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5 RESULTS OF THE SEARCH FOR LABORATORY SIGNS OF AUTOIMMUNE REACTIONS TO BRAIN AND EXTRACEREBRAL AUTOANTIGENS IN CHILDREN WITH AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

INTRODUCTION

Genetic deficiency of the folate cycle (GDFC) is an important associated factor in autism spectrum disorders (ASD) in children, as evidenced by the accumulated evidence base from meta-analyses of randomized controlled trials [1–5]. Biochemical abnormalities caused by GDFC have been shown to lead to immune system damage with induction of immunodeficiency and associated immune dysregulation [6]. Data from a systematic review by H. K. Hughes et al. clearly outline a range of representative pathological changes in the immune status in children with ASD, in particular, a pronounced cytokine imbalance with a predominance of pro-inflammatory mediators, aberrant subpopulation composition of blood lymphocytes, increased serum and cerebrospinal fluid concentrations of laboratory markers of neuroinflammation, multidirectional abnormal deviations in the functioning of the adaptive and innate immune systems, impaired ratios of immunoglobulins of different classes and subclasses in blood serum, and autoimmune reactions to a number of cerebral and extracerebral autoantigens [7].

At least 3 independent immune-mediated mechanisms of CNS damage in GDFC are currently known, caused by persistent immune dysfunction, which significantly contribute to the formation of encephalopathy with the clinical picture of ASD. These include the development of neurotropic opportunistic and conditionally pathogenic infections [8], autoimmune reactions to neurons and myelin of the cerebral hemispheres [9–12], and systemic and associated intracerebral aseptic inflammation caused by immune dysregulation [13, 14]. Inhibition or elimination of immune-dependent mechanisms of CNS damage appears to be a promising strategy for the treatment of ASD in children with GDFC [15].

A special role in the pathogenesis of encephalopathy in children with ASD is assigned to autoimmune mechanisms. Such ideas are based on a number of scientific evidence.

First, the results of a number of controlled clinical studies indicate the abnormal detection in patients with ASD of autoantibodies to CNS neurons, previously validated as markers of autoimmune encephalitis, which are not observed in healthy children [16]. Thus, U. K. Rout et al. found autoantibodies to the brain antigen GAD65 (GADA) among children with autism in 15 % of cases, autism spectrum disorders in 27 % of cases and in no healthy child in the control group [17]. These autoantibodies are a recognized laboratory marker of the so-called autoimmune anti-GAD65 limbic encephalitis, which leads to the development of a number of severe mental disorders in children and adults [16, 18]. At the same time, R. E. Frye et al. identified antibodies to folic acid receptors of brain neurons in children with ASD, indicating the heterogeneity of manifestations of anti-brain autoimmunity in such cases [10]. M. Cabanlit et al. established an association of ASD and the presence of autoantibodies to autoantigens of hypothalamic and thalamic neurons [9].

Second, there are several descriptions of the acute development of clinical manifestations of ASD after the onset of verified acute autoimmune limbic encephalitis in children and the achievement of clinical

improvement as a result of specific treatment of the autoimmune disease. Thus, M. C. González-Toro et al. reported two cases of autoimmune anti-NMDA limbic encephalitis in children, the clinical manifestations of which were consistent with ASD symptoms [19]. R. Kiani et al. also reported rapid autistic regression in the development of autoimmune anti-NMDA limbic encephalitis in a child [20].

Third, animal experiments have shown that anti-brain autoantibodies found in children with ASD can damage the brain in experimental rats, rabbits, and monkeys, inducing behavioral disorders that resemble the manifestations of ASD in humans. Thus, M. Gonzalez-Gronow et al. showed that catalytic IgG and IgA autoantibodies isolated from the blood of patients with autism disrupt the processes of hippocampal neuroplasticity in rats, inducing a pathological pathomorphological phenomenon similar to mesial temporal sclerosis [21], which is observed in many children with ASD according to the results of a clinical study by L. Monge-Galindo et al. [22]. Other studies have shown that after the introduction of autoantibodies obtained from the blood of children with ASD, rhesus macaques develop pronounced behavioral disorders that closely resemble those in autism in humans [23]. G. A. Mostafa and L. Y. Al-Ayadhi not only found an increased titer of autoantibodies to ganglioside M1 of nervous tissue in children with ASD, but also demonstrated a correlation between the titer of these autoantibodies and the severity of mental disorders in patients [24].

Fourth, several drugs with proven anti-inflammatory and immunomodulatory effects have demonstrated clinical efficacy in ASD, the data on which are summarized in a systematic review by J. Marchezan et al. [15], including infliximab [25] and human normal intravenous immunoglobulin [26], the mechanism of their therapeutic effect is associated precisely with the suppression of antineuronal autoimmunity and the associated intracerebral inflammation in the patient's body.

