DOI: 10.15587/978-617-8360-21-4.CH7

7 NEURORADIOLOGICAL SIGNS OF ENCEPHALOPATHY IN CHILDREN WITH AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

JUSTIFICATION

One of the important advances in psychiatry and neurology in recent years is the elucidation of the association between genetic deficiency of the folate cycle (GDFC) and autism spectrum disorders (ASD) in children. The evidence for such an association is based on the results of at least five meta-analyses of randomized controlled clinical trials [15, 20, 27, 28, 30] and a number of additional randomized controlled trials [11], the results of which are still not systematized. It has been shown that GDFC leads to the development of a number of typical biochemical disorders [10], which cause a state of a special form of immunodeficiency [19] and associated persistent oxidative stress [4], systemic inflammation, including hyperproduction of tumor necrosis factor alpha (TNF-alpha) and other pro-inflammatory cytokines with neurotoxic effects [18], opportunistic neurotropic infections, including those caused by herpes viruses 6 (HHV-6) and 7 types (HHV-7) [23], and anti-brain autoimmune reactions to neuronal autoantigens [2] and myelin [33]. It seems obvious that these three currently known immune-dependent mechanisms of cerebral damage are important in the development of encephalopathy in GDFC, one of the clinical manifestations of which is ASD, but the general concept of the pathogenesis of the disease is still not properly formulated. Since most of the studied pathways of CNS damage in GDFC are immune-mediated, they suggest a specific violation of the neuroimmune interface as a model for forming encephalopathy in such cases, which can be used in planning and conducting further clinical studies in the outlined direction.

It would be useful for clinical practice to describe typical radiological signs of such encephalopathy, which would improve the detection of the disease and optimize the assessment of the severity of the patient's condition and the effectiveness of the applied therapeutic interventions. Accordingly, Hegarty J.P. et al. in a specially designed clinical study recently showed that radiological data can be potentially informative for predicting the results of rehabilitation of children with ASD [13].

Given the multicomponent pathogenesis of encephalopathy in GDFC, one should expect the detection of a whole complex of heterogeneous neuroimaging signs, which theoretically should correlate with the implementation of certain mechanisms of cerebral damage that are the cause of their development, and the appearance of certain clinical symptoms that are a consequence of their occurrence.

The aim of the study: to describe typical neuroimaging signs of encephalopathy in children with GDFC suffering from ASD, and to search for correlations between clinical signs, mechanisms of nervous system damage and neuroimaging data to optimize the algorithm for diagnosis, monitoring and treatment.

Materials and methods. To achieve the goal, the medical data of 225 children aged 2 to 9 years with GDFC, who had clinical manifestations of ASD type (183 boys and 42 girls), were retrospectively analyzed. All of them were patients of the specialized neuroimmunological clinic Vivere (registration dossier dated 12/22/2018 No. 10/2212-M). Obtaining data for the study and processing the material was carried out in accordance with contract No. 150221 dated February 15, 2021, and the conclusion of the bioethical

examination commission (protocol No. 140 dated December 21, 2020, Bogomolets NMU). The clinical diagnosis of ASD was made by child psychiatrists according to the criteria of DSM–IV–TR (Diagnostic and Statistical Manual of mental disorders) and ICD–10 (The International Statistical Classification of Diseases and Related Health Problems). Pathogenic polymorphic variants of folate cycle genes were determined by restriction PCR based on the detection of the MTHFR C677T nucleotide substitution in monoform (68 patients), as well as – in combination with other nucleotide substitutions – MTHFR A1298C, MTRR A66G and/or MTR A2756G (157 people). These individuals constituted the study group (SG). The control group (CG) included 51 clinically healthy children (37 boys and 14 girls) of similar age distribution who did not suffer from GDFC.

The severity of clinical symptoms of ASD among SG patients was assessed according to the specialized Aberrant Behavior Checklist (ABC) scale.

