD. From a general point of view, the greater number of pathogenic polymorphic variants of folate cycle genes are present in the carrier genome, the higher is the risk of ASD.

The results of clinical studies suggest that pathogenic polymorphic variants of folate cycle genes may contribute to the development of encephalopathy with a clinical picture of ASD in at least three ways:

- metabolic, closely related to the phenomenon of hyperhomocysteinemia and oxidative stress induction in the central nervous system (CNS) tissue (9-11);
- immune-dependent, caused by neurotropic opportunistic infections, antineuronal autoimmunity, and persistent systemic/intracerebral inflammation (12-14);
- geno-regulatory, mediated by de-repression of other pathogenic mutations/polymorphisms in the carrier genome due to disruption of the DNA methylation process (15-17).

The results of several meta-analyses of randomized controlled clinical trials indicate an association between hyperhomocysteinemia, a disorder of one-carbon metabolism specific to GDFC, and ASD phenotype in children (3,10-11), confirming the results of studies on the association between pathogenic polymorphic nucleotide substitutions in folate cycle enzyme genes and ASD phenotype. Currently, neurotoxic effects of homocysteine have been identified (1,9), which sheds light on one of the mechanisms of CNS damage in children with ASD who have GDFC. Other biochemical abnormalities have been described in children with ASD; among these deficiencies of vitamins B6, B9, B12, D3, and hypercreatininemia have the greatest evidence base and decreased serum concentrations of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) (18-20), which may be involved in both biochemical and immune-dependent pathways of GDFC-induced CNS damage in children with ASD. However, it is currently unclear whether these disorders are specifically related to GDFC, as it happens with hyperhomocysteinemia, or are the result of other biochemical pathways that have been reported to be associated with ASD in controlled clinical trials. The ratio of frequencies of these biochemical abnormalities in the population of children with ASD is not sufficiently understood, which makes it difficult to understand the diagnostic value of the different biomarkers, and to determine the clinical utility of certain therapeutic interventions for biochemical correction in ASD.

The scope of this paper is to explore the biochemical abnormalities in children with ASD associated with genetic deficiency of the folate cycle to search for biomarkers that can be used for monitoring the condition and adaptation of biochemical correction approaches. The use of the above-mentioned biomarkers will help to optimize the diagnostic testing algorithm; the introduction of new biochemical correction approaches will help to enhance the treatment effectiveness.

METHODS

225 children (183 boys and 42 girls) aged 2 to 9 years (average age: 4.26±0.31 years) with a genetic folate cycle deficiency and diagnosed with ASD were enrolled in the study group. All of them attended Vivere Specialized Neuroimmunology Clinic (registration dossier No. 10/2212-M dated December 22, 2018, Kyiv, Ukraine). The study data were obtained and processed under the agreement No. 150221 dated February 15, 2021 based on the Conclusion of the Bioethics Commission at the O'Bogomolets National Medical University (protocol No. 140 dated December 21, 2020, Kyiv, Ukraine). ASD was diagnosed by pediatric psychiatrists using the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) and International Statistical Classification of Diseases and Related Health Problems (ICD-10).

The children assigned to the experimental group (EG) had the pathogenic polymorphic variants of nucleotide substitutions in the folate cycle enzyme genes determined by real-time restriction PCR (detecting amplifier "DT-96"; "DNA-Technology", Synevo, Ukraine) (21) based on the detection of MTHFR C677T nucleotide substitutions in monoform (68 patients; 30% of cases) and in combination with other major pathogenic nucleotide substitutions, such as MTHFR A1298C, MTRR A66G and/or MTR A2756G (157 people; 70% of cases). The data reflecting the number of EG children with the respective genome are listed here: MTHFR C677T + MTHFR A1298C – 26 (12.5%); MTHFR C677T + MTRR A66G – 19 (8.5%); MTHFR C677T + MTR A2756G – 25 (11%); MTHFR C677T + MTHFR A1266G + MTR – 21 (9%); MTHFR C677T + MTHFR A1298C + MTR A2756G – 22 (9.5%); MTHFR C677T + MTRR A66G + MTR A2756G – 23 (10.5%); MTHFR C677T + MTHFR A1298C + MTR A2756G A66G – 21 (9%). The data are illustrated in Figure 1.