Opportunistic and conditionally pathogenic that are reactivated in the context of GDFC-induced immunodeficiency [6] may be involved in the induction of anti-brain autoimmunity in children with ASD through the phenomenon of molecular mimicry [27]. M. Mora et al. in a controlled study found abnormally high titers of antibodies to herpes simplex virus type 2 in children with ASD, which were associated with autoantibodies to brain antigens (77 % – anti-amygdala, 70 % – anti-caudate nucleus, 47.5 % – anti-cerebellum and brainstem, 45 % – anti-hippocampus, 40 % – anti-corpus callosum and 17.5 % – anti-cortex) [28]. V. K. Singh et al. established an association between high seropositivity to measles virus and human herpesvirus type 6 and abnormally high titers of autoantibodies to myelin basic protein and axonal filament protein of CNS neurons in children with ASD [12]. In another study, the authors showed cross-reactivity between anti-corrosion and anti-worm antibodies and autoantibodies against the myelin basic protein of the cerebral hemispheres in children with autism syndrome [11]. Induction of cross-reactive antibodies can be provoked by various superantigens of microorganisms in conditions of immunodeficiency caused by GDFC [6]. A. Vojdani et al. showed that in children with ASD, Chlamydia pneumoniae peptides, M-protein of streptococci and milk butyrophyllin lead to the production of defective specific antibodies with cross-reactivity, capable of recognizing not only microbial and food antigens, but also some molecules of nervous tissue, in particular – myelin basic protein, myelin-associated glycoprotein, myelin oligodendrocyte protein, proteins of neurofilaments and tubulin [29].

Therefore, the search for signs of autoimmunity in children with ASD associated with GDFC is an important task of modern neuroimmunology, the solution of which can provide useful information about immune-dependent pathways of CNS damage with the formation of the clinical phenotype of ASD and rational

approaches to immunomodulatory treatment to suppress anti-brain autoimmune reactions with potential neuroprotective effects in these patients.

The aim of the research: to study the structure of autoimmune reactions in children with ASD associated with GDFC, according to the evidence base accumulated to date, and to study the association of signs of autoimmunity with identified microorganisms to improve understanding of the pathogenesis of encephalopathy and improve diagnostic, monitoring, and treatment algorithms.

MATERIALS AND METHODS OF THE RESEARCH

Data on the selection of patients for the study and control groups, the principles of making a clinical diagnosis of ASD, ethical and organizational aspects, the diagnosis 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**. Data on the principles and approaches to laboratory assessment of the immune status of patients in the observation groups are given in the **Section Materials and methods of the research Section Materials and methods of the research**. The methods used to assess the microbial profile in individuals participating in the study are given in the **Section Materials and methods of the research in Chapter 4**.

We evaluated the known mechanisms of immune-mediated CNS damage in children with ASD according to the evidence accumulated to date in controlled clinical trials published in peer-reviewed medical journals cited in the electronic scientometric databases PubMed and Embase. Accordingly, 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), which meets modern requirements for laboratory diagnostics of PANS/PITANDS/PANDAS (pediatric acute-onset neuropsychiatric syndrome / pediatric infection–triggered autoimmune neuropsychiatric disorder / pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections) in children according to the review data of D. Dop et al. [30]. The results of a recent controlled clinical study by C. Shimasaki et al. prove the relevance of the results of the Cunningham panel in the diagnosis of autoimmune subcortical encephalitis in children [31]. 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, neuronal NMDA receptors, GABA, CV2, Yo, Ri, Ma, Hu, AMPAR 1 and 2 (ELISA; MDI Limbach Berlin GmbH, Germany), which corresponds to modern approaches to the diagnosis of autoimmune limbic encephalitis in humans [18]. In particular, such diagnostics were performed according to the data of a systematic review by A. Budhram et al., devoted to a comprehensive analysis of the informativeness of validated methods of paraclinical diagnostics of autoimmune limbic encephalitis in the modern population [16].

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), as recommended by V. K. Singh et al. [11, 12]. Autoantibodies to extracerebral autoantigens were measured by Western blotting in the Sinevo laboratory (Ukraine), which is in accordance with generally accepted approaches in modern rheumatology [32, 33]. In particular, the results of the "ANA profile" were analyzed, which included the determination of specific IgG to the autoantigens of the connective tissue cell nuclei 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 the serum concentration of TNF-alpha (*N* up to 8.1 pg/ml; ELISA; Sinevo, Ukraine) according to the data of the systematic review by A. Masi et al. [13].

Statistical processing of the material was carried out by comparative and structural analyses. To determine the probability of differences between indicators in the observation groups, the parametric student's T-test with the confidence probability indicator p and the non-parametric criterion – the number of signs Z according to Yu. Urbach were used. Differences were considered probable at $p < 0.05$ and $Z < Z_{0.05}$. To study the associations between the studied indicators, the odds ratio (OR) and 95 % confidence interval (95 % CI) were used. Microsoft Excel was used to perform statistical calculations.

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

In SG patients, laboratory signs of all five types of autoimmunity studied to cerebral and extracerebral autoantigens with different mechanisms of development and targets of autoaggression were identified, which were previously reported in the results of separate and unsystematic clinical studies evaluating autoimmunity in both children with ASD and patients with GDFC.

Laboratory signs of autoimmunity were detected among SG children in 68 % of cases, with at least two-thirds of them having a combination of several different autoimmune reactions, and in one-third – a combination of three or four different manifestations of autoimmunity. These data differ from similar results in CG, where signs of autoimmune reactions were detected in only 13 % of cases ($p < 0.05$; $Z < Z_{0.05}$), and combinations of different autoimmune reactions – in 3 % of cases ($p < 0.05$; $Z < Z_{0.05}$). Thus, children with ASD associated with GDFC generally exhibit laboratory signs of autoimmunity to the studied brain and extracerebral autoantigens, which are not typical for healthy children of similar gender and age. We can speak of an abnormal syndrome of impaired maintenance of immune tolerance to self-antigens in SG patients.