Special laboratory paraclinical examination of children in the observation groups was carried out taking into account modern ideas about the mechanisms of CNS damage in ASD associated with GDFC. Thus, the diagnosis of reactivated herpesvirus infections was carried out by PCR of blood leukocytes (Department of Neurobiochemistry of the Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine) according to the data of the study by Nicolson G.L. et al. [23]. Detection of beta-hemolytic streptococcus group A was carried out 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, Germany), as stated in the systematic review by Dop D. et al., devoted to autoimmune subcortical encephalitis in children [6]. Accordingly, the Cunningham Panel™ was additionally performed to identify autoantibodies to neurons of the subcortical ganglia (ELISA, cell–based assay; Moleculera Labs, Inc, USA). The results of serological studies of blood serum were evaluated for the detection of specific antineuronal autoantibodies that are validated as 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, Ro, Hu, AMPAR 1 and 2 (enzyme-linked immunosorbent assay; MDI Limbach Berlin GmbH, Germany), which corresponds to modern approaches to the diagnosis of autoimmune limbic encephalitis [22]. Serum TNF-alpha concentration was also measured by enzyme immunoassay (N up to 8.1 pg/ml) (Sinevo, Ukraine).

Neuroimaging was performed by MRI of the brain in conventional modes (T1- and T2-weighted, FLAIR) on tomographs with a magnetic induction value of the coil of 1.5 T. Video-EEG monitoring was carried out by 30-minute recording of the bioelectric activity of the child's cerebral cortex in standard leads with photo-stimulation and hyperventilation tests.

Statistical processing of the material was carried out by comparative and structural analyses. To determine the probability of differences between the indicators in the observation groups, the parametric Student's T-test with a confidence probability indicator p and the non-parametric criterion – the number of signs Z according to Urbach Yu.V. 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 their discussion. Analysis of MR images in children with ASD associated with GDFC, as expected, revealed a number of characteristic neuroimaging signs that were typical for this category of patients and were not observed in healthy children. All detected neuroradiological signs can be combined into at least 5 heterogeneous groups according to their nature. First, manifestations of leukoencephalopathy of varying severity were noted with a predominant violation of myelination in the white matter of the parietal lobes of the cerebral hemispheres periventricularly – in the so-called peritrigonal zones, areas of terminal myelination (89 % of cases in SG and only 17 % of cases in CG; $p < 0,05$; $Z < Z_{0,05}$). Since radiological manifestations of leukoencephalopathy were noted in almost all SG patients, we can speak of these changes as a class feature for children with ASD associated with GDFC. Secondly, there were manifestations of temporal mesial sclerosis with lesions of the hippocampi, parahippocampal gyri, amygdalae and insulae (67 % of cases in SG and only 12 % of cases in CG; $p < 0.05$; $Z < Z_{0.05}$). Thirdly, symptoms of hypertrophy of the subcortical ganglia of the cerebral hemispheres, mainly the caudate nuclei, with compression of the anterior horns of the lateral ventricles were detected (39 % of cases in SG and only 7 % of cases in CG; $p < 0.05$; $Z < Z_{0.05}$). Fourth, neuroradiological signs of congenital CMV neuroinfection were diagnosed (7 % of cases in SG and only 2 % of cases in CG; $p < 0.05$; $Z < Z_{0.05}$) and residual phenomena of postnatally transferred viral encephalitis (16 % of cases and no case in CG; $p < 0.05$; $Z < Z_{0.05}$). And, finally, fifth, manifestations of the so-called small anomalies of brain development were identified (48 % of cases in SG and only 22 % of cases in CG; $p < 0,05$; $Z < Z_{0,05}$) (**Fig. 7.1**).