The control group (CG) included 51 children (37 boys and 14 girls) who followed the same age distribution

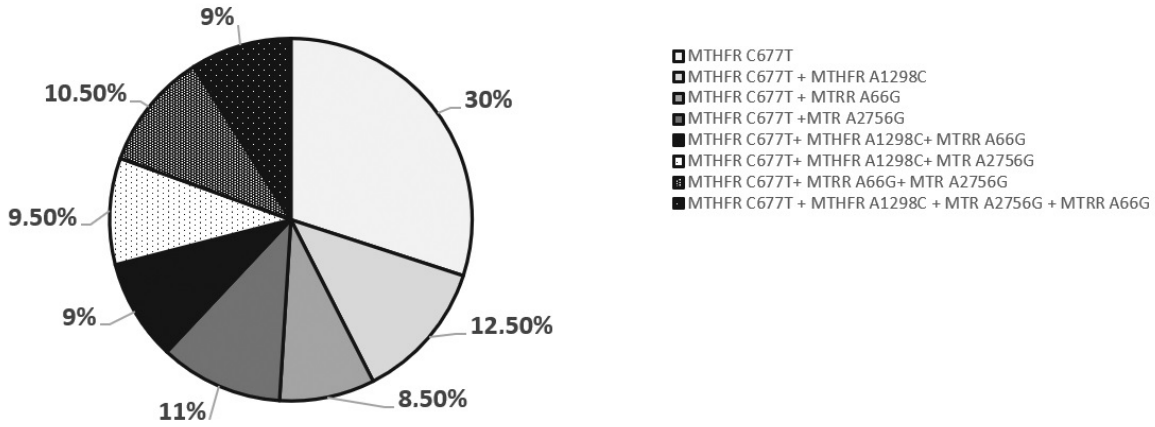

Figure 1
Patient group (n=225): results of the genetic testing for genetic folate deficiency.

pattern (average age 4.11±0.39 years). None of them suffered from genetic deficiency of the folate cycle (real-time PCR with restriction; detecting amplifier "DT-96"; "DNA-Technology"; Synevo, Ukraine) and ASD.

A range of biochemical parameters were analyzed, which had been recently identified as informative biomarkers of genetic deficiency of the folate cycle (Table 1).

The tests were performed in the Synevo laboratory (Kyiv, Ukraine).

Statistical processing of the data was performed using comparative and structural analyses. To determine whether the difference between the groups is statistically significant, the Student's T-test with the confidence

Table 1
Details of the measured analytes

Analytes	Units of measurements	Reference values	Analytical methods	Reagents and Analyzers
Homocysteine	µmol/L	<5.2	Electrochemiluminescence immunoassay (ECLIA)	Homocysteine Enzymatic Assay Cobas 6000 / Cobas 8000, Roche Diagnostics (Switzerland)
Vitamin B6	µg/L	8.7-27.2	High-performance liquid chromatography (HPLC)	Fluorescent detection on HPLC-System 1100. Agilent technologies (Germany)
Vitamin B12	pg/mL	197-771	Electrochemiluminescence immunoassay (ECLIA)	Elecsys reagent Cobas 6000 Roche Diagnostics (Switzerland)
Vitamin D3	ng/mL	30-60	Enzyme-linked immunosorbent assay (ELISA)	25-OH Vitamin D Euroimmun (Germany)
Folic acid	ng/mL	3.89-26.8	Electrochemiluminescence immunoassay (ECLIA)	Elecsys reagent o Cobas 6000 Roche Diagnostics (Switzerland)
Creatinine	U/L	3 years: 21-36; 3-5 years: 27-42; 5-8 years: 28-5	Kinetic method	Creatinine Jaffè Cobas 6000 Roche Diagnostics (Switzerland)
Creatine kinase	U/L	39-308	Kinetic method	Cobas 6000 Roche Diagnostics (Switzerland)
Lactate dehydrogenase	U/L	135-225	Kinetic method	Cobas 6000 Roche Diagnostics (Switzerland)