Although the reported cases of laboratory signs of autoimmunity to extracerebral autoantigens in SG were somewhat more frequent than to cerebral autoantigens, there was no significant difference between the specific gravity of these types of autoimmunity ($p > 0.05$; $Z > Z_{0.05}$).

The distribution of SG patients (n = 225) compared with CG (n = 51) according to the detected laboratory signs of autoimmunity to various studied autoantigens of the patient's body is shown in **Fig. 5.1**. According to the detected 5 types of autoimmunity, SG was divided into 5 subgroups. The 1st subgroup of SG included patients with laboratory signs of autoimmunity to subcortical ganglia (n = 72), the 2nd – neurons of the mesolimbic system (n = 81), the 3rd – myelin of the cerebral hemispheres (n = 97), the 4th – nuclei of connective tissue cells (n = 119), and the 5th – lumbar striated muscles (n = 108).

The appearance of signs of autoimmune reactions can be explained by a state of immune dysregulation caused by immunodeficiency, which, as previously shown, is observed in children with ASD associated with GDFC [6]. These data are consistent with numerous reports and results of controlled clinical studies on the detection of laboratory signs of immunization to various autoantigens in children with ASD [9–12], as well as with the data of a recent systematic review, which summarized the accumulated data on autoimmunity among this category of patients [7]. At the same time, the obtained results are consistent with the current evidence base on the association of GDFC with an increased risk of developing a number of autoimmune diseases during ontogenesis, including autoimmunity to CNS [34] and connective tissue autoantigens [35].

Thus, according to the results of separate analysis accumulated so far, both ASD and GDFC are separately associated with signs of autoimmunity to brain and extracerebral autoantigens, therefore, the detection in this study of a similar association in the combined analysis in children with ASD associated with GDFC seems logical and consistent with the current evidence base of the results of randomized controlled clinical trials in this area.

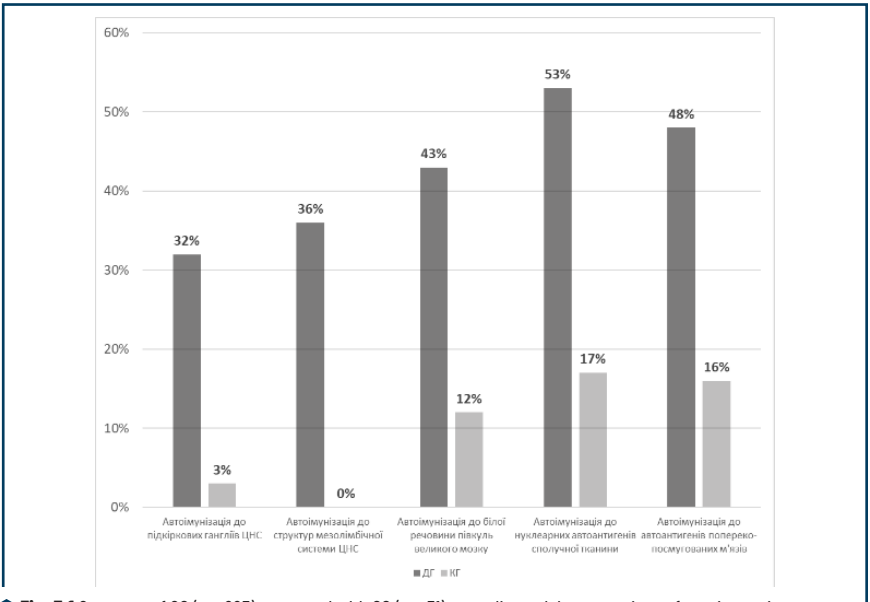


Fig. 5.1 Structure of SG (n = 225) compared with CG (n = 51) according to laboratory signs of autoimmunity to various autoantigens of the patient's body

Positive results of the Cunningham panel occurred in 32 % of cases among SG children ($p < 0.05$; $Z < Z_{0.05}$). Positive results of measuring calmodulin kinase activity prevailed (65 % of cases in this subgroup), autoantibodies to dopamine receptors type 1 and tubulin were less common (47 % and 42 %, respectively). Autoantibodies to lysoganglioside and dopamine receptors type 2 were detected only in 23 % and 17 % of children in this subgroup. Combinations of different autoantibodies to subcortical ganglia autoantigens were noted in almost all cases (94 % of cases in this subgroup) (**Fig. 5.2**).

The presence of anti-antibodies to dopamine receptors of types 1 and 2 in the blood serum was associated with signs of pronounced hyperactivity and hyperexcitability, to tubulin – with manifestations of obsessive-compulsive syndrome, to lysoganglioside – with hyperkinesis in the form of tics, myoclonus and/or dystonia, and abnormally increased activity of calmodulin kinase – with clinical signs of activation of the sympathetic autonomic nervous system ($p < 0.05$; $Z < Z_{0.05}$), which indicates the unequal influence of different autoantibodies to autoantigens of the subcortical ganglia of the CNS on the clinical symptoms of mental illness in SG children and can be used in the diagnostic and prognostic plan in the clinical management of such children.