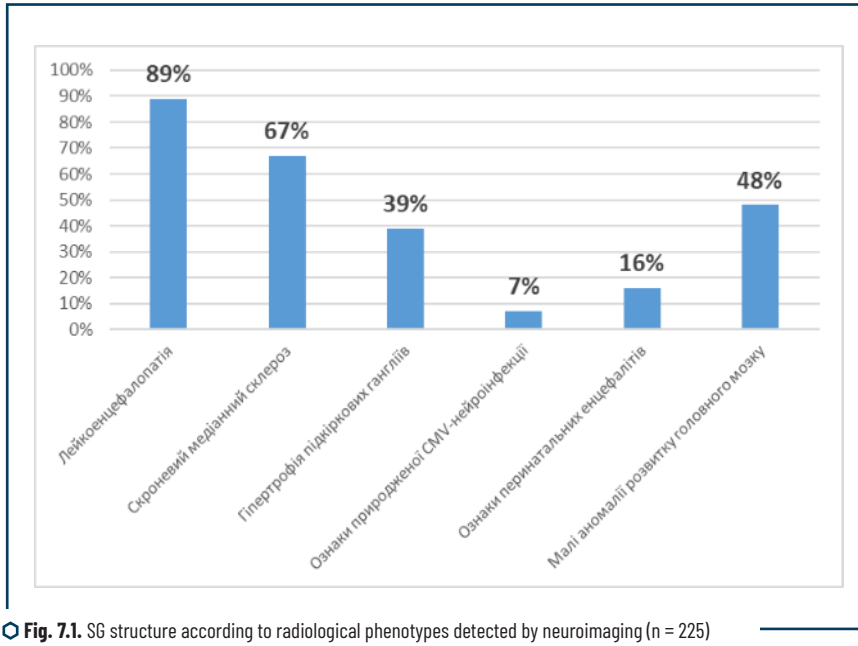


Fig. 7.1. SG structure according to radiological phenotypes detected by neuroimaging (n = 225)

The combination of 4–5 groups of neuroradiological signs of encephalopathy was considered as complete, while the presence of only 1–3 groups of instrumental signs of brain damage was considered as an incomplete neuroimaging phenotype of the disease. The complete phenotype was noted in at least 40 % of cases, and the incomplete phenotype in 60 % of cases. It should be noted that the complete neuroradiological phenotype of encephalopathy was associated with a more severe clinical condition of the patient on the ABC scale compared to the patient with an incomplete phenotype ($p < 0,05$; $Z < Z_{0,05}$).

Leukoencephalopathy, which appeared on MR images as patchy or mosaically unevenly distributed hyperintense signal of moderate or weak intensity with fuzzy contours in T2-weighted and FLAIR modes, varied in severity and prevalence in different SG patients (**Fig. 7.2**).

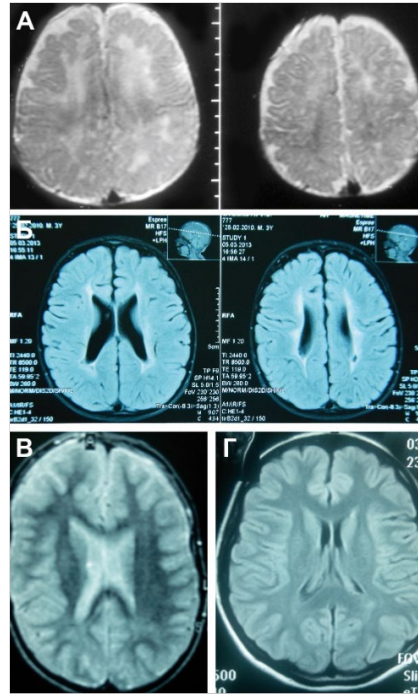


Fig. 7.2. Heterogeneity of MR manifestations of leukoencephalopathy in SG children (n = 225)
A – immaturity of the brain and diffuse myelination disorder in the white matter of the cerebral hemispheres (T2-weighted mode, axial projection);
B – a large zone of periventricular demyelination, resembling signs of leukodystrophy (FLAIR mode, axial projection);
C – pronounced bilateral myelination disorder in the parietal lobes periventricularly, brain dysgenesis, deformation of the ventricular system (T2-weighted mode, axial projection),
D – limited in size bilateral myelination disorder of the white matter in the parietal lobes periventricularly in the zone of terminal myelination (FLAIR mode, axial projection) (own observations).