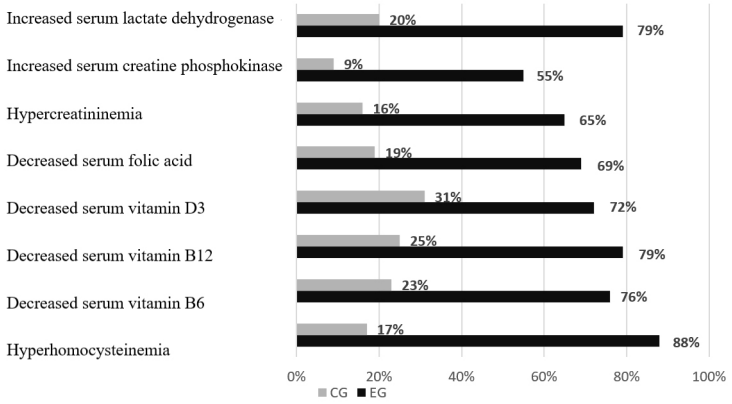

Figure 2
Percentages of individuals with abnormal biochemical parameters in the patient (EG, n=225) and control group (CG, n=51)

probability p (parametric criterion) and the number of Urbach Z signs (non-parametric criterion) were used. To evaluate the correlation between pathogenic polymorphic variants of folate cycle genes and biochemical profile parameters, the odd ratio (OR) and the 95% confidence intervals (95% CI) were used. Microsoft Excel was used for statistical calculations.

RESULTS

The study revealed multidirectional pathological abnormalities in the serum concentrations of the studied metabolites in children with ASD compared to healthy peers. These data are illustrated in Figure 2. The structural and comparative analyses revealed the following pattern of biochemical abnormalities characteristic for the EG patients: hyperhomocysteinemia, decreased serum concentrations of vitamins B6, B12, D3, and folic acid, hypercreatininemia, increased serum concentrations of CK and LDH. These biomarkers were statistically different from those obtained from the CG group ($p<0,05$; $Z<Z_{0,05}$). In particular, at the time of examination there were 88% of children in the EG with an increase in the serum concentration of homocysteine, 76% of children with a decrease in the serum concentration of vitamin B6, 79% of children with a decrease in the serum concentration of vitamin B12, 72% of children with a decrease in the serum concentration of vitamin D3, and 69% of children with a decrease in the serum concentration of folic acid. Hypercreatininemia was noted in 65% of cases. An increase in the serum concentration of CK was documented for 57% of the EG patients, and an increase in the serum concentration of LD was reported in 79% of cases.

As expected, hyperhomocysteinemia was the most common feature in the EG: it was 5 times more common than in the CG. Decreased serum concentrations of vitamins B6 and B12 were reported three times more often, and low concentration of vitamin D3 was reported twice as often in the EG as in the CG. Elevated serum concentration of CK was observed in the EG less frequently than changes in other studied parameters, but five times more often than in the CG.

The results of variation analysis in the two groups indicate that the studied biochemical alterations are typical for children with ASD associated with GDFC and are not so frequent in healthy children. Table 2 compares the average values of the studied serum parameters describing the biochemical profile of the EG and CG patients.