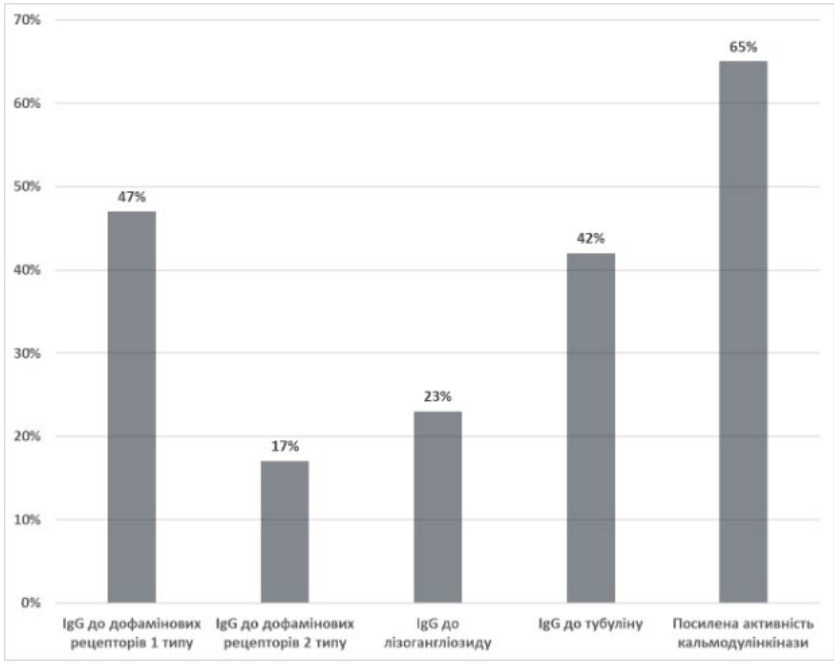


Fig. 5.2 Structure of subgroup 1 SG ($n = 72$) by type of serum autoantibodies to autoantigens of the subcortical ganglia of the CNS

Autoantibodies to autoantigens of neurons of the mesolimbic system of the temporal lobes of the cerebral hemispheres in the blood serum were noted in 81 of 225 SG patients (36 % of cases), while in the CG such autoantibodies were not identified in any case ($p < 0.05$; $Z < Z_{0.05}$). Autoantibodies to GADA occurred in SG in 48 % of cases among children of this subgroup, to potassium channels of neurons – in 39 % of cases. Autoantibodies to amphiphysin (3 individuals, 5 %), NMDA-receptors of neurons (3 individuals, 5 %) and the CV2 molecule (2 individuals, 3 % of cases) were also rarely found (**Fig. 5.3**). There was no patient in SG who had a combination of these autoantibodies, which is different from the results of the study of autoantibodies to autoantigens of the subcortical ganglia of the CNS, where combinations of autoantibodies to different autoantigens of subcortical neurons were characteristic ($p < 0.05$; $Z < Z_{0.05}$).

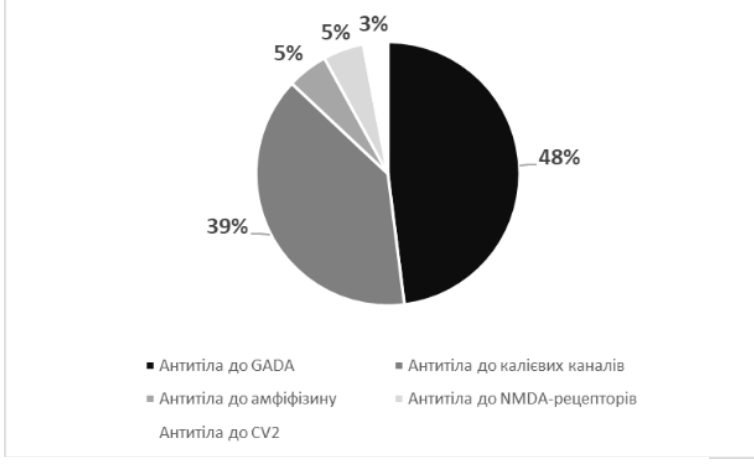


Fig. 5.3 Structure of subgroup 2 SG ($n = 81$) by type of serum autoantibodies to autoantigens of neurons of the mesolimbic system of the temporal lobes of the cerebral hemispheres

The presence of such autoantibodies in the blood serum was associated with more pronounced manifestations of hyperactivity and hyperexcitability in the child at the time of presentation, anamnestic indications of episodes of psychosis, the presence of an epileptic syndrome, and a deeper cognitive decline in the individual ($p < 0.05$; $Z < Z_{0.05}$).

Laboratory signs of autoimmunity to autoantigens of the white matter of the cerebral hemispheres were noted in 43 % of cases among SG children, which was significantly more common than in CG ($p < 0.05$; $Z < Z_{0.05}$). Autoantibodies to the basic myelin protein were most common (69 % of cases in this subgroup), which are currently considered the leading factor in damage to the white matter of the hemispheres in patients with multiple sclerosis [36]. Positive results of the assessment of cellular mechanisms of autoaggression to myelin autoantigens of the white matter of the cerebral hemispheres were less common – signs of sensitization of CD8+ cytotoxic T-lymphocytes to myelin and neurosensitization of neutrophils (24 % and 32 % of cases, respectively) (**Fig. 5.4**).

