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3 RESULTS OF THE ASSESSMENT OF IMMUNE STATUS IN CHILDREN WITH ASD: IMMUNODEFICIENCY ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

INTRODUCTION

The results of the last 5 meta-analyses of randomized controlled clinical trials indicate an association between genetically determined deficiency of folate cycle enzyme (GDFC) and autism spectrum disorders (ASD) in children [1–5], however, the mechanism of this relationship, as well as the association with additional clinical manifestations of a broad disease phenotype, has not yet been fully explained. Since there are frequent reports of the association of ASD with immunodeficiency diseases [6, 7] and signs of immune dysregulation [8, 9], and immune-dependent mechanisms of CNS damage in children with ASD have been described, including opportunistic infections [10–12], cerebral autoimmunity [8, 13], and intracerebral inflammation [14], there is reason to assume that impaired immune status is a key link in the pathogenesis of ASD in children with GDFC.

Indeed, there is now accumulating evidence that the immune system plays an important role in brain development, participating in the regulation of neuronal proliferation and synapse formation, as well as influencing neuroplasticity processes, so disruption of its functioning may be important in the formation of encephalopathy in children with ASD [15].

There are indirect signs of compromised immune systems in children with ASD, including the following: an abnormally high frequency of congenital cytomegalovirus infection [16], high microbial burden on the body [17], frequent episodes of infections and antibiotic use [18], hyperproduction of proinflammatory cytokines [14], a state of oxidative stress [19], production of anti-brain autoantibodies [8], association with certain HLA histocompatibility molecule loci [20], immunoinflammatory intestinal lesions [21], hypersensitivity to food antigens and other forms of allergic reactions [9, 22], predisposition to the formation of malignant neoplasms [23], poor tolerance of vaccines [24], and the effectiveness of a number of immunomodulatory, anti-inflammatory, and immunotherapeutic interventions [25].

Various forms of primary immunodeficiencies have been described in children with ASD, and studies of some primary immune dysfunctions indicate an increased risk of developing ASD in immunocompromised individuals.

In particular, unclassified hypogammaglobulinemia [6] and deficiencies of NK cells [26], CD8+ cytotoxic T lymphocytes [60], CD4+ T helper cells [27], neutrophil myeloperoxidase [28], IgA molecules [29, 30], IgG subclasses [6], complement component C4b [31], specific antibodies [7], and specific T lymphocytes [32] to certain infectious agents have been described in children with ASD.

Autistic traits have been described in primary immunodeficiencies such as common variable immunodeficiency [33], type II adhesion molecule deficiency [34], ataxia telangiectasia [35], DiGeorge syndrome [36], CaV1.2 channelopathy [37], and hyper-IgE syndrome [38]. Results of a population-based national study J. Isung et al. involving 8378 patients showed that humoral immunodeficiencies (common variable immunodeficiency, selective IgG subclass deficiency, and specific antibody deficiency) are associated

with an increased risk of any psychiatric disorder (adjusted odds ratio (AOR) = 1.91; 95 % CI = 1.81–2.01), and the closest association among other psychiatric illnesses was with ASD (AOR = 2.99; 95 % CI = 2.42–3.70) [39].

A systematic review by J. Mead et al. suggests evidence of immune dysregulation in children with ASD, including neuroinflammation, autoantibodies, enhanced T-cell responses, and abnormal natural killer and monocyte activity. These immune aberrations have been associated with worsening clinical features of ASD, including impaired social interaction, stereotyped behavior, and communication deficits. Furthermore, animal models have demonstrated resolution of ASD symptoms after removal of immune factors implicated in aberrant immune responses [15]. D. B. Noriega and H. F. J. Savelkoul in a systematic review devoted to ASD in children, indicate signs of immune dysregulation in such patients, including hyperproduction of pro- and suppression of anti-inflammatory cytokines, increased permeability of the blood-brain barrier, abnormal synthesis of anti-brain autoantibodies and modification of the functional activity of natural killer cells [40]. As noted by H. K. Hughes et al. in a systematic review devoted to the phenomenon of immune system dysfunction in children with ASD, in such cases, an aberrant cytokine profile, deviations in the absolute and relative number of immunocompetent cells and their subpopulations, signs of neuroinflammation, dysfunction of the adaptive and innate immune systems, imbalance of immunoglobulins of different classes and signs of autoimmunity are noted [17].

Indirect evidence of compromised immune system in patients with GDFC has been accumulated, since in such cases an association with immune-dependent complications has been demonstrated, which in classical immunology are described as manifestations of immunodeficiency diseases, in particular with autoimmune diseases [41, 42], various types of allergies [43], immunoinflammatory syndromes [44] and oncological lesions [45].

In experimental and clinical studies, various disorders of the immune status have already been reported in patients with both verified GDFC and folic acid deficiency. In particular, van der M. B. Weyden et al. established inhibition of lymphoblast metabolism in folate deficiency, which includes disorders of deoxynucleotide metabolism and the thymidylate cycle [46]. T. Partearroyo et al. showed that folic acid and vitamin B12 imbalances, typical of the GDFC phenotype, disrupt NK cells, B-lymphocyte activity, and lymphoproliferation [47]. C. Courtemanche et al. demonstrated that folate deficiency leads to inhibition of proliferation of primary CD8+ cytotoxic T lymphocytes [48]. I. Abe et al. showed that folic acid deficiency leads to a decrease in the number of NK cells, T lymphocytes and B cells, but not basophils and granulocytes [49]. A. M. Troen et al. found that unmetabolized folic acid in serum, which is noted in GDFC, causes inhibition of NK cell cytotoxicity in postmenopausal women [50]. Accordingly, Bhatnagar N. et al. described pancytopenia in severe folate deficiency [51].

Thus, the current evidence base indicates signs of impaired immune function in both patients with GDFC and children with ASD. Establishing the association of GDFC and ASD should attract additional attention to the problem of studying the immune status in children with autism. Currently, there is a lack of systematization of knowledge, a comprehensive analysis of immune status, a single solid concept of the involvement of the immune system in the pathogenesis of ASD in children with GDFC, which could not only deepen fundamental knowledge in the field of autism neuroimmunology, but also provide informative diagnostic markers and potentially useful targets for immunotherapeutic interventions for clinical practice.

In this regard, we conducted this clinical study, dedicated to an in-depth comprehensive assessment of the immune status in patients with ASD associated with GDFC, taking into account the connections with biochemical profile data and clinical syndromes of the extended phenotype.

The aim of the research: to carry out a comprehensive analysis of immune status indicators in children with ASD associated with GDFC, in connection with specific biochemical disorders and immune-dependent clinical manifestations.

MATERIALS AND METHODS OF THE RESEARCH

Data on the selection of patients for the study and control groups, the principles of clinical diagnosis of ASD, ethical and organizational aspects, diagnostics of pathogenic polymorphic nucleotide substitutions in the genes of folate cycle enzymes, and the laboratory methods used to study associated biochemical disorders are given in the **Section Materials and methods of the research in Chapter 2**.

All patients underwent a comprehensive immunological examination at the Institute of Immunology and Allergology of the Bogomolets National Medical University and/or the Sinevo laboratory (Ukraine), which, in addition to a general blood test, included the study of the subpopulation composition of lymphocytes using laser flow cytofluorimetry (Epics XI cytofluorimeter, USA) and the indirect immunofluorescence method with monoclonal antibodies to CD markers with two or three labels (CD3+, CD3+CD4+, CD3+CD8+, CD3-CD19+, CD3-CD16+CD56+, CD3+CD16+CD56+) (Beckman Coulter reagents, USA). Phagocytosis was assessed using a latex test to determine the phagocytosis index, phagocytic index, number of active phagocytes and phagocytic capacity of the blood, as well as the activity of the enzymes myeloperoxidase (flow cytofluorimetry) and NADPH oxidase (NST test). Serum concentrations of immunoglobulins of the main classes (M, G, A) were determined using the results of simple radial immunodiffusion according to Mancini and solid-phase ELISA. The concentration of IgE, IgD and IgG subclasses (IgG1, IgG2, IgG3, IgG4) in serum was measured using a home-made solid-phase enzyme-linked immunosorbent assay (VectorBEST, the Russian Federation; MDI Limbach Berlin GmbH, the Federal Republic of Germany).