It was possible to determine a diffuse form of leukoencephalopathy, in which myelination disorders were almost evenly distributed between different lobes of the cerebral hemispheres (22 % of cases). Focal forms of damage to the white matter of the hemispheres were more often noted, with the parietal lobe being most often involved, especially in the periventricular zone, where, as is known, myelin maturation during ontogenesis occurs the latest. Frontotemporoparietal lesions of the white matter of the cerebral hemispheres occurred in 13 %, temporoparietal lesions in 16 % of cases, frontoparietal lesions in 11 %, frontotemporal lesions in 3 %, and isolated lesions of the white matter of the parietal lobes in 15 % of cases. There were less pronounced radiological manifestations of leukoencephalopathy in children of the older age group (over 4 years) compared to children aged 2–4 years ($p < 0.05$; $Z < Z_{0.05}$), which probably reflects the process of delayed myelin maturation in children with ASD associated with GDFC.

Myelinopathy in children with GDFC has been reported previously. Strunk T et al. reported the phenomenon of facilitated demyelination in the white matter of the cerebral hemispheres in GDFC, describing subacute leukoencephalopathy in a patient with a heterozygous pathological polymorphic nucleotide substitution MTHFR C677T when using methotrexate at a low therapeutic dose [34]. Accordingly, Marseglia L.M. et al. in a controlled clinical study showed the association of the MTHFR A1298C and A1298C/C677T genotypes with the appearance of pathological hyperintense MR signal in the T2-weighted and FLAIR modes in the white matter of the cerebral hemispheres in full-term newborns, namely periventricular demyelination and loss of white matter volume around the ventricles with the development of vicarious ventriculomegaly [17]. Hardan A.Y. et al. conducted a specially designed clinical study to study the state of the white matter of the cerebral hemispheres in children with ASD based on the analysis of the results of brain MR spectroscopy ((1)H-MRS) with the acquisition of multivoxel echo-temporal in vivo (1)H-MRS data. Proton MR spectroscopy demonstrated a specific pattern of metabolic disorders, including an abnormal decrease in the N-acetylaspartate/creatine ratio, which indicated extensive multifocal unevenly distributed myelin damage and abnormal axonal development in the white matter of the cerebral hemispheres in children with ASD [12].

Temporal median sclerosis (**Fig. 7.3**), which appeared on MRI in the form of a hyperintense signal from the main structures of the mesolimbic system of the temporal lobes of the cerebral hemispheres in the T2-weighted and FLAIR modes, in SG children also differed in the severity and prevalence of pathological neuroimaging changes in different patients. The total form of the lesion with the involvement of all four main structures of the mesolimbic system occurred in 43 %, while partial forms with the involvement of only 1–3 of these structures occurred in 57 % of cases. Bilateral lesions (76 %) prevailed over unilateral ones (34 % of cases). In partial lesions of the mesolimbic system structures, the hippocampus was predominantly involved (64 % of cases). Hyperintense MR signal from the insula was less common (53 % of cases), and even less common – from the parahippocampal gyri (47 % of cases) and amygdalae (36 % of cases). Signs of atrophy of the mesolimbic system structures were recorded in the majority of SG patients (76 %), and the manifestations of atrophy and the prevalence of hyperintense signal were greater in patients of the older age group (over 4 years) compared to children aged 2–4 years ($p < 0.05$; $Z < Z_{0.05}$), which probably reflected the slowly progressive nature of the course of temporal median sclerosis in children with ASD associated with GDFC. In children with radiological signs of temporal median sclerosis, epileptiform bioelectric activity

was more often recorded during EEG, mainly in frontotemporal leads ($p < 0.05$; $Z < Z_{0.05}$), characteristic of mesial temporal lobe epilepsy associated with hippocampal sclerosis (MTLE–HS) [36].