Combinations of various autoimmune reactions occurred in every fourth patient of this subgroup, which is probably less than in patients with signs of autoimmunity to autoantigens of the subcortical ganglia of the CNS ($p < 0.05$; $Z < Z_{0.05}$), but more than in children from the subgroup of autoimmunity to autoantigens of the mesolimbic system of the temporal lobes of the cerebral hemispheres ($p < 0.05$; $Z < Z_{0.05}$).

The presence of such autoantibodies in the blood serum was associated with more pronounced MR manifestations of leukoencephalopathy and physical signs of damage to the pyramidal and cerebellar conduction pathways, which clinically manifested themselves as symptoms of central paresis and/or pyramidal insufficiency and static-dynamic cerebellar ataxia, respectively ($p < 0.05$; $Z < Z_{0.05}$).

The obtained data are consistent with the results of controlled clinical studies indicating the association of GDFC with an increased risk of developing multiple sclerosis associated with autoimmunity to myelin autoantigens of the cerebral hemispheres [34], as well as with the data of scientific works on the abnormally frequent detection of laboratory manifestations of antimyelin autoimmunity in children with ASD [11, 12].

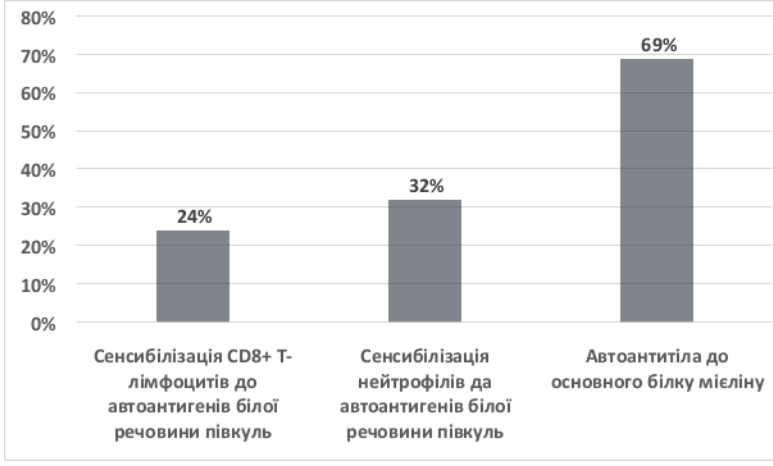


Fig. 5.4 Structure of subgroup 3 SG ($n = 97$) by type of serum autoantibodies to autoantigens of myelin of the white matter of the cerebral hemispheres

Determining different types of autoantibodies to nuclear proteins showed laboratory signs of autoimmunity to connective tissue autoantigens in 53 % of cases among SG children ($p < 0.05$; $Z < Z_{0.05}$). Positive results of autoantibodies to RNP-70 -A and -C, PM-Scl, PCNA and AMA-M-2 prevailed, which occurred in most cases among patients in this subgroup ($p < 0.05$; $Z < Z_{0.05}$). Autoantibodies to nRMP/Sm, Smith antigen, Ro-52, SS-B, Scl-70, Jo-1, CEN-pB, dsDNA, Nucleosomes, Histones, Rib P-protein were less common, each of which was registered in no more than a third of cases among patients in this subgroup (**Fig. 5.5**). Combinations of different autoantibodies to connective tissue nuclear autoantigens were almost always present in the same patient.

These serum autoantibodies were associated with anamnestic evidence of past arthritis and/or persistent arthralgias and myalgias in SG children ($p < 0.05$; $Z < Z_{0.05}$). It was among patients in this subgroup that all 11 cases of MR signs of diffuse small cerebral artery vasculopathy that were noted in SG were registered.

The obtained data are consistent with the results of randomized controlled clinical trials on the association of GDFC with an increased risk of developing rheumatic diseases, including systemic lupus erythematosus, which is characterized by the production of antinuclear autoantibodies [35].