The results of the diagnosis of reactivated viral infection were evaluated based on the results of quantitative PCR of blood leukocytes with species-specific primers for herpesviruses (herpes simplex viruses 1 and 2 types (HSV-1 and HSV-2), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesviruses 6, 7 and 8 types (HHV-6, HHV-7, HHV-8)), measles and rubella viruses (DNA-Technology reagents, RF). Group A beta-hemolytic streptococcus was detected by bacteriological culture from the oropharyngeal mucosa on a selective nutrient medium or by specific antitoxic immunity in blood serum (antistreptolysin-O, antistreptodornase, antihyaluronidase) (ELISA; MDI Limbach Berlin GmbH, the Federal Republic of Germany). Infection caused by Candida albicans was diagnosed based on specific IgM in serum (ELISA; MDI Limbach Berlin GmbH, the Federal Republic of Germany). Infections caused by Mycoplasma and Chlamydia pneumoniae were detected based on specific IgM in serum (ELISA, Sinevo, Ukraine) [47]. Borreliosis and yersiniosis were identified based on the Wersten blot analysis with simultaneous detection of IgM and IgG to a number of surface and deep antigens of the indicated pathogens (Sinevo, Ukraine). Toxoplasmosis was diagnosed based on specific IgA in serum (ELISA, Sinevo, Ukraine). Transferred congenital

CMV neuroinfection was identified based on the data of anamnestic studies of the newborn's serum (PCR, Department of Neurobiochemistry of the Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine and other laboratory centers).

The results of the Cunningham Panel™ (Moleculera Labs, Inc, United States of America) were analyzed to identify autoantibodies to antigens of CNS subcortical ganglia neurons in serum, including measurement of specific IgG to dopamine receptors types 1 and 2, lysoganglioside, and tubulin (ELISA) and assessment of Ca-dependent calmodulin kinase activity in neurons of diagnostic culture after contact with patient serum (cell-based assay; CBA). The results of serological studies of blood serum were evaluated for the detection of specific antineuronal autoantibodies to hippocampal autoantigens, which are currently validated as laboratory markers of autoimmune limbic encephalitis in children and adults, namely autoantibodies to glutamic acid decarboxylase (GADA), neuronal potassium channels, amphiphysin, NMDA-neuronal receptors, GABA, CV2, Yo, Ri, Ma, Hu, AMPAR 1 and 2 (ELISA; MDI Limbach Berlin GmbH, the Federal Republic of Germany). Autoimmunization to myelin was determined by serum concentrations of autoantibodies to myelin basic protein (ELISA) and signs of neurosensitization to myelin by neutrophils and CD8+ cytotoxic T lymphocytes (CBA; Department of Neuroimmunology, Institute of Neurosurgery, NAMS of Ukraine). Autoantibodies to extracerebral autoantigens were measured by Western blotting in the Sinevo laboratory (Ukraine). In particular, the results of the "ANA profile" were analyzed, which included the determination of specific IgG to the autoantigens of the nuclei of connective tissue cells nRMP/Sm, Smith antigen, RNP-70 -A and -C, SS-A, Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, CEN-pB, PCNA, dsDNA, Nucleosomes, Histones, Rib P-protein, AMA-M-2, and the "Myositis profile" with the measurement of specific IgG to the autoantigens of the lumbar striated muscles Mi-2, Ku, PM-Scl, Jo-1, PL-7, PL-12, and Ro-52 in blood serum. Signs of systemic inflammation were assessed by serum concentrations of TNF-alpha (N up to 8.1 pg/ml; ELISA), IL-6 (N up to 7 pg/ml), and tumor M2 pyruvate kinase (N up to 20 U/ml) (Sinevo, Ukraine).

Statistical analysis of the obtained information was processed by methods of structural and comparative analyses using the electronic program Microsoft Excel. To study the distribution of variants in the variation series, the Shapiro-Wilk test was used. The average values of the studied immunity indicators (X), standard deviations (σ) and errors of the mean value (m) were calculated. In order to establish the reliability of the differences in the results, the student's T-test was used with the calculation of the confidence probability coefficient p (parametric criterion) and the number of signs Z according to Urbach (non-parametric criterion). Differences were considered reliable at $p < 0.05$ and $Z < Z_{0.05}$.

To study the relationship between folate cycle gene polymorphisms and immune status indicators, Pearson's chi-square (χ^2) test was used, comparing the obtained value with the tabulated value at a given number of degrees of freedom and probability levels $p = 0.05$ and $p = 0.01$. For actual values from 5 to 9, Yates' correction was additionally applied, and for values less than 5, Fisher's exact test was used. The odds ratio and the associated 95 % CI were calculated to clarify the association between folate cycle deficiency and immune status indicators.

To assess the strength of the relationships between the studied phenomena, the φ criterion was used, taking into account that for four-dimensional tables, the φ, Chuprov and Kramer criteria take the same value, as well as the Pearson correlation coefficient (C) and the normalized value of this indicator (C').

The study was carried out as a fragment of research work commissioned by the Ministry of Health of Ukraine (state registration number 0121U107940).

RESULTS AND DISCUSSION OF THE RESEARCH

The following pattern was characteristic for the general blood analysis of SG patients: normal leukocyte count or leukopenia ($p > 0.05$; $Z > Z_{0.05}$), neutropenia ($p < 0.05$; $Z < Z_{0.05}$), lymphocytosis ($p < 0.05$; $Z < Z_{0.05}$), eosinophilia ($p < 0.05$; $Z < Z_{0.05}$) and monocytosis ($p < 0.05$; $Z < Z_{0.05}$), normal ESR ($p > 0.05$; $Z > Z_{0.05}$).

As a result of the assessment of the immune status, it was found that almost all children with GDFC were immunocompromised individuals. Although the severity of immunological disorders varied widely, some similar types of immune disorders were noted. The results of the comparative analysis of the mean values of the studied laboratory indicators of immune status in the observation groups are given in **Table 3.1**.

Table 3.1 Comparative analysis of the average values of the studied immune status indicators in patients of the observation groups

Cell/factor	X SG	X CG	Statistical significance
NK, ×109/l	0.08 ± 0.004	0.27 ± 0.09	$p < 0.05$; $Z < Z0.05$
NKT, ×109/l	0.03 ± 0.009	0.19 ± 0.08	$p < 0.05$; $Z < Z0.05$
CD8+ T lymphocytes, ×109/l	0.19 ± 0.09	0.54 ± 0.07	$p < 0.05$; $Z < Z0.05$
CD4+ T lymphocytes, ×109/l	3.39 ± 1.21	3.44 ± 1.82	$p > 0.05$; $Z > Z0.05$
CD19+ B lymphocytes, ×109/l	1.78 ± 0.23	0.35 ± 0.09	$p > 0.05$; $Z > Z0.05$
Myeloperoxidase, %	54.22 ± 4.25	89.8 ± 2.37	$p < 0.05$; $Z < Z0.05$
IgM, g/l	0.90 ± 0.42	1.22 ± 0.64	$p > 0.05$; $Z > Z0.05$
IgA, g/l	0.63 ± 0.24	0.82 ± 0.73	$p > 0.05$; $Z > Z0.05$
IgG, g/l	8.81 ± 1.29	11.94 ± 2.46	$p > 0.05$; $Z > Z0.05$
IgE, IU/ml	27.16 ± 8.85	38.83 ± 4.89	$p > 0.05$; $Z > Z0.05$
IgG1, g/l	5.13 ± 2.29	5.32 ± 0.57	$p > 0.05$; $Z > Z0.05$
IgG2, g/l	1.81 ± 0.87	1.69 ± 0.73	$p > 0.05$; $Z > Z0.05$
IgG3, g/l	0.76 ± 0.43	0.55 ± 0.11	$p > 0.05$; $Z > Z0.05$
IgG4, g/l	0.32 ± 0.12	0.28 ± 0.07	$p > 0.05$; $Z > Z0.05$

The main finding when assessing the immune status in SG children was a sharply reduced number of lymphocyte subpopulations with the immunophenotype CD3-CD16+CD56+, known as natural killers (NK), immunophenotype CD3+CD16+CD56+, or natural killer T cells (NKT), and immunophenotype CD3+CD8+, or cytotoxic T-lymphocytes, or T killers, in the peripheral blood. The average number of NK, NKT cells and CD8+ cytotoxic T lymphocytes in the peripheral blood of SG children was lower than the reference values and significantly lower than in CG children ($p < 0.05$; $Z < Z_{0.05}$), while the number of CD4+ T-helpers did not significantly differ from that in mentally healthy children ($p > 0.05$; $Z > Z_{0.05}$), and the number of CD3-CD19+

B-lymphocytes was even significantly higher than in CG ($p < 0.05$; $Z < Z_{0.05}$). A significant decrease in myeloperoxidase content in peripheral blood phagocytes was also recorded in SG children compared to CG ($p < 0.05$; $Z < Z_{0.05}$). Although serum concentrations of immunoglobulins of different classes and subclasses were mostly lower in children with autism spectrum disorders, no significant differences were found in peers without ASD ($p > 0.05$; $Z > Z_{0.05}$) due to the large proportion of cases of increased serum antibody concentrations, which most likely reflected the implementation of autoimmune reactions.