Table 2
Mean values (SD) of the studied biochemical parameters calculated for the patients (EG, n=225) and control group (CG, n=51)

Parameter, units of measurements	EG	CG	t-criterion	Z
Homocysteine, μmol/L	9.63 (5.36)	3.42 (0.78)	$p<0.05$	$Z<Z_{0.05}$
Vitamin B12, pg/mL	112.64 (374.2)	337.78 (291.9)	$p<0.05$	$Z<Z_{0.05}$
Vitamin B6, μg/L	6.32 (3.58)	18.11 (12.16)	$p<0.05$	$Z<Z_{0.05}$
Vitamin D3, ng/mL	13.98 (20.41)	35.65 (20.79)	$p<0.05$	$Z<Z_{0.05}$
Folic acid, pg/mL	2.97 (6.85)	17.19 (21.14)	$p<0.05$	$Z<Z_{0.05}$
Creatinine, μmol/L	69.13 (64.21)	32.36 (14.99)	$p<0.05$	$Z<Z_{0.05}$
Creatine kinase, U/L	314.12 (443.42)	52.17 (138.08)	$p<0.05$	$Z<Z_{0.05}$
Lactate dehydrogenase, U/L	378.47 (443.72)	178.24 (126.06)	$p<0.05$	$Z<Z_{0.05}$

Table 3
The association between alterations in the studied biochemical parameters (OR; 95%CI) and a combination of folate cycle genes polymorphisms obtained in the patient group (n=225)

Genotype	Homocysteine	Vitamin B12	Vitamin B6	Vitamin D3	Vitamin B9	Creatinine	Creatine kinase	Lactate dehidrogenase
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
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
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

There are important issues concerning the correlation between biochemical profile abnormalities and pathogenic polymorphic variants of nucleotide substitutions in the folate cycle enzymes genes. Moreover, it is interesting to reveal differences in the associations between different genotypes of folate deficiency and certain biochemical alterations. Table 3 demonstrates the relationship between alterations in the studied biochemical parameters (OR; 95% CI) and results of genetic testing conducted in the EG patients.

As shown in Table 3, all studied genotypes are associated with a distinct pattern of biochemical changes discussed above and not limited to fluctuations in the levels of individual biochemical parameters. The expected frequency of certain biochemical abnormalities in the presence of a certain genotype increases from 2 to 7 times depending on the biochemical parameter and pathogenic polymorphic variant of nucleotide substitution in the gene of the folic acid cycle enzyme. This is indicative of the complex and interrelated nature of the detected biochemical alterations in EG patients. Each identified genotype was characterized deviations in the levels of different biochemical parameters; it is actually possible, to identify differences in biochemical status depending on the genotype (i.e. genotype-associated biochemical profiles). Thus, the carriers of genotype MTHFR C677T have the frequency of biochemical deviations increased at least 2-4 times, while for the genotype MTHFR C677T + MTHFR A1298C the deviation frequency is increased 3-6 times. MTHFR A1298C was characterized by a closer association with deviations in the levels of the studied biochemical parameters as compared to MTRR A66G and MTR A2756G. The study has also shown that the accumulation of pathogenic polymorphic variants of nucleotide substitutions in the genes of folic acid cycle enzymes is associated with more pronounced biochemical changes, indicating the cumulative effect of identified genotype-associated biochemical EG profiles. Accordingly, the most severe in terms of serum biochemical status is the most widespread genotype MTHFR C677T + MTHFR A1298C + MTR A2756G + MTRR A66G, which includes all major pathogenic nucleotide substitutions, and the mildest is the monoform MTHFR C677T. The MTHFR C677T + MTHFR A1298C genotype is characterized by biochemical abnormalities of moderate severity and is associated with more pronounced metabolic disorders than the genotypes MTHFR C677T + MTRR A66G and MTHFR C677T + MTR A2756G. Meanwhile, genotypes MTHFR C677T + MTHFR A1298C + MTRR A66G, MTHFR C677T + MTHFR A1298C + MTR A2756G and MTHFR C677T + MTRR A66G + MTR A2756G are accompanied by deeper changes in the studied indicators of the biochemical profile, yielding in severity of metabolic disorders only to the genotype MTHFR C677T + MTHFR A1298C + MTR A2756G + MTRR A66G. Among the studied biochemical parameters, homocysteine was found to be more closely associated with GDFC, and changes in its serum concentration better reflect the changes in the genetic status of the patient. Thus, homocysteine is the most representative biochemical parameter of GDFC, which should be considered in the algorithms of laboratory screening of GDFC in children with ASD. This feature can be directly related to the phenomenon of hyperhomocysteinemia with a specific metabolic block found in GDFC, while, for example, serum vitamin concentrations are expected to be affected by some other factors, such as the quality of absorption in the small intestine and dietary characteristics of the patient.