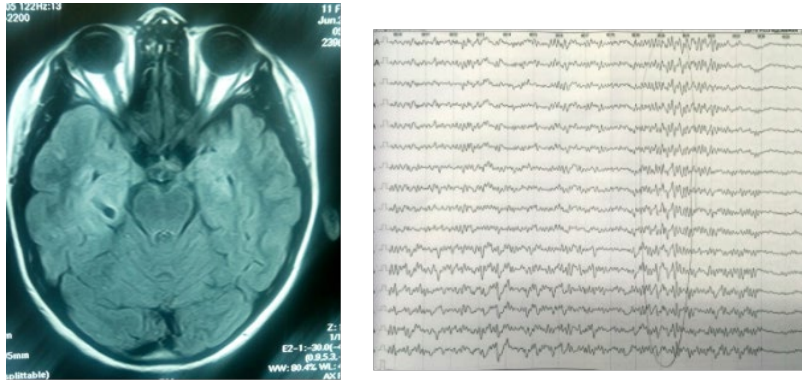


Fig. 7.3. Photo of a brain MRI image (left) in the FLAIR mode in the axial projection of a child with ASD associated with GDFC, demonstrating bilateral hyperintensity of the MRI signal in the hippocampal area and signs of atrophy of these structures due to vicarious expansion of local CSF pathways and photo of an EEG (right) of the same patient, showing epileptiform activity (pathological waves are circled) associated with the indicated neuroimaging changes (own observation)

The obtained data correspond to the results of a specially planned retrospective clinical study by Monge–Galindo L. et al. The authors presented the experience of diagnosing temporal median sclerosis in one clinical center in children with symptoms of impaired development over the past 19 years. The causes of the specified cerebral lesion were herpesvirus infection, cytomegalovirus, prenatal cerebral pathology. In 5 patients, there was an isolated epileptic syndrome, in 1 – a delay in psychomotor and intellectual development, in 1 – ASD, in 3 – an epileptic syndrome with a delay in psychospeech development, in another 1 – ASD with an epileptic syndrome, in 2 – ASD with a delay in psychospeech development, in 2 – ASD with an epileptic syndrome and a delay in psychospeech development, and finally, in another 1 – severe migraine cephalgic paroxysms [21].

Hypertrophy of the subcortical ganglia of the cerebral hemispheres was mostly bilateral and almost symmetrical (67 % of cases) (**Fig. 7.4**). Unilateral lesions were found in only 33 % of cases. The caudate nuclei were mainly involved (89 % of cases) as an isolated radiological syndrome or in combination with lesions of other subcortical ganglia (putamen, pallidum and lenticular nuclei; 27 % of cases). Complete compression of the anterior horns of the lateral ventricles due to an increase in the size of the caudate nuclei occurred in 47 %, while partial compression occurred in 53 % of cases. There were no differences in the neuroimaging manifestations of hypertrophy of the subcortical nodes in patients of different age groups ($p>0,05$; $Z>Z_{0,05}$).

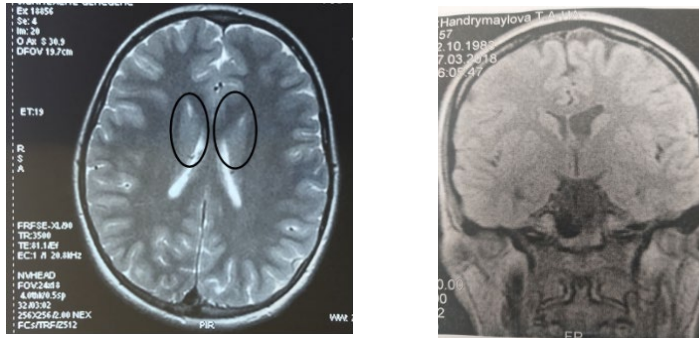


Fig. 7.4. Photo of T2-weighted axial MR images of the brain showing bilateral hypertrophy of the caudate subcortical nuclei with compression of both anterior horns of the lateral ventricles (right; lesions are circled by an oval) and FLAIR coronal images showing unilateral hypertrophy of the caudate nucleus with compression of the ipsilateral anterior horn of the lateral ventricle (left) in children with ASD associated with GDFC (own observations)

The EEG results obtained in this category of patients fit into three patterns of pathological disorders of the bioelectric activity of the cerebral cortex. With the initial MR signs of hypertrophy of the caudate subcortical ganglia, manifestations of local hyperexcitation of bioelectric activity in the projection of the subcortical nodes of the cerebral hemispheres were noted (**Fig. 7.5**). In patients with manifestations of a more pronounced increase in the size of nn. caudati, signs of bilateral lateralized synchronous electrical discharges were recorded (**Fig. 7.6**). And, finally, with MR signs of severe hypertrophy of the caudate subcortical nuclei with complete compression of the anterior horns of the lateral ventricles, diffuse hypersynchronization of bioelectric cortical rhythms took place (**Fig. 7.7**).