It is known that NK cells carry out antiviral and antitumor responses through spontaneous and antibody-dependent cell-mediated cytotoxicity reactions, and NKT cells carry out target selection using an invariant receptor that interacts with individual non-classical histocompatibility molecules, including the CD1 antigen. CD8+ cytotoxic T lymphocytes perform similar functions to NK and NKT cells, but they exert specific immune cytotoxicity after dual recognition of an immunogenic peptide in complex with the HLA I molecule of the target cell. Although these three types of cytotoxic lymphocytes have some overlapping functions, the role of NK cells and CD8+ cytotoxic T lymphocytes in antiviral immunity appears to be much more important than that of NKT cells. At the same time, the latter have a more pronounced immunoregulatory effect, for example, due to the production of the anti-inflammatory cytokine IL-10, which is necessary for the prevention of autoimmunity and allergy.

If we talk about the results of the structural analysis, the deficiency of NK and/or NKT cells was the most frequent finding in the assessment of the immune status and was noted among the SG participants in 82 % of cases, that is, it was a specific feature of children with ASD associated with GDFC, while in CG children signs of a similar immunological phenotype occurred only in 32 % of cases, and usually a slight decrease in the number of cells in the peripheral blood was recorded ($p < 0.05$; $Z < Z_{0.05}$). In general, the deficiency of NKT cells occurred in 73 %, the deficiency of NK lymphocytes in 65 %, and the combined disorder, which included the simultaneous deficiency of both of these lymphocyte subpopulations, in 56 % of cases. Accordingly, isolated NK cell deficiency was noted in 9 %, and isolated NKT lymphocyte deficiency in 17 % of cases (**Fig. 3.1**).

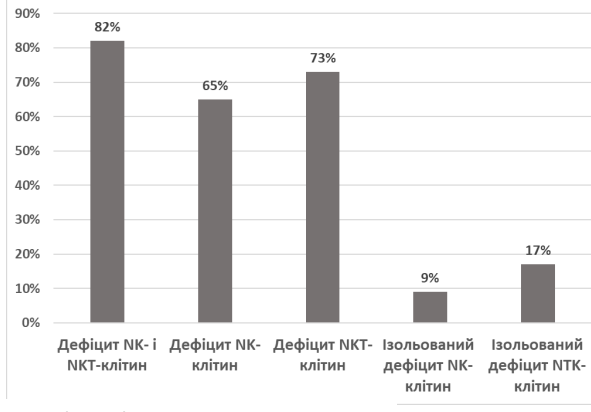

Fig. 3.1 Structure of SG (n = 225) by NK and/or NKT cell deficiency

The decrease in the number of CD8+ T lymphocytes in SG occurred in 49 %, while in CG – only in 18 % of cases ($p < 0.05$; $Z < Z_{0.05}$), and was always combined with a deficiency of NK and/or NKT cells.

Thus, in 49 % of cases there was a total deficiency of all major antiviral subpopulations of effector cytotoxic lymphocytes: T-killers, natural killers and natural killer T-lymphocytes, and it was in such children that a higher microbial load was noted at the time of entry into the study, formed mainly by intracellular pathogens compared to other SG children ($p < 0.05$; $Z < Z_{0.05}$). Combined NK and NKT cell deficiency without CD8+ T cell deficiency was observed in only 7 % of cases, while overall NK cell deficiency without a decrease in CD8+ T cell numbers occurred in 16 %, and corresponding NKT cell deficiency occurred in 24 % of cases.

All three of the effector lymphocyte subpopulations that were found to be reduced in SG patients are minority in number in the peripheral blood, so such cellular immunodeficiency usually did not lead to lymphopenia in the complete blood count. Only every tenth participant in the study group had a total decrease in all studied lymphocyte subpopulations, which was reflected in the form of lymphopenia in the complete blood count. However, a specific form of immunodeficiency with a predominant involvement of cytotoxic lymphocytes of various subpopulations, characteristic of SG, could be easily identified in the complete blood count, recording an abnormally small number of cells with the phenotype of large granular lymphocytes.

Abnormalities in the immunological examination of SG patients were also noted in the humoral link of adaptive immunity. Dysimmunoglobulinemia, which included isolated and combined deficiencies of individual classes and/or subclasses of immunoglobulins without the phenomenon of hypoimmunoglobulinemia, was detected in 47 % of cases in children of different ages and usually had a stable nature, reproducing in serial studies during follow-up ($p < 0.05$; $Z < Z_{0.05}$). IgE deficiency was most often noted in SG, which occurred in 57 % of cases. IgG subclass deficiency and combined disorders of antibody genesis were more common than in every third SG participant. IgM and IgA deficiencies were less frequently recorded, occurring in every fourth and fifth child with ASD associated with GDFC, respectively (**Fig. 3.2**).

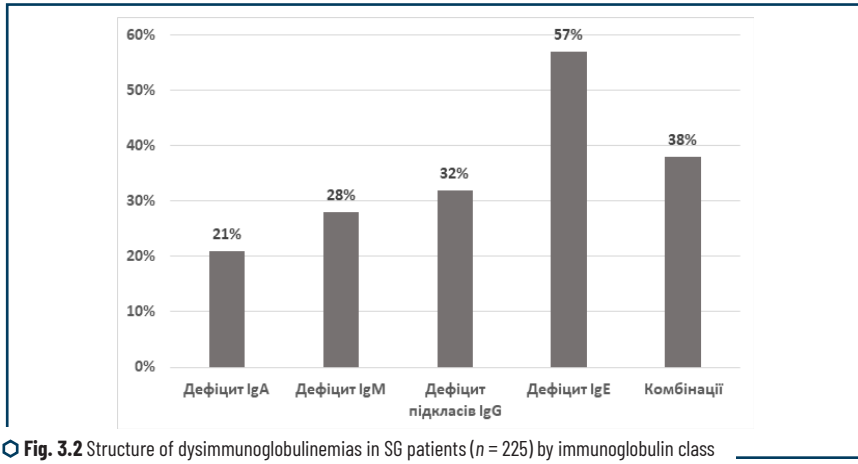

Fig. 3.2 Structure of dysimmunoglobulinemias in SG patients ($n = 225$) by immunoglobulin class and subclass deficiencies

Hypoimmunoglobulinemia, which included a simultaneous deficiency of immunoglobulins of all classes in the blood serum with a total concentration of immunoglobulins less than 7 g/l, was registered in SG only in 12 % of cases, mainly in children aged 2–3 years and was often transient. No significant differences with the CG data were found ($p > 0.05$; $Z > Z_{0.05}$).

Deficiency of the microbicidal enzyme myeloperoxidase of peripheral blood phagocytes in SG children was observed in 39 % of cases among patients of different ages and was combined with disorders in other parts of the immune system in a variable form (**Fig. 3.3**), and significant differences with CG were observed ($p < 0.05$; $Z < Z_{0.05}$). This disorder was mostly persistent, as was the deficiency of killer cells of innate immunity.

In addition, other immune status disorders were observed much less frequently in children with ASD associated with GDFC: deficiencies of CD3+CD4+ T cells (13 %), CD3-CD19+ B lymphocytes (11 % of cases), mainly in children aged 2–3 years, although no significant differences from CG were obtained ($p > 0.05$; $Z > Z_{0.05}$) (**Fig. 3.3**).

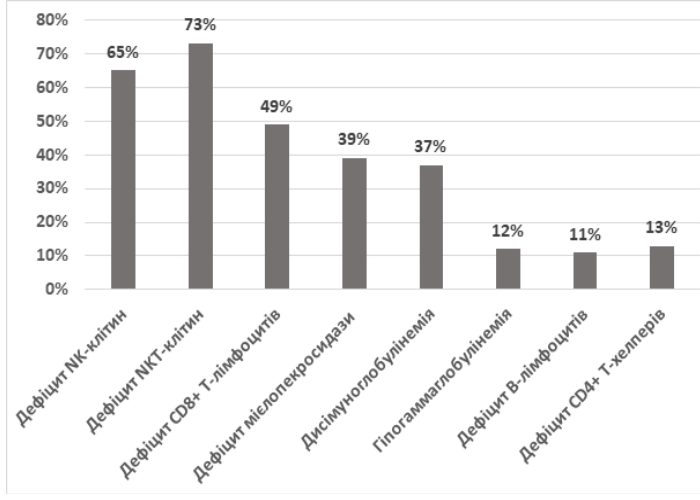

Fig. 3.3 Structure of SG ($n = 225$) by detected disorders in immune status

Based on the data of the immune status analysis, the main immunological phenotypes in children with GDFC can be distinguished. The main immunological phenotype was a deficiency of the antiviral link of cellular innate immunity, which included deficiencies of NK and NKT cells (82 % of cases). A total deficiency of the studied indicators of the cellular link of innate immunity, which included deficiencies of NK, NKT cells and myeloperoxidase of phagocytes, occurred in 35 % of cases, reducing resistance not only to viral, allergens and to a number of bacterial and fungal microbial agents due to impaired phagocytosis.