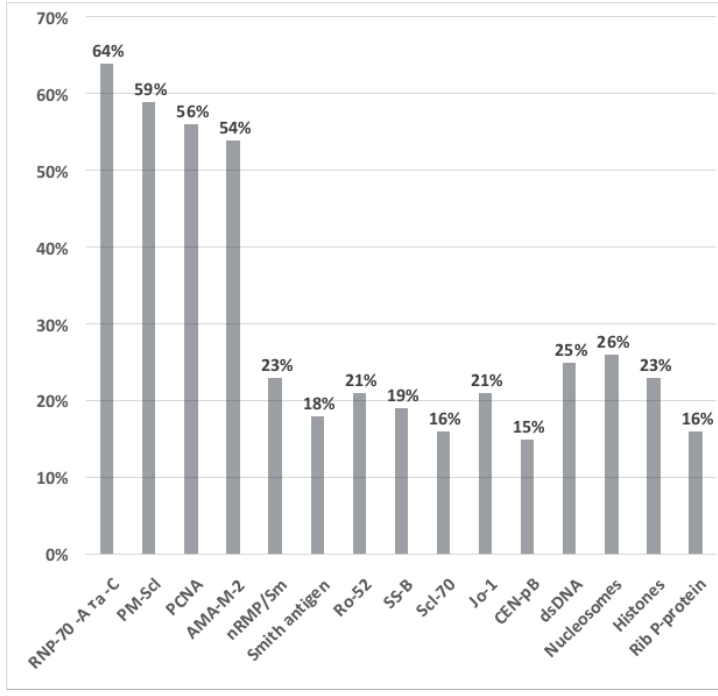
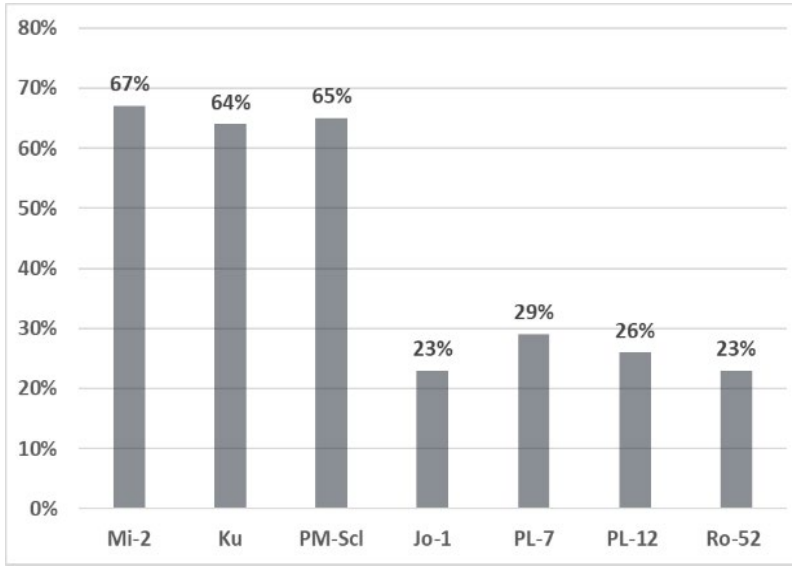


Fig. 5.5 Structure of subgroup 4 SG ($n = 119$) by type of serum autoantibodies to connective tissue nuclear autoantigens

Laboratory signs of autoimmunity to autoantigens of lumbar striated muscles occurred in 48 % of cases among SG patients, almost always in the form of combinations of different antimuscle autoantibodies, while in CG positive results of the assessment of autoimmunity to muscles were registered only in 15 % of cases, mostly in the form of single positive results ($p < 0.05$; $Z < Z_{0.05}$). Serum autoantibodies to Mi-2, Ku and PM-Scl prevailed, while autoantibodies to Jo-1, PL-7, PL-12 and Ro-52 were found almost twice as rarely in SG ($p < 0.05$; $Z < Z_{0.05}$) (**Fig. 5.6**).



⬡ **Fig. 5.6** Structure of subgroup 5 SG (n = 108) by type of serum autoantibodies to lumbar striated muscle autoantigens

The appearance of such autoantibodies in the blood serum was associated with anamnestic indications of transient periods of lethargy and limitation of mobility and/or manifestations of myositis of various groups of lumbar striated muscles, as well as with deeper disorders of the development of fine motor skills of the hands in SG children ($p < 0.05$; $Z < Z_{0.05}$). In this subgroup, there was a more pronounced decrease in skeletal muscle strength in individuals with signs of damage to the pyramidal tracts of the CNS ($p < 0.05$; $Z < Z_{0.05}$).

If we talk about the mechanism of autoimmune reactions in patients with ASD associated with GDFC, then according to the evidence accumulated so far, it is believed that the triggers of autoimmunity are mainly some opportunistic and conditionally pathogenic microbial agents, the control over which is abnormally weakened due to the presence of immune dysfunction caused by GDFC [6]. There are reasons to believe that different microorganisms have different effects on the development of autoimmune reactions, and the identification of relationships between the type of microorganism and the type of associated autoimmune lesion may be useful for rational planning of laboratory and instrumental paraclinical examinations, monitoring and prognosis, as well as the selection of adequate therapy. The results of the study of associations between microbial agents observed in children with ASD associated with GDFC and registered laboratory signs of autoimmunity are presented in **Table 5.1**.

Table 5.1 Results of the study of the association (OR; 95 % CI) of microbial agents and laboratory signs of autoimmune reactions among SG patients (*n* = 225)

Indicator	Autoimmunization to subcortical ganglia	Ат до мезолімбічної системи мозку	Autoimmunization to the white matter of the hemispheres	Ат до нуклеарних антигенів сполучної тканини	Ат до попереково–посмугованих м'язів
EBV	1.0643; 0.5112–2.2156	2.3061; 1.2038–4.4177*	5.1506; 2.4717–10.7329*	3.1157; 1.6156–6.0087*	3.0172; 1.5664–5.8118*
HHV-6	0.9903; 0.4773–2.0546	3.2356; 1.6637–6.2926*	3.1130; 1.5662–6.1873*	2.9229; 1.5192–5.6237*	2.4596; 1.2851–4.7076*
HHV-7	1.1922; 0.5693–2.4968	2.6248; 1.3633–5.0535*	2.8632; 1.449–5.6576*	2.5750; 1.3417–4.9421*	2.3861; 1.2478–4.5628*
Streptococcus	13.2407; 6.2118–28.223*	1.7667; 0.911–3.4262	1.6045; 0.8461–3.0426	1.7961; 0.9453–3.4125	1.7855; 0.9398–3.3923
Borrelia	5.9325; 2.8312–12.4308*	1.9146; 0.9902–3.7021	2.5071; 1.3093–4.8007*	3.3750; 1.7376–6.5555*	4.7884; 2.4216–9.4686*
Toxoplasma	1.7113; 0.8933–3.2785	2.2475; 1.1773–4.2907*	1.3977; 0.7383–2.6462	1.7457; 0.9193–3.3150	1.9436; 1.0215–3.6982
TTV	1.8214; 0.9487–3.4967	2.1202; 1.0915–4.1184*	2.1656; 1.1350–4.1321*	2.0165; 1.0586–3.8412*	2.1821; 1.1439–4.1627*