DISCUSSION

Children with ASD associated with GDFC have a specific pattern of pathological serum biochemical changes that is determined by GDFC, not found in healthy children; this may be an important component of the pathogenesis of immunodeficiency and encephalopathy that usually develops in such cases. Hyperhomocysteinemia, deficiency of some vitamins and signs of mitochondrial dysfunction are noted. The mechanism of development of these serum biochemical disturbances can be complex and multicomponent, but all studied pathological biochemical abnormalities are closely associated with pathogenic polymorphic variants of nucleotide substitutions of folate enzyme genes. Their severity depends on the type of pathogenic polymorphic variants of nucleotide substitutions in the genes of folic acid cycle enzymes as well as on the number and composition of these pathogenic nucleotide substitutions in the patient's genome. The most biochemically favorable genotype is MTHFR C677T, which involves only one pathogenic nucleotide substitution, while the most severe is the broadest genotype MTHFR C677T + MTHFR A1298C + MTR A2756G + MTRR A66G combining all major polymorphisms of folate cycle enzyme genes.

As mentioned above, sufficient evidence has now been accumulated for an association between ASD and GDFC in children. Data from the first meta-analysis of randomized controlled clinical trials conducted by Pu et al. (2), who analyzed the results of 8 trials involving 1 672 children with ASD and 6 760 healthy children, demonstrated that the pathogenic polymorphic variant MTHFR C677T is associated with ASD in children. Further meta-analysis of randomized controlled clinical trials conducted by Shaik et al. (3) involved 1 361 children with ASD and 6 591 healthy children. It showed that MTHFR C677T and related hyperhomocysteinemia were associated with ASD. Additionally, the synergism of MTHFR C677T and MTRR A66G in the induction of hyperhomocysteinemia and increasing the risk of ASD in the carrier were demonstrated. A subsequent meta-analysis of randomized controlled clinical trials conducted by Rai et al. (4), who included data from 13 trials involving 1 978 children with ASD and 7 257 healthy children, established an association between MTHFR C677T and ASD in both European and Asian children. MTHFR C677T increased the risk of ASD in all 4 genetic models used (OR 95% CI): T (thymine) *versus* C (cytosine)=1.48 (1.18-1.86), p=0.0007; TT + CT *versus* CC=1.70 (0.96-2.9), p=0.05; TT *versus* CC=1.84 (1.12-3.02), p=0.02; CT *versus* CC=1.60 (1.2-2.1), p=0.003; TT *versus* CT +

CC=1.5 (1.02-2.2), p=0.03. Another recent meta-analysis of randomized controlled clinical trials conducted by Sadeghiyeh et al. (5), who analyzed the results of 25 case-control clinical trials, found an association between MTHFR 677C>T and ASD in general population and an association between MTHFR 1298A>C and ASD in European children. In particular, MTHFR 677C>T increased the risk of ASD in children in 5 genetic models (OR 95% CI) (5): T *versus* C=1.483, (1.188-1.850), p≤0.001; TT *versus* CC=1.834, (1.155-2.913), p=0.010; TC *versus* CC=1.512, (1.101-2.078), p=0.011; TT + TC *versus* CC=1.632, (1.261-2.113), p≤0.001; TT *versus* TC + CC=1.427, (1.002-2.032), p=0.049 (5). The latest meta-analysis of 15 randomized controlled clinical trials conducted by Li et al. (6) indicated an association between MTHFR C677T and ASD in children in 5 genetic models (viz, allelic, dominant, recessive, heterozygous, homozygous). A subgroup analysis confirmed a link between both MTHFR C677T and MTHFR A1298C and ASD in children (6).