An immunological phenotype with a total lesion of the studied subpopulations of cytotoxic lymphocytes was also noted, which included deficiencies of all three subpopulations of cytotoxic cells in the peripheral blood (deficiency of large granular lymphocytes, or a combined impairment of the effector link of cellular adaptive and innate immunity), which covered almost half of the cases in SG. Almost as often, the phenomenon of dysimmunoglobulinemia was observed, reflecting a partial deficiency of the humoral component of adaptive immunity and combined with deficiencies of various subpopulations of cytotoxic lymphocytes in a variable manner in almost two-thirds of the registered cases, forming a phenotype of combined immunodeficiency with the involvement of both the cellular component of innate and/or acquired immunity and the humoral component of acquired immunity. Only in 10 % of cases was an immunological phenotype resembling a common variable immunodeficiency with hypogammaglobulinemia and deficiency of certain subpopulations of lymphocytes noted. In 13 % of cases, a phenotype corresponding to idiopathic CD4+ T-cell lymphopenia was noted in SG (**Fig. 3.4**).

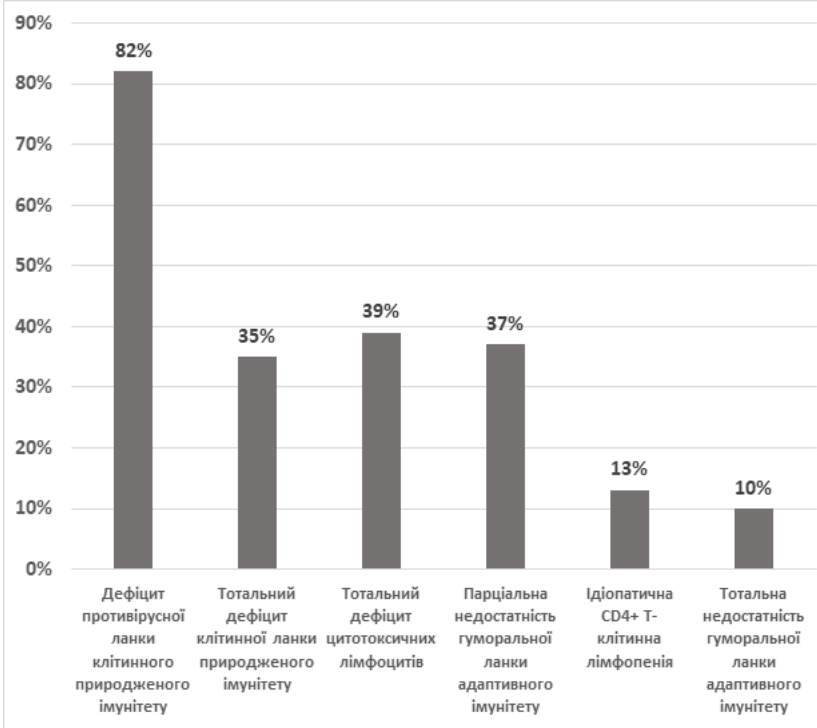

Fig. 3.4 Structure of SG (*n* = 225) according to the identified immunological phenotypes when assessing immune status

Thus, the immune status of SG children was significantly different from that of CG children, but it was important to study whether the immunological differences were associated with GDFC. The results of the calculations of the chi-square test to assess the relationship between the presence of folate cycle gene polymorphisms and impaired immune status indicators in SG and CG patients are given in **Table 3.2**.

● **Table 3.2** Results of calculations of the chi-square test of immune status indicators when comparing patients SG (n = 225) and CG (n = 51)

Cell/factor deficiency	χ^2	χ^2 with Yates' correction	χ^2 with a plausibility adjustment	Statistical significance
NK cells	32.758	30.893	30.876	$p < 0.001$
NKT cells	42.856	40.649	39.238	$p < 0.001$
CD3 + CD8 + T lymphocytes	14.625	13.458	15.540	$p < 0.001$
Myeloperoxidase	10.749	9.743	11.475	$p = 0.002$

As can be seen from the data in **Table 3.2**, the deficiency of NK, NKT cells and CD8+ cytotoxic T-lymphocytes is associated with the presence of polymorphisms in the genes of folate cycle enzymes ($p < 0.001$; $\alpha = 0.05$; **Table 3.2**).

A less pronounced, but statistically significant association of the studied genetic disorders with a deficiency of the microbicidal enzyme of phagocytes, myeloperoxidase, was also demonstrated ($p = 0.002$).

It is important not only to clarify the association between GDFC and disorders in immune status, but also to assess the strength of the relationship between the studied phenomena, the results of which are presented in **Table 3.3**.

● **Table 3.3** Assessment of the φ criterion and other indicators of the strength of the relationship between GDFC and indicators of immune status in patients with SG (n = 225) and CG (n = 51)

Indicator	NK cells		NKTcells		CD8 + T lymphocytes		Myeloperoxidase	
	value	bond strength	value	bond strength	value	bond strength	value	bond strength
criterion φ	0.345	average	0.395	average	0.230	average	0.197	weak
Pearson's correlation coefficient (C)	0.361	average	0.367	average	0.224	average	0.194	weak
normalized value of Pearson's correlation coefficient (C')	0.461	relatively strong	0.519	relatively strong	0.317	average	0.274	average

As can be seen from the data in **Table 3.3**, there was a medium-strength relationship according to the φ criterion and Pearson's correlation coefficient and a relatively strong relationship according to the normalized value of the Pearson's correlation coefficient between GDFC and NK and NKT cell deficiency in SG children. At the same time, all three studied statistical coefficients demonstrated the average strength of the relationship between GDFC and CD8+ cytotoxic T-lymphocyte deficiency. On the other hand, the φ criterion and Pearson's correlation coefficient indicated a weak relationship between GDFC and phagocyte myeloperoxidase deficiency, but the normalized value of the Pearson's correlation coefficient indicated a medium-strength relationship between the studied indicators. The results obtained are consistent with the data of the structural analysis of SG by the specific gravity of deficiencies of various factors of the immune system and the results of comparing the average values of the studied immune indicators in the observation groups.

Since the obtained data on the presence of specific immune dysfunction are a cornerstone for understanding the pathogenesis of immune-dependent disorders in children with ASD associated with GDFC, an additional verification of the obtained data was carried out using the estimation of the odds ratio and 95 % confidence interval, demonstrating the association of the studied phenomena. (**Table 3.4**).

● **Table 3.4** Results of measuring odds ratio (OR) and 95 **%** confidence interval (95 **%** CI) in studying the association of GDFC and immune status indicators among SG (*n* **=** 225) and CG patients (*n* **=** 51)

Cell/factor deficiency	OR	95 % CI	S
NK cells	6.011	3.113–11.563*	0.335
NKT cells	7.739	3.975–15.069*	0.340
CD3 + CD8 + T lymphocytes	3.802	1.857–7.784*	0.366
Myeloperoxidase	3.182	1.554–6.516*	0.366

*Note: * $\alpha = 0.05$*

As can be seen from the data in **Table 3.4**, the presence of ASD associated with GDFC increased the chance of NKT cell deficiency by 7 times, and NK cell deficiency by 6 times, indicating a close association between these phenomena. At the same time, the closeness of the association between ASD associated with GDFC and deficiencies of CD8+ cytotoxic T lymphocytes and myeloperoxidase of phagocytes was half as low, since the appearance of the specified clinical and genetic phenotype increased the chance of detecting the specified disorders of the immune system by 3 times. These results are generally consistent with the data obtained regarding the values of Pearson's chi-square, emphasizing the greater representativeness of NK and NKT cell deficiencies than deficiencies of CD8+ cytotoxic T lymphocytes and myeloperoxidase of phagocytes for characterizing the immunological phenotype in children with ASD associated with GDFC.

Thus, children with ASD associated with GDFC have clear signs of immune dysfunction with specific features that are not characteristic of mentally healthy children without GDFC. The question of the origin of signs of immune dysfunction in children with ASD associated with GDFC is important. In order to determine

whether the identified disorders of the immune status in SG children are a consequence of GDFC itself, an additional study of the associations between the most specific for GDFC violations of the biochemical status and the most characteristic pathological deviations in the indicators of the state of the immune system among SG children was conducted, the results of which are presented in **Table 3.5**.