*Note: *α = 0.05*

As can be seen from the data in **Table 5.1**, different microorganisms were differently associated with laboratory signs of certain autoimmune reactions among SG children. No associations were found for Candida albicans, Yersinia enterocolitica, Mycoplasma pneumoniae, and Chlamydia pneumoniae, so these data were not included in the table. EBV, among other studied microbial agents, was most closely associated with the development of autoimmune reactions in SG children. Thus, EBV was associated with laboratory signs of autoimmune reactions to autoantigens of the mesolimbic system of the cerebral hemispheres, white matter of the cerebral hemispheres, nuclei of connective tissue cells, and lumbar striated muscles. In particular, identification of EBV increases the chances of detecting positive laboratory signs of autoimmunity to myelin of the white matter of the cerebral hemispheres by at least 5 times, to nuclear and muscle autoantigens by 3 times, and to proteins of neurons of the mesolimbic system of the brain by 2 times. This is consistent with the evidence accumulated so far regarding the participation of this virus as a trigger in the development of autoimmunity in the human body. The leading role of this virus can be associated with a wider arsenal of mechanisms for inducing the breakdown of immune tolerance to the antigens of the body's own body. In particular, EBV uses not only the mechanism of molecular mimicry, like most other studied microorganisms, but also polyclonal activation of B lymphocytes [36].

The results of a meta-analysis of randomized controlled clinical trials by Z. X. Li et al. indicate that EBV is a trigger for the development of an autoimmune reaction in systemic lupus erythematosus in humans, the laboratory marker of which is antinuclear autoantibodies, which, according to the results of this study, are observed in the majority of children with ASD associated with GDFC [37]. The data of a meta-analysis and systematic review of randomized controlled clinical trials by Y. H. Almohmeed et al. confirm that EBV is associated with the development of an autoimmune reaction in multiple sclerosis, when laboratory signs of autoimmunity to autoantigens of myelin of the white matter of the cerebral hemispheres are noted, which correspond to the results of the assessment of the corresponding autoimmunity in SG children [36]. Controlled clinical studies have implicated EBV as a trigger for autoimmune reactions in autoimmune limbic encephalitis [38] and dermatomyositis [33], in which positive autoantibody tests for autoantigens of CNS mesolimbic neurons and serum antimuscle autoantibodies have been identified in SG patients. These data fully explain the observed pattern of association of EBV with laboratory signs of autoimmunity in SG children.

Similar evidence has been accumulated regarding the associations of other studied microorganisms and the specific microbial spectrum characteristic of GDFC-associated immunodeficiency with autoimmune reactions to cerebral and extracerebral autoantigens.

Thus, HHV-6 and HHV-7 had a similar distribution of associations with laboratory signs of autoimmunity to autoantigens to EBV, which can be explained by the biological affinity of these microbial agents, but the chances of detecting certain autoimmune disorders with positive results of virological tests for the identification of HHV-6 and HHV-7 were lower than in the case of EBV reactivation. The presence of these viruses in a reactivated state increased the chances of detecting serological laboratory signs of autoimmunity to cerebral autoantigens at least 3 times, and to extracerebral autoantigens – 2–2.5 times. Accordingly, the results of a meta-analysis of randomized controlled clinical trials by A. Pormohammad et al. indicate that HHV-6 is a trigger for the breakdown of immune tolerance to autoantigens of the white matter of the cerebral hemispheres in multiple sclerosis, in which laboratory markers of autoimmunity to CNS myelin are similar to those observed among SG children [37]. Broccolo F. et al., having discovered selective reactivation of HHV-6 among patients with autoimmune diseases of connective tissue and muscles, which are characterized by abnormal synthesis of antinuclear and antimuscle autoantibodies, respectively, substantiated the role of this as a trigger for the development of an autoimmune reaction in these cases [39]. It has been established that transolfactory migration of HHV-6 to hippocampal neurons [40] and subsequent expression of viral proteins on these cells contributes to autoimmunization to autoantigens of neurons of the mesolimbic system of the temporal lobes of the cerebral hemispheres with the development of autoimmune limbic encephalitis [41], although HHV-6-induced forms of infectious limbic encephalitis in humans have been described [42]. J. J. Linnoila et al. in a specially designed clinical study indicated the simultaneous detection of HHV-6 DNA and autoantibodies to NMDA and GABA receptors of hippocampal neurons in cerebrospinal fluid in patients with signs of limbic encephalitis, indicating a mixed mechanism of CNS damage in many patients [43]. Accordingly, P. Venâncio et al. described an illustrative clinical case of autoimmune limbic encephalitis, in which the trigger for the development of an autoimmune reaction to hippocampal neurons was reactivated HHV-7 infection [44].