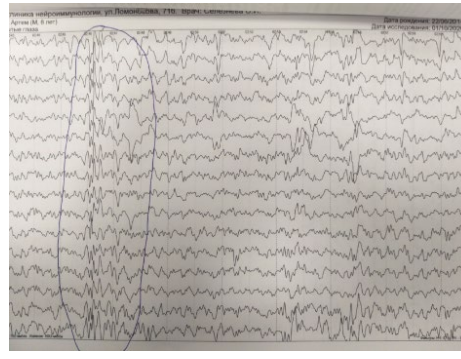


Fig. 7.5. EEG signs of excitation in the subcortical ganglia of the cerebral hemispheres in a child with GDFC associated with ASD, who had MRI signs of subcortical ganglia hypertrophy and positive results of the Cunningham panel (PANDAS; pathological complexes are circled) (own observation)

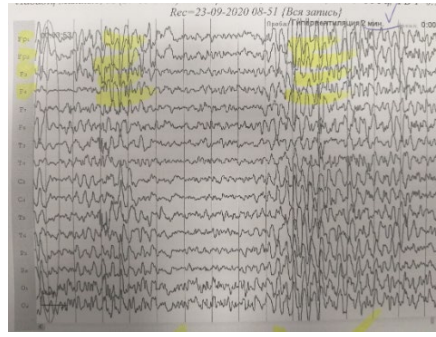


Fig. 7.6. EEG signs of bilateral lateralized synchronous electrical discharges (pathological complexes highlighted) in a child with GDFC associated with ASD, who had MRI signs of subcortical ganglia hypertrophy and positive results of the Cunningham panel (PANDAS; pathological complexes circled) (own observation)

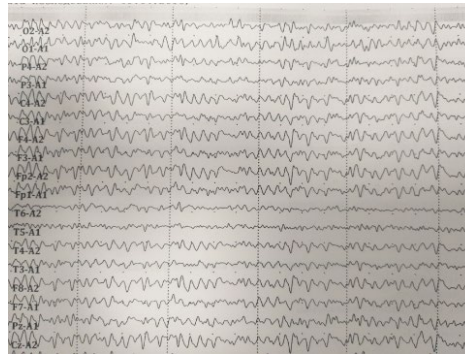
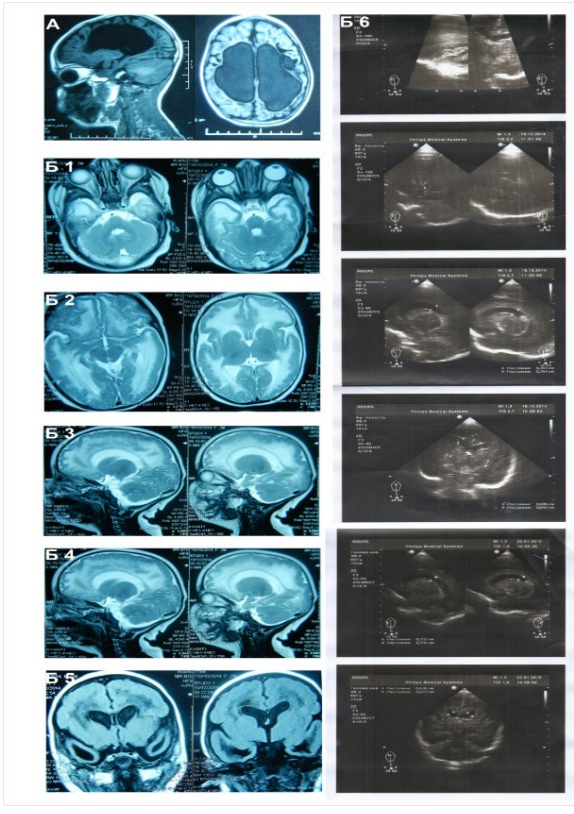