Table 3.5 Results of the study of associations (OR; 95 % CI) between biochemical abnormalities and immune factor deficiencies in SG patients (n = 225)

Indicator	NK cells	NKT cells	CD3 + CD8 + + T lymphocytes	Myeloperoxidase
Homocysteine	8.938; 4.388–18.202*	11.375; 5.541–23.353*	7.196; 3.553–14.578*	6.632; 3.279–13.412*
Vit B6	7.727; 3.747–15.934*	7.129; 3.462–14.679*	6.853; 3.331–14.101*	5.402; 2.719–10.735*
Vit B12	7.289; 3.467–15.322*	6.465; 3.145–13.290*	6.372; 3.153–12.877*	5.181; 2.640–10.168*
Vit D	5.702; 2.779–11.702*	5.768; 2.810–11.841*	5.989; 2.916–12.300*	5.877; 2.862–12.068*
Folic acid	4.144; 2.119–8.104*	5.097; 2.531–10.264*	3.764; 1.947–7.275*	4.584; 2.313–9.086*
Creatinine	5.731; 2.881–11.399*	5.846; 2.938–11.633*	4.790; 2.444–9.388*	4.698; 2.398–9.205*
LDH	3.667; 1.897–7.088*	3.694; 1.912–7.139*	3.470; 1.796–6.702*	3.09; 1.714–6.388*
CPK	2.947; 1.527–5.685*	2.296; 1.191–4.427*	2.509; 1.301–4.838*	2.743; 1.422–5.290*

*Note: *α = 0.05*

As can be seen from the data in **Table 3.5**, all the main biochemical disorders characteristic of GDFC are associated with all the identified typical disorders of the immune status observed among children with the indicated genetic pathology. Different biochemical disorders have different closeness of association with the studied immunological abnormalities. Hyperhomocysteinemia turned out to be the most closely associated among other biochemical phenomena with all disorders of the immunological phenotype in SG children, since its presence increased the chance of identifying a deficiency of NKT cells by 11 times, NK cells by at least 9 times, CD8+ cytotoxic T lymphocytes by 7 times, and myeloperoxidase deficiency of phagocytes by at least 6 times. Deficiencies of various vitamins were associated with specific immune status disorders somewhat weaker than hyperhomocysteinemia, increasing the chance of detecting immunological abnormalities by 4–7 times. Laboratory signs of mitochondrial dysfunction were associated with the weakest of all studied indicators of biochemical status with immune status disorders among SG patients, increasing the chance of detecting an immunological abnormality only by 2–5 times. The obtained results are consistent with the data of the structural analysis of SG by the specific weight of various biochemical disorders and deficiencies of immune system factors, as well as the results of comparing the average values of the studied laboratory parameters in the observation groups, indicating a close association of biochemical and immunological disorders in children with ASD associated with GDFC.

Thus, it is shown that children with ASD associated with GDFC not only have immune dysfunction with specific features, but also that the main immunological abnormalities characteristic of the detected immune dysfunction are associated with typical biochemical disorders that represent GDFC as a pathology of metabolism in the human body.

An important question is whether the existing metabolically induced immune dysfunction in children with ASD associated with GDFC leads to clinical manifestations. From the study of immunodeficiency diseases, it is known that immunodeficiencies appear mainly in the form of 5 main syndromes – infectious, autoimmune, allergic, immunoinflammatory and oncological. Accordingly, a structural analysis of SG was performed according to the clinical manifestations of the indicated immune-dependent syndromes in SG patients both at the time of admission and in the anamnesis of the disease. The results of the specified structural analysis are shown in **Fig. 3.5**.

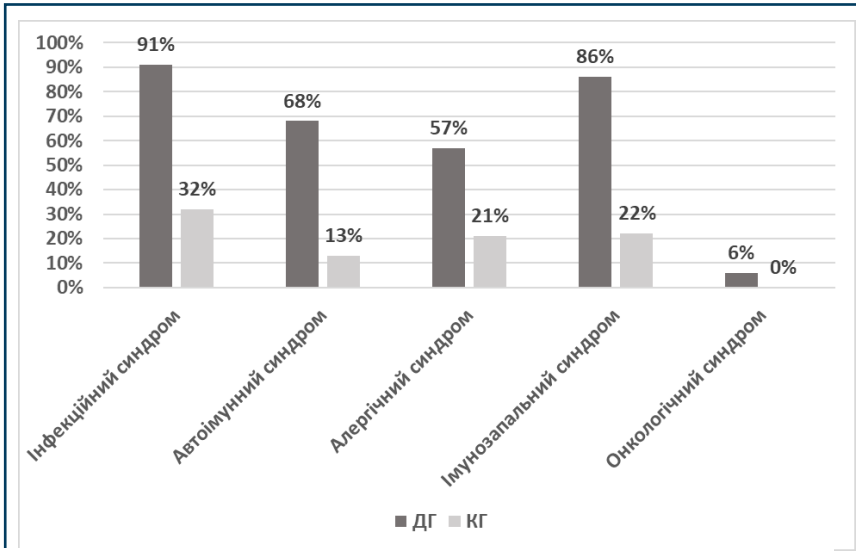

Fig. 3.5 Results of structural analysis of SG (n = 225) and CG (n = 51) by major immune-dependent syndromes

Comparison of SG results with clinical data of CG patients indicates a significantly higher frequency of all studied immune-dependent clinical syndromes in children with ASD associated with GDFC ($p > 0.05$; $Z > Z_{0.05}$). This is an indirect sign of immunocompromise of SG patients, which is consistent with the previously obtained results of the assessment of immune status. It is important to find out whether the identified immune-dependent clinical syndromes are a consequence of GDFC and the associated biochemical and immunological disorders. For this purpose, a study of associations between immune-dependent clinical syndromes and the main disorders of immune status identified in SG children was conducted, the results of which are presented in **Table 3.6**.

● **Table 3.6** Results of measuring odds ratio (OR) and 95 % confidence interval (95 % CI) in studying the association of GDFC and immune status indicators among SG patients (n = 225)

Indicator	NK cells	NKT cells	CD3+CD8+ T lymphocytes	Myeloperoxidase
Infectious syndrome	2.878; 1.576–5.255*	2.361; 1.547–5.155*	2.162; 1.824–6.163*	3.487; 1.896–6.412*
Autoimmune syndrome	8.400; 4.258–16.571*	7.556; 3.927–14.535*	10.286; 5.254–20.136*	8.816; 4.609–16.865*
Allergic syndrome	4.271; 2.312–7.892*	3.823; 2.078–7.036*	4.457; 2.409–8.244*	3.749; 2.038–6.895*
Immunoinflammatory syndrome	3.429; 1.872–6.281*	3.107; 1.699–5.681*	4.714; 2.551–8.711*	4.408; 2.391–8.128*
Oncological syndrome	1.234; 0.675–2.258	1.957; 0.991–3.862	2.023; 1.025–3.994*	1.796; 0.917–3.518

*Note: *α = 0.05*

As can be seen from **Table 3.6**, almost all identified immune-dependent clinical syndromes were associated with specific disorders of the immune status in SG children, but the strength of the associations differed in different cases. Thus, syndromes associated with immune dysregulation (autoimmune, immunoinflammatory, allergic) were more closely associated with disorders in the immune status than syndromes caused by reduced immune resistance (infectious and oncological). These data indicate that immune dysfunction in SG children has clinical significance, being associated with the induction of a number of immune-dependent complications that may be involved in the pathogenesis of encephalopathy and the development of ASD symptoms.

Previously, fragments of the immunological phenotype identified in this study were reported in patients with ASD. Thus, NK cell deficiency was reported by A. M. Enstrom et al. [52], and cytotoxic T lymphocyte deficiency by R. P. Warren et al. [53], myeloperoxidase deficiency –A. J. Russo et al. [28], and IgA deficiency as a manifestation of dysimmunoglobulinemia –M. L. Santaella et al. [29], however, only in this work was a comprehensive analysis of the immune status carried out, and – with the study of the relationship with biochemical status indicators and clinical syndromes, which allows us to recreate a holistic picture of immunopathological changes in ASD patients associated with GDFC.

NK and NKT cells are minority lymphocyte subpopulations that are extremely important for the implementation of antiviral and antitumor immunity, which can largely explain the selective impairment of antiviral resistance in SG children, as well as the increased susceptibility to the development of neoplasia, mainly virus-induced forms of oncological pathology, in patients with GDFC [45] and ASD [23]. In addition, the deficiency of NK and NKT cells is associated with an increased susceptibility to the development of autoimmune complications [54, 55] and delayed-type hypersensitivity [56], which is consistent with the repeatedly recorded phenomenon of abnormally increased production of autoantibodies to brain antigens [8, 51] and intolerance to many food antigens [57] in children with ASD.