Streptococcus pyogenes was associated only with laboratory signs of autoimmune subcortical encephalitis, with a narrow range of associations among other pathogens studied, but the association found

was the strongest of all those found in this clinical study. In particular, the identification of Streptococcus pyogenes was associated with an increase in the odds of detecting autoantibodies to neurons of the subcortical nuclei of the cerebral hemispheres in the serum of the SG patient by at least 13 times. These data are consistent with the current concept of PANDAS in children [30].

Borrelia showed a combined pattern of associations, showing some common features with herpesviruses, increasing the odds of identifying signs of autoimmune reactions to autoantigens of myelin white matter of the cerebral hemispheres, connective tissue cell nuclei and proteins of lumbar striated muscles by 2, 3 and 4 times, respectively. However, unlike herpesviruses, there was no association with laboratory signs of autoimmune limbic encephalitis. At the same time, Borrelia, like Streptococcus pyogenes, showed an association with laboratory signs of PANS/PITANDS/PANDAS, increasing the odds of detecting positive Cunningham panel results by at least 5 times. These data are consistent with the results of a recent systematic review prepared by H. Rhee et al., in which borreliosis is positioned as the second most frequent trigger of the development of antineuronal autoimmune reaction in subcortical autoimmune encephalitides in humans, designated by the acronyms PANS/PITANDS/PANDAS [45].

Toxoplasma gondii had the narrowest and weakest association pattern with the studied signs of autoimmunity, increasing the chances of detecting autoantibodies to CNS hippocampal autoantigens in the serum of the SG patient by at least 2 times. Accordingly, X. Cai et al. reported the development of acute limbic anti-NMDA encephalitis with impaired mental activity during reactivation of toxoplasma infection, and complete resolution of clinical symptoms of autoimmune disease occurred after a 10-day course of azithromycin to suppress Toxoplasma reproduction without the use of immunosuppressive therapy for the autoimmune reaction against CNS neurons [46].

TTV demonstrated a distribution of associations similar to that of herpesviruses, which was associated with the simultaneous detection of these viruses in one patient in most cases and, possibly, some interaction between these infectious agents. Thus, S. S. Borkosky et al. showed that EBV stimulates the reproductive activity of TTV, which contributes to a stronger effect of this virus on the disruption of immune tolerance to myelin of the white matter of the cerebral hemispheres in multiple sclerosis [47]. However, the detected associations of TTV with laboratory signs of autoimmunity were weak – at the level of a two-fold increase in the risk of developing autoimmunity upon reactivation of this opportunistic agent from a persistent state. While the role of TTV as a trigger in multiple sclerosis [48] and autoimmune limbic encephalitis [41] has been previously reported, we consider the data on the association of this virus with autoimmunity to extracerebral autoantigens – connective tissue cell nuclei and lumbar striated muscle proteins – to be new, as we did not find relevant information in the available scientific literature.

Thus, serological signs of autoimmunity to autoantigens of the subcortical ganglia of the cerebral hemispheres were associated only with Streptococcus pyogenes and Borrelia, demonstrating close links between the phenomena under study, while laboratory signs of other autoimmune reactions had a broader pattern of associations with microbes of the studied spectrum, which were generally weaker than with positive results of the Cunningham panel. Thus, laboratory signs of autoimmune limbic encephalitis were associated with EBV, HHV-6, HHV-7, Toxoplasma and TTV, and autoimmune demyelination in the white matter of the cerebral hemispheres – with EBV, HHV-6, HHV-7, Borrelia and TTV. Laboratory signs of autoimmunity

to extracerebral autoantigens generally demonstrated a broader pattern of associations with the studied microorganisms compared to brain autoantigens, but the closeness of such associations was less. Thus, autoantibodies to autoantigens of connective tissue cell nuclei and lumbar striated muscle proteins were associated with EBV, HHV-6, HHV-7, Borrelia, and TTV, but not with Toxoplasma gondii and Streptococcus pyogenes.

CONCLUSIONS TO THE SECTION 5

The obtained data suggest the presence of laboratory signs of a syndrome of impaired immune tolerance in children with ASD associated with GDFC, which can be explained by the known state of immune dysregulation in such cases [6]. Five different types of autoimmunity with distinct mechanisms and targets of autoaggression have been identified, three of which are directed against autoantigens of the gray and white matter of the CNS, including antigens of the hippocampus, subcortical ganglia and myelin of the cerebral hemispheres, and two – against extracerebral proteins of the nuclei of connective tissue cells and lumbar striated muscles. In the development of encephalopathy in children with ASD associated with GDFC, not only autoimmune reactions to cerebral autoantigens, but also extracerebral autoimmunity may contribute. In particular, immunization to nuclear autoantigens of connective tissue can lead to the development of cerebral artery vasculopathy, and autoimmune damage to the lumbar striated muscles can exacerbate the manifestations of pyramidal insufficiency. Each of the identified types of autoimmunity is associated with distinct clinical and radiological symptoms, most likely resulting in the level of physical and mental health of children. Association studies shed light on potential mechanisms of autoimmunity in children with ASD associated with GDFC, demonstrating links between certain types of autoimmunity and reactivated opportunistic and conditionally pathogenic microorganisms of a specific spectrum with known properties to disrupt immune tolerance to self-antigens in conditions of immunosuppression. The identified signs of autoimmune reactions may be the object of effective therapeutic interventions aimed at achieving progress in the mental and physical development of children with ASD associated with GDFC by achieving neuroprotection by suppressing anti-brain autoimmunity, which should be tested in specially designed clinical trials.

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