Fig. 7.7. EEG signs of diffuse hypersynchronization of cortical rhythms in a child with GDFC associated with ASD, who had MRI signs of subcortical ganglia hypertrophy and positive results of the Cunningham panel (PANDAS; own observation)

Subcortical ganglionic hypertrophy as a radiological biomarker has been discussed in the context of subcortical autoimmune encephalitides in children and adults (PANS/PITANDS/PANDAS; pediatric acute-onset neuropsychiatric syndrome/pediatric infection-triggered autoimmune neuropsychiatric disorder/ediatric autoimmune neuropsychiatric disorders associated with streptococcal infections) [6]. It has previously been reported that at least one third of children with ASD associated with GDFC have clinical and radiological signs of subcortical autoimmune encephalitis, which may explain the neuroimaging findings in SG children [1].

Neuroimaging signs of congenital CMV neuroinfection were understood as a pentad of instrumental symptoms: ventriculomegaly, hypoplasia of the corpus callosum, symmetrical cysts in the poles of the temporal lobes, extensive fields of demyelination in the white matter of the cerebral hemispheres, and periventricular

calcifications (**Fig. 7.8**). The complete neuroradiological phenotype was found in 79 % of cases, while the partial (3–4 signs) was found in 21 % of cases. Only bilateral and exclusively asymmetric lesions occurred. These changes were usually combined with signs of striatal artery vasculopathy, which were detected during neurosonographic examination in the antenatal period and/or during the first year after birth.



⬡ **Fig. 7.8.** Typical neuroimaging signs of congenital cytomegalovirus neuroinfection in children with ASD associated with GDFC (own observations)

A (FLAIR mode; sagittal (left) and horizontal (right) projections) – severe CNS malformation with pronounced ventriculomegaly and profound hypogenesis of the cerebral hemispheres (porencephaly), probably due to infection with the virus in the early period of intrauterine ontogenesis;

B – complex of classical signs of late antenatal cytomegalovirus infection: symmetrical bilateral cysts in the poles of the temporal lobes (B1, T2-weighted mode, horizontal projections), leukoencephalopathy (B2, T2-weighted mode, horizontal projections; B3, B4, T2-weighted mode, sagittal projections), agenesis of the corpus callosum (B5, FLAIR mode, coronal projections), ventriculomegaly (B2, T2-weighted mode, horizontal projections; B3, B4, T2-weighted mode, sagittal projections projections) and vasculopathy of the striatal arteries according to EchoEG (B6)

The data obtained on neuroimaging signs of congenital CMV neuroinfection in SG children correspond to the results of a controlled clinical trial by Pinillos-Pisón R. et al. The authors presented the results of an 18-year longitudinal study using CMV DNA detection on filter paper and identified the following manifestations of intrauterine CMV infection: antenatal developmental delay, microcephaly, sensorineural hearing loss, chorioretinitis, mental retardation, behavioral disorders (especially autism spectrum disorders), ventriculomegaly, intracranial calcifications, encephaloclastic disorders, leukoencephalopathy, cortical dysplasia, temporal lobe and hippocampal malformations, including cysts in the poles of the temporal lobes [25].

The data on the prevalence of congenital CMV infection obtained in this study correspond to the results of a controlled clinical trial by Sakamoto A. et al. The authors showed that congenital CMV infection with CNS involvement in children with ASD was significantly more common (7.4 %) than in the general population (0.31 %) ($p = 0.004$). CMV was identified by real-time PCR of dried neonatal blood samples and cord blood samples obtained immediately after birth [31].

Among the postnatal encephalitis cases, residual signs of temporal partial hemorrhagic-necrotic encephalitis of HSV-1/2 etiology prevailed (52 % of cases) (**Fig. 9**). Residual phenomena after limbic encephalitis (14 %) (**Fig. 10**), multifocal leukoencephalitis (15 %), stem encephalitis (9 %) and cerebellitis (10 % of cases) were less frequently recorded.