For the first time, a deficiency of natural killer cells in children with ASD was reported by R. P. Warren et al. in 1987, and already in 1990, a selective deficiency of cytotoxic T lymphocytes in this cohort of patients was reported. In particular, a deficiency of natural killer cells was noted in 12 of 31 participants in a clinical study [26]. Recently, A. Vojdani et al. found reduced NK cell activity in 45 % of cases among 1027 children with ASD, and functional disorders were often combined with quantitative ones [58]. Later, A. M. Enstrom et al. characterized in more detail the disorders of the functional activity of natural killer cells in children with ASD. There was an abnormally increased spontaneous production of perforin, granzyme B and proinflammatory cytokines, including interferon-gamma ($p < 0.01$), but there was a sharply reduced cytotoxicity against K562 cells compared with CG ($p < 0.001$) [59]. A. R. Torres et al. in a controlled clinical study showed an abnormally increased expression of the killing-activating receptor of natural killers 2DS1 and the associated ligand HLA-C2 in children with ASD, which deepened the understanding of the mechanism of increased proinflammatory potential of NK cells in such cases [59]. However, according to the results of the work of P. Ashwood et al. in children with the ASD phenotype with severe neuropsychiatric manifestations, the number of NK cells was 40 % higher than in CG [60].

A. J. Russo et al. in a controlled clinical study showed that neutrophil myeloperoxidase deficiency is a specific feature in children with ASD, which is associated with persistent gastrointestinal symptoms related to immunoinflammatory damage to the intestine [28]. Primary myeloperoxidase deficiency occurs in the population with a frequency of 1 case per 2000–4000 inhabitants, and candidal infection is the most frequent clinical manifestation of this immunodeficiency [56]. Accordingly, H. K. Hughes and P. E. Ashwood in a controlled clinical study not only showed an abnormally high frequency of candidal infection in children with ASD, but also the fact that candidiasis was the leading factor in the damage to the gastrointestinal tract [61].

GDFC has previously been associated with several autoimmune diseases, the pathogenesis of which is dominated by cellular immunopathological reactions, including multiple sclerosis [62] and rheumatoid arthritis [41]. It is important to note that these autoimmune lesions are also prevalent in patients with primary NK and NKT cell deficiencies [54, 55]. In addition, polymorphisms of folate cycle genes [45], as well as autism itself [23], have been associated with malignant neoplasms, which are known to be characteristic manifestations of primary NK and NKT cell deficiencies due to reduced antitumor immune surveillance. Thus, the data obtained allow us to find a missing link in the pathogenesis of infectious, autoimmune, allergic and neoplastic lesions in patients with GDFC, which is a primary immunodeficiency associated with a predominant lesion of killer cells of innate and acquired immunity.

We have shown the heterogeneous impact of biochemical disorders caused by GDFC on various factors of the immune status of children with ASD. At the moment, another immunodeficiency is known, in which the metabolic defect is present in all cells of the body, but a clinically significant disorder is formed only in certain subpopulations of lymphocytes, which determines the development of immune dysfunction, and not a classic metabolic genetic disease. We are talking about primary adenosine deaminase deficiency, in which a selective deficiency of T lymphocytes develops, although the mutant gene is expressed in many cells of the human body [63].

In addition, a primary immunodeficiency is already known and well characterized, caused not by classic Mendelian mutations, but by pathogenic polymorphic substitutions in the gene encoding a component of the immune system. Thus, in hereditary mannose-binding lectin deficiency, there are combinations of polymorphisms

of structural genes and the promoter region, which cause abnormally low production of this immune factor. The clinical picture of this immunodeficiency is also dominated by various infectious, allergic, autoimmune, immunoinflammatory, oncological and neuropsychiatric lesions, which closely resemble those in GDFC [64].

Thus, similar forms of primary immunodeficiencies have already been described and studied in clinical immunology, which makes it easier to understand the nature of a new immunodeficiency disease associated with a genetically determined violation of the activity of folate cycle enzymes.

It should be emphasized that some other mental disorders are now associated with GDFC, including major depression, bipolar disorder, and schizophrenia. The data we obtained suggest an immune-dependent component of pathogenesis in such cases.

CONCLUSIONS TO THE SECTION 3

All these data allow us to conclude that GDFC leads to the development of a special form of primary immunodeficiency with a variable immunological phenotype, with the predominant involvement of NK, NKT cells, CD8+ cytotoxic T lymphocytes, myeloperoxidase phagocytes, which are often combined with dysimmunoglobulinemia in an arbitrary manner. This immunodeficiency has a dysmetabolic nature, since its main components are associated with specific biochemical disorders characteristic of GDFC, and most likely determines a sharp decrease in resistance to intracellular microorganisms and tumors, signs of systemic inflammation, autoimmune and allergic reactions, which are typical of children with ASD. We propose to call this new primary immunodeficiency as *immunodeficiency associated with a genetic disorder of the folate cycle*.

Immunodeficiency allows us to combine into a common phenotype seemingly disparate clinical syndromes that often develop sequentially or simultaneously in children with ASD, including various infectious lesions, immune-mediated leukoencephalopathy, PANS/PANDAS, temporal median epilepsy associated with herpesvirus infections, allergies and immunoinflammatory intestinal disorders, autoimmune connective tissue lesions, etc. Although there are obviously also direct metabolic effects, the various clinical neuropsychiatric manifestations observed in children with GDFC are mostly associated not with the direct toxic effects of homocysteine and other harmful metabolic products directly related to methylation disorders on nerve and glial cells, but with the development of immune-dependent complications mediated by the combined (cellular, humoral and phagocytic) immunodeficiency in GDFC, including neuroinfectious lesions, systemic and intracerebral inflammation, cerebral histamine-mediated processes and induction of autoimmune reactions to antigens of nervous tissue and cerebral vessels.

REFERENCES

1. Li, Y., Qiu, S., Shi, J., Guo, Y., Li, Z., Cheng, Y., Liu, Y. (2020). Association between MTHFR C677T/A1298C and susceptibility to autism spectrum disorders: a meta-analysis. BMC Pediatrics, 20 (1). https://doi.org/10.1186/s12887-020-02330-3

2. Shaik Mohammad, N., Sai Shruti, P., Bharathi, V., Krishna Prasad, C., Hussain, T., Alrokayan, S. A. et al. (2016). Clinical utility of folate pathway genetic polymorphisms in the diagnosis of autism spectrum disorders. Psychiatric Genetics, 26 (6), 281–286. https://doi.org/10.1097/ypg.0000000000000152
3. Pu, D., Shen, Y., Wu, J. (2013). Association between MTHFR Gene Polymorphisms and the Risk of Autism Spectrum Disorders: A Meta-Analysis. Autism Research, 6 (5), 384–392. https://doi.org/10.1002/aur.1300
4. Rai, V. (2016). Association of methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism with autism: evidence of genetic susceptibility. Metabolic Brain Disease, 31 (4), 727–735. https://doi.org/10.1007/s11011-016-9815-0
5. Sadeghiyeh, T., Dastgheib, S. A., Mirzaee-Khoramabadi, K., Morovati-Sharifabad, M., Akbarian-Bafghi, M. J., Poursharif, Z. et al. (2019). Association of MTHFR 677C>T and 1298A>C polymorphisms with susceptibility to autism: A systematic review and meta-analysis. Asian Journal of Psychiatry, 46, 54–61. https://doi.org/10.1016/j.ajp.2019.09.016
6. Heuer, L., Ashwood, P., Schauer, J., Goines, P., Krakowiak, P., Hertz-Picciotto, I. et al. (2008). Reduced levels of immunoglobulin in children with autism correlates with behavioral symptoms. Autism Research, 1 (5), 275–283. https://doi.org/10.1002/aur.42
7. Jyonouchi, H., Geng, L., Streck, D. L., Toruner, G. A. (2012). Immunological characterization and transcription profiling of peripheral blood (PB) monocytes in children with autism spectrum disorders (ASD) and specific polysaccharide antibody deficiency (SPAD): case study. Journal of Neuroinflammation, 9 (1). https://doi.org/10.1186/1742-2094-9-4
8. Frye, R. E., Sequeira, J. M., Quadros, E. V., James, S. J., Rossignol, D. A. (2012). Cerebral folate receptor autoantibodies in autism spectrum disorder. Molecular Psychiatry, 18 (3), 369–381. https://doi.org/10.1038/mp.2011.175
9. Lau, N. M., Green, P. H. R., Taylor, A. K., Hellberg, D., Ajamian, M., Tan, C. Z. et al. (2013). Markers of Celiac Disease and Gluten Sensitivity in Children with Autism. PLoS ONE, 8 (6), e66155. https://doi.org/10.1371/journal.pone.0066155
10. Binstock, T. (2001). Intra-monocyte pathogens delineate autism subgroups. Medical Hypotheses, 56 (4), 523–531. https://doi.org/10.1054/mehy.2000.1247
11. Nayeri, T., Sarvi, S., Moosazadeh, M., Hosseininejad, Z., Sharif, M., Amouei, A., Daryani, A. (2020). Relationship between toxoplasmosis and autism: A systematic review and meta-analysis. Microbial Pathogenesis, 147, 104434. https://doi.org/10.1016/j.micpath.2020.104434
12. Nicolson, G. L., Gan, R., Nicolson, N. L., Haier, J. (2007). Evidence for Mycoplasma ssp., Chlamydia pneunomiae, and human herpes virus-6 coinfections in the blood of patients with autistic spectrum disorders. Journal of Neuroscience Research, 85 (5), 1143–1148. https://doi.org/10.1002/jnr.21203
13. Brimberg, L., Sadiq, A., Gregersen, P. K., Diamond, B. (2013). Brain-reactive IgG correlates with autoimmunity in mothers of a child with an autism spectrum disorder. Molecular Psychiatry, 18 (11), 1171–1177. https://doi.org/10.1038/mp.2013.101
14. Saghazadeh, A., Ataeinia, B., Keynejad, K., Abdolalizadeh, A., Hirbod-Mobarakeh, A., Rezaei, N. (2019). A meta-analysis of pro-inflammatory cytokines in autism spectrum disorders: Effects of age, gender, and latitude. Journal of Psychiatric Research, 115, 90–102. https://doi.org/10.1016/j.jpsychires.2019.05.019