The results of a controlled clinical study performed by Haghiri et al. (7), which involved 103 children with ASD and 130 healthy children assigned to the control group, showed a close association between MTR A2756G and ASD in children. A 1.6-fold increase in the risk of developing ASD was demonstrated in MTR A2756G carriers (7).

Thus, all 4 major polymorphic variants of folate cycle enzyme genes are associated with ASD in children. However, the available evidence regarding this association is more abundant for MTHFR C677T and MTHFR A1298C and less conclusive for MTR A2756G and MTRR A66G.

Similar data for most of the studied biochemical parameters have already been demonstrated in other clinical trials published in PubMed, though still no comprehensive analysis of the condition-specific biochemical profile has been performed (18-20,22). Thus, the results of meta-analysis of controlled clinical trials by Wang et al. (19), who covered the results of 34 trials involving 20580 children, indicate that low serum vitamin D levels are a typical response of children with ASD [mean difference (95%CI): -7.46 ng/mL, (-10.26-4.66) p<0.0001, I2=98%]. Accordingly, a systematic review and meta-analysis of controlled clinical trials performed by Li et al. (23), who included the results of 5 trials involving 349 people, indicate that vitamin D supplementation in ASD children (having a deficiency of this nutrient) helps to significantly reduce the severity of hyperactivity [pooled MD: -3.20; (-6.06, -0.34)] with low heterogeneity (I2=10%, p=0.33).

The results of a controlled clinical study enrolling 118 children, which was conducted by Yektaş et al. (24), showed a significant increase in serum homocysteine and a decrease in vitamin B12 (but not folic acid) in children with ASD and attention deficit hyperactivity disorder as compared to healthy individuals. However, data from a controlled clinical trial involving 120 patients, by Belardo et al. (20), indicate a significant decrease in serum concentrations of vitamins B6 and B12, as well as folic acid in children with ASD as compared to healthy children.

The results of controlled clinical trials performed independently by Lv et al. (25) and Al-Mosalem et al. (26) indicate a significant increase in CK serum concentration associate with hyperactivity in children with ASD as compared to healthy individuals. Data from a controlled clinical study obtained by El-Ansary et al. (27) show that increased serum concentrations of LDH and CK are biomarkers of ASD in children, along with some other metabolic profile parameters.

Previous research focused exclusively on certain metabolic parameters and did not establish links to other parameters. Often, the diagnosis of GDFC was not confirmed, and enrollment of a patient was based solely on the clinical manifestations of ASD, which might be of heterogeneous origin (23-27). In this article presents the first comprehensive analysis of the key indicators of biochemical status in children with ASD associated with GDFC, which allows reproducing a holistic picture of the metabolic profile typical for such pediatric patients.

The problem of the pathogenetic significance of the detected biochemical abnormalities in children with ASD associated with GDFC is of great importance (28). There are reports of direct neurotoxic effects, such as those produced by homocysteine and creatinine, in particular, the agonistic effect of homocysteine on N-methyl-D-aspartate receptors and, on the contrary, antagonistic effect on glycine receptors of neurons, which disrupts the balance of activation and inhibition processes in the CNS (9). Vitamin deficiency can affect metabolism in the CNS. In particular, it can affect the metabolism of key neurotransmitters, including dopamine, GABA, norepinephrine, and serotonin (29). According to Gevi et al. (18), vitamin B6 deficiency slows 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 hyperactivity and hyperexcitability symptoms in children with ASD. In addition, biochemical alterations can adversely affect the development and functioning of a child's immune system, contributing to the development of immunodeficiency and related immune dysregulation, which can mediate a number of severe immune-dependent complications in the pathogenesis of encephalopathy in children with ASD (30,31). As noted in the results of a systematic review by Mikkelsen et al. (30), deficiency of vitamin B12 and folic acid in humans can induce lymphopenia, changes in the level of CD4/CD8 index, impaired proliferation of T- and B-lymphocytes, decreased number and weakening of the functional activity of natural killer cells and neutrophils, which leads to the formation of secondary immunodeficiency. Accordingly, a specific form of immunodeficiency has been described in children with ASD who have GDFC, with a predominant affect on natural killer cells, natural killer T cells, CD8+ cytotoxic T cells and neutrophil myeloperoxidase (31).