15. Mead, J., Ashwood, P. (2015). Evidence supporting an altered immune response in ASD. Immunology Letters, 163 (1), 49–55. https://doi.org/10.1016/j.imlet.2014.11.006
16. Sakamoto, A., Moriuchi, H., Matsuzaki, J., Motoyama, K., Moriuchi, M. (2015). Retrospective diagnosis of congenital cytomegalovirus infection in children with autism spectrum disorder but no other major neurologic deficit. Brain and Development, 37 (2), 200–205. https://doi.org/10.1016/j.braindev.2014.03.016
17. Hughes, H. K., Mills Ko, E., Rose, D., Ashwood, P. (2018). Immune Dysfunction and Autoimmunity as Pathological Mechanisms in Autism Spectrum Disorders. Frontiers in Cellular Neuroscience, 12. https://doi.org/10.3389/fncel.2018.00405
18. Matarazzo, E. B. (2002). Treatment of Late Onset Autism as a Consequence of Probable Autommune Processes Related to Chronic Bacterial Infection. The World Journal of Biological Psychiatry, 3 (3), 162–166. https://doi.org/10.3109/15622970209150618
19. Chen, L., Shi, X.-J., Liu, H., Mao, X., Gui, L.-N., Wang, H., Cheng, Y. (2021). Oxidative stress marker aberrations in children with autism spectrum disorder: a systematic review and meta-analysis of 87 studies (N = 9109). Translational Psychiatry, 11 (1). https://doi.org/10.1038/s41398-020-01135-3
20. Puangpetch, A., Suwannarat, P., Chamnanphol, M., Koomdee, N., Ngamsamut, N., Limsila, P., Sukasem, C. (2015). Significant Association ofHLA-BAlleles and Genotypes in Thai Children with Autism Spectrum Disorders: A Case-Control Study. Disease Markers, 2015, 1–7. https://doi.org/10.1155/2015/724935
21. Furlano, R. I., Anthony, A., Day, R., Brown, A., McGarvey, L., Thomson, M. A. et al. (2001). Colonic CD8 and γδ T-cell infiltration with epithelial damage in children with autism. The Journal of Pediatrics, 138 (3), 366–372. https://doi.org/10.1067/mpd.2001.111323
22. Xu, G., Snetselaar, L. G., Jing, J., Liu, B., Strathearn, L., Bao, W. (2018). Association of Food Allergy and Other Allergic Conditions With Autism Spectrum Disorder in Children. JAMA Network Open, 1 (2), e180279. https://doi.org/10.1001/jamanetworkopen.2018.0279
23. Crawley, J. N., Heyer, W.-D., LaSalle, J. M. (2016). Autism and Cancer Share Risk Genes, Pathways, and Drug Targets. Trends in Genetics, 32 (3), 139–146. https://doi.org/10.1016/j.tig.2016.01.001
24. Jefferson, T., Price, D., Demicheli, V., Bianco, E. (2003). Unintended events following immunization with MMR: a systematic review. Vaccine, 21 (25-26), 3954–3960. https://doi.org/10.1016/s0264-410x(03)00271-8
25. Melamed, I. R., Heffron, M., Testori, A., Lipe, K. (2018). A pilot study of high-dose intravenous immunoglobulin 5% for autism: Impact on autism spectrum and markers of neuroinflammation. Autism Research, 11 (3), 421–433. https://doi.org/10.1002/aur.1906
26. Warren, R. P., Foster, A., Margaretten, N. C. (1987). Reduced Natural Killer Cell Activity in Autism. Journal of the American Academy of Child & Adolescent Psychiatry, 26 (3), 333–335. https://doi.org/10.1097/00004583-198705000-00008
27. Yonk, L. J., Warren, R. P., Burger, R. A., Cole, P., Odell, J. D., Warren, W. L. et al. (1990). CD4+ helper T cell depression in autism. Immunology Letters, 25 (4), 341–345. https://doi.org/10.1016/0165-2478(90)90205-5

28. Russo, A., Krigsman, A., Jepson, B., Wakefield, A. (2009). Low serum myeloperoxidase in autistic children with gastrointestinal disease. Clinical and Experimental Gastroenterology, 2, 85–94. https://doi.org/10.2147/ceg.s6051
29. Santaella, M. L., Varela, Y., Linares, N., Disdier, O. M. (2008). Prevalence of autism spectrum disorders in relatives of patients with selective immunoglobulin A deficiency. Puerto Rico Health Sciences Journal, 27 (3), 204–208.
30. Wasilewska, J., Kaczmarski, M., Stasiak-Barmuta, A., Tobolczyk, J., Kowalewska, E. (2012). Low serum IgA and increased expression of CD23 on B lymphocytes in peripheral blood in children with regressive autism aged 3-6 years old. Archives of Medical Science, 2, 324–331. https://doi.org/10.5114/aoms.2012.28561
31. Warren, R. P. (1994). Decreased Plasma Concentrations of the C4B Complement Protein in Autism. Archives of Pediatrics & Adolescent Medicine, 148 (2), 180–183. https://doi.org/10.1001/archpedi.1994.02170020066011
32. Reinert, P., Moulias, R., Goust, J. M. (1972). Demonstration of cellular immunity deficiency limited to measles virus in 20 cases of subacute sclerosing leukoencephalitis. Archives Francaises de Pediatrie, 29 (6), 655–665.
33. Salehi Sadaghiani, M., Aghamohammadi, A., Ashrafi, M. R., Hosseini, F., Abolhassani, H., Rezaei, N. (2013). Autism in a child with common variable immunodeficiency. Iranian Journal of Allergy, Asthma and Immunology, 12 (3), 287–289.
34. Gazit, Y., Mory, A., Etzioni, A., Frydman, M., Scheuerman, O., Gershoni-Baruch, R., Garty, B.-Z. (2010). Leukocyte Adhesion Deficiency Type II: Long-Term Follow-Up and Review of the Literature. Journal of Clinical Immunology, 30 (2), 308–313. https://doi.org/10.1007/s10875-009-9354-0
35. Vinck, A., Verhagen, M. M. M., Gerven, M. van, de Groot, I. J. M., Weemaes, C. M. R. et al. (2011). Cognitive and speech-language performance in children with ataxia telangiectasia. Developmental Neurorehabilitation, 14 (5), 315–322. https://doi.org/10.3109/17518423.2011.603368
36. Shin, S., Yu, N., Choi, J. R., Jeong, S., Lee, K.-A. (2015). Routine Chromosomal Microarray Analysis is Necessary in Korean Patients With Unexplained Developmental Delay/Mental Retardation/Autism Spectrum Disorder. Annals of Laboratory Medicine, 35 (5), 510–518. https://doi.org/10.3343/alm.2015.35.5.510
37. Liao, P., Soong, T. W. (2009). CaV1.2 channelopathies: from arrhythmias to autism, bipolar disorder, and immunodeficiency. Pflügers Archiv – European Journal of Physiology, 460 (2), 353–359. https://doi.org/10.1007/s00424-009-0753-0
38. Grimbacher, B., Dutra, A. S., Holland, S. M., Fischer, R. E., Pao, M., Gallin, J. I., Puck, J. M. (1999). Analphoid marker chromosome in a patient with hyper-IgE syndrome, autism, and mild mental retardation. Genetics in Medicine, 1 (5), 213–218. https://doi.org/10.1097/00125817-199907000-00008
39. Isung, J., Williams, K., Isomura, K., Gromark, C., Hesselmark, E., Lichtenstein, P. et al. (2020). Association of Primary Humoral Immunodeficiencies With Psychiatric Disorders and Suicidal Behavior and the Role of Autoimmune Diseases. JAMA Psychiatry, 77 (11), 1147–1154. https://doi.org/10.1001/jamapsychiatry.2020.1260

40. Noriega, D. B., Savelkoul, H. F. J. (2013). Immune dysregulation in autism spectrum disorder. European Journal of Pediatrics, 173 (1), 33–43. https://doi.org/10.1007/s00431-013-2183-4
41. Bagheri-Hosseinabadi, Z., Imani, D., Yousefi, H., Abbasifard, M. (2020). MTHFR gene polymorphisms and susceptibility to rheumatoid arthritis: a meta-analysis based on 16 studies. Clinical Rheumatology, 39 (8), 2267–2279. https://doi.org/10.1007/s10067-020-05031-5
42. Dimitroulas, T., Sandoo, A., Hodson, J., Smith, J., Douglas, K., Kitas, G. (2015). Associations between asymmetric dimethylarginine, homocysteine, and the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism (rs1801133) in rheumatoid arthritis. Scandinavian Journal of Rheumatology, 45 (4), 267–273. https://doi.org/10.3109/03009742.2015.1086433
43. Li, M., Tang, Y., Zhao, E. Y., Chen, C.-H., Dong, L.-L. et al. (2021). Relationship between MTHFR gene polymorphism and susceptibility to bronchial asthma and glucocorticoid efficacy in children. Zhongguo Dang Dai Er Ke Za Zhi., 23 (8), 802–808. https://doi.org/10.7499/j.issn.1008-8830.2105035
44. Mao, N., Chen, J., Wang, J., Xie, X., Li, F., Liu, Y. et al. (2020). Correlations of Methylenetetrahydrofolate Reductase Gene c and Genomic DNA Hypomethylation Level with Ankylosing Spondylitis. Zhongguo Yi Xue Ke Xue Yuan Xue Bao, 42 (3), 307–312. https://doi.org/10.3881/j.issn.1000-503X.11516
45. Chen, F., Wen, T., Lv, Q., Liu, F. (2019). Associations between Folate Metabolism Enzyme Polymorphisms and Lung Cancer: A Meta-Analysis. Nutrition and Cancer, 72 (7), 1211–1218. https://doi.org/10.1080/01635581.2019.1677924
46. van der Weyden, M. B., Hayman, R. J., Rose, I. S., Brumley, J. (1991). Folate-deficient human lymphoblasts: Changes in deoxynucleotide metabolism and thymidylate cycle activities. European Journal of Haematology, 47 (2), 109–114. https://doi.org/10.1111/j.1600-0609.1991.tb00131.x
47. Partearroyo, T., Úbeda, N., Montero, A., Achón, M., Varela-Moreiras, G. (2013). Vitamin B12 and Folic Acid Imbalance Modifies NK Cytotoxicity, Lymphocytes B and Lymphoprolipheration in Aged Rats. Nutrients, 5 (12), 4836–4848. https://doi.org/10.3390/nu5124836
48. Courtemanche, C., Elson-Schwab, I., Mashiyama, S. T., Kerry, N., Ames, B. N. (2004). Folate Deficiency Inhibits the Proliferation of Primary Human CD8+ T Lymphocytes In Vitro. The Journal of Immunology, 173 (5), 3186–3192. https://doi.org/10.4049/jimmunol.173.5.3186
49. Abe, I., Shirato, K., Hashizume, Y., Mitsuhashi, R., Kobayashi, A., Shiono, C. et al. (2012). Folate-deficiency induced cell-specific changes in the distribution of lymphocytes and granulocytes in rats. Environmental Health and Preventive Medicine, 18 (1), 78–84. https://doi.org/10.1007/s12199-012-0286-6
50. Troen, A. M., Mitchell, B., Sorensen, B., Wener, M. H., Johnston, A., Wood, B. et al. (2006). Unmetabolized Folic Acid in Plasma Is Associated with Reduced Natural Killer Cell Cytotoxicity among Postmenopausal Women. The Journal of Nutrition, 136 (1), 189–194. https://doi.org/10.1093/jn/136.1.189
51. Bhatnagar, N., Wechalekar, A., McNamara, C. (2012). Pancytopenia due to severe folate deficiency. Internal Medicine Journal, 42 (9), 1063–1064. Portico. https://doi.org/10.1111/j.1445-5994.2012.02849.x
52. Enstrom, A. M., Lit, L., Onore, C. E., Gregg, J. P., Hansen, R. L., Pessah, I. N. et al. (2009). Altered gene expression and function of peripheral blood natural killer cells in children with autism. Brain, Behavior, and Immunity, 23 (1), 124–133. https://doi.org/10.1016/j.bbi.2008.08.001

53. Warren, R. P., Yonk, L. J., Burger, R. A., Cole, P., Odell, J. D., Warren, W. L. et al. (1990). Deficiency of Suppressor-Inducer (Cd4+Cd45ra+) T Cells in Autism. Immunological Investigations, 19 (3), 245–251. https://doi.org/10.3109/08820139009041839
54. O'Keeffe, J., Gately, C. M., Counihan, T., Hennessy, M., Leahy, T., Moran, A. P., Hogan, E. L. (2008). T-cells expressing natural killer (NK) receptors are altered in multiple sclerosis and responses to α-galactosylceramide are impaired. Journal of the Neurological Sciences, 275 (1-2), 22–28. https://doi.org/10.1016/j.jns.2008.07.007
55. Villanueva, J., Lee, S., Giannini, E. H., Graham, T. B., Passo, M. H., Filipovich, A., Grom, A. A. (2004). Natural killer cell dysfunction is a distinguishing feature of systemic onset juvenile rheumatoid arthritis and macrophage activation syndrome. Arthritis Res Ther, 7 (1). https://doi.org/10.1186/ar1453
56. Nauseef, W. M. (2014). Diagnostic Assays for Myeloperoxidase and Myeloperoxidase Deficiency. Neutrophil Methods and Protocols. Totowa: Humana Press, 537–546. https://doi.org/10.1007/978-1-62703-845-4_32
57. de Magistris, L., Picardi, A., Siniscalco, D., Riccio, M. P., Sapone, A., Cariello, R. et al. (2013). Antibodies against Food Antigens in Patients with Autistic Spectrum Disorders. BioMed Research International, 2013, 1–11. https://doi.org/10.1155/2013/729349
58. Vojdani, A., Mumper, E., Granpeesheh, D., Mielke, L., Traver, D., Bock, K. et al. (2008). Low natural killer cell cytotoxic activity in autism: The role of glutathione, IL-2 and IL-15. Journal of Neuroimmunology, 205 (1-2), 148–154. https://doi.org/10.1016/j.jneuroim.2008.09.005
59. Torres, A. R., Westover, J. B., Gibbons, C., Johnson, R. C., Ward, D. C. (2012). Activating killer-cell immunoglobulin-like receptors (KIR) and their cognate HLA ligands are significantly increased in autism. Brain, Behavior, and Immunity, 26 (7), 1122–1127. https://doi.org/10.1016/j.bbi.2012.07.014
60. Ashwood, P., Corbett, B. A., Kantor, A., Schulman, H., Van de Water, J., Amaral, D. G. (2011). In Search of Cellular Immunophenotypes in the Blood of Children with Autism. PLoS ONE, 6 (5), e19299. https://doi.org/10.1371/journal.pone.0019299
61. Hughes, H. K., Ashwood, P. (2018). Anti-Candida albicans IgG Antibodies in Children With Autism Spectrum Disorders. Frontiers in Psychiatry, 9. https://doi.org/10.3389/fpsyt.2018.00627
62. Naghibalhossaini, F., Ehyakonandeh, H., Nikseresht, A., Kamali, E. (2015). Association Between MTHFR Genetic Variants and Multiple Sclerosis in a Southern Iranian Population. International Journal of Molecular and Cellular Medicine, 4 (2), 87–93.
63. Watanabe, N., Gao, S., Wu, Z., Batchu, S., Kajigaya, S., Diamond, C. et al. (2021). Analysis of deficiency of adenosine deaminase 2 pathogenesis based on single-cell RNA sequencing of monocytes. Journal of Leukocyte Biology, 110 (3), 409–424. https://doi.org/10.1002/jlb.3hi0220-119rr
64. Hayes, B., Stanley, J., Peppers, B. P. (2021). COVID-19 Recurrence Without Seroconversion in a Patient With Mannose-Binding Lectin Deficiency. Allergy & Rhinology, 12. https://doi.org/10.1177/21526567211024140