Another critical issue is the origin of the detected biochemical disturbances. It has been recently established that the mechanism of biochemical imbalance in ASD children is complex and multicomponent (22). Thus, some disorders are a direct consequence of pathogenic polymorphic nucleotide substitutions in the genes of folic

acid cycle enzymes (i.e., disorders are directly related to folate cycle dysfunction). In particular, the above refers to the phenomenon of hyperhomocysteinemia (9). Other disorders may develop through indirect mechanisms. For example, a deficiency of a number of vitamins, in addition to GDFC, is explained by both impaired absorption of nutrients in the small intestine due to the development of persistent enterocolitis in ASD children and behavioral disorders involving dietary restrictions due to pathological food selectivity in ASD (22).

Signs of mitochondrial dysfunction, including increased serum concentrations of creatinine, LDH and CK, are the result of oxidative stress, which develops due to the direct action of homocysteine on the antioxidant system enzymes and indirect effects of immune dysregulation caused by biochemical alterations (10). Immune dysregulation is associated with an abnormal increase in the production of prooxidant compounds during persistent systemic inflammation (11).

The findings pave the way for testing therapeutic interventions based on biochemical correction in children with ASD. In 2018, Li et al. (32) published a systematic review of the results of randomized controlled clinical trials devoted to the correction of micronutrient disorders observed in children with ASD. The results of 7 studies indicate that vitamin B6 supplementation is ineffective in correcting mental disorders in children with autism. Data from two other trials have shown that the use of the methyl form of vitamin B12 leads to some improvement in mental status in children with ASD. The results of three studies on the use of vitamin D3 indicate poor effectiveness of this approach in terms of correction of mental disorders in children with autism. Data from another trial showed the benefit of folic acid in children with ASD (32). The results of a recent meta-analysis and systematic review of controlled clinical trials by Rossignol et al. (22) prove the clinical efficacy of specific metabolic therapy with methylcobalamin at a dose of 64.5-75 mg/kg for the correction of specific biochemical disorders induced by GDFC, and the associated reduction of clinical manifestations of ASD in children. The data of another systematic review and meta-analysis of randomized controlled clinical trials indicate the effectiveness of long-term use of d,l-leucovorin (folinic acid) at a dose of 0.5-1.0 to 6.0-9.0 mg/kg/day in reducing the severity of the main clinical ASD manifestations in children (33).

The obtained data allow a better understanding of the pathogenesis of the disease in children with ASD associated with GDFC. The detected pattern of laboratory biochemical alterations in blood serum may be used in the diagnostic process when screening the children with ASD for GDFC, assessing the condition severity, making prognosis, and monitoring pediatric patients with ASD associated with GDFC. In addition, these GDFC-associated serum biochemical alterations may be subjected to targeted therapeutic interventions to correct the patient's metabolic status, reduce the manifestations of GDFC-induced immunosuppression, and reduce the severity of related mental disorders in children with ASD.

CONCLUSIONS

Children with ASD associated with GDFC have a specific pattern of pathological biochemical changes that is not usual in healthy children and may explain the pathogenesis of immune and nervous system disorders and be the target of corrective therapy. The obtained data confirm and expand modern scientific hypothesis about the range of pathological biochemical changes associated with GDFC in children with ASD. The study results may be used by medical geneticists, clinical immunologists, pediatric neurologists, and psychiatrists in planning and implementing algorithms for diagnostic laboratory tests in children with ASD associated with GDFC. In particular, the results may be useful for assessing the condition severity, monitoring, and predicting further course of the disease. Besides, the data obtained can be used in planning clinical trials aimed at testing experimental drugs for biochemical correction of the detected metabolic disorders. Further clinical research is needed to increase our knowledge of the biochemical spectrum of pathological disorders in children with ASD associated with GDFC and find new approaches to the correction of biochemical status to enable mental development of children.

CONFLICT OF INTEREST

None.

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