There was a decrease in neuroimaging manifestations of the encephalitis cases and their transformation due to the increase in cystic, atrophic and glial changes in the brain parenchyma in children of the older age group ($p < 0.05$; $Z < Z_{0.05}$), which reflected the natural evolution of postencephalitic foci in the human brain over time.

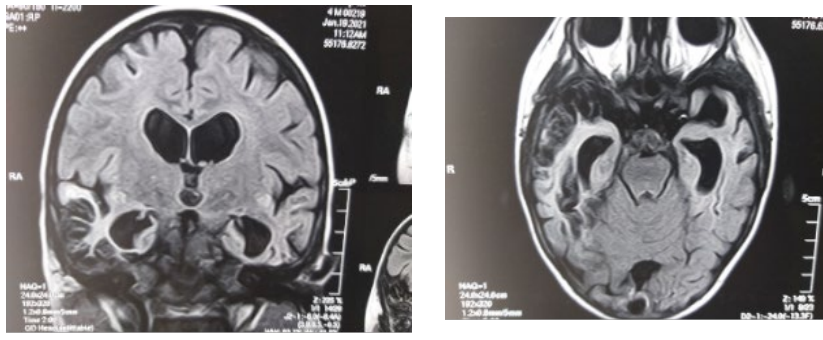


Fig. 7.9. Photo of MR images of the brain in the FLAIR mode in coronal (left) and axial (right) projections in a child with ASD associated with GDFC, demonstrating signs of bilateral atrophy and cystic-gliotic transformation in the temporal lobes of the cerebral hemispheres in the areas of bilateral temporal partial necrotic-hemorrhagic encephalitis of HSV1 etiology suffered in the first year after birth (own observation)

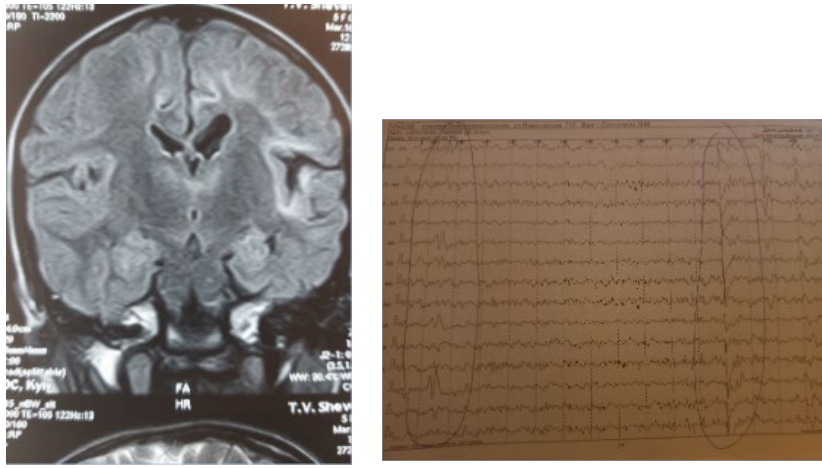


Fig. 7.10. Photo of a FLAIR MRI of the brain in axial projection (right) demonstrating signs of bilateral autoimmune limbic encephalitis caused by autoantibodies to neuronal potassium channels, with asymmetric hyperintensity of the MR signal and manifestations of atrophy of both hippocampi and insula with vicarious expansion of local CSF pathways and EEG data (left) demonstrating epileptiform activity (abnormal waves circled) in a child with ASD associated with GDFC (own observation)

The prevalence of cases of temporal partial necrotizing hemorrhagic encephalitis of HSV1 etiology among ASD children associated with GDFC is consistent with previously published clinical case reports that have repeatedly described the appearance of the clinical phenotype of ASD in children and adults shortly after transmission of this form of neuroinfection [5, 7, 8].

Among the so-called minor brain developmental anomalies in SG children, neuroradiological manifestations of pineal cyst (52 %), retrocerebellar cyst (27 %), unilateral temporal lobe pole cyst (25 %), Arnold-Chiari malformation of the first degree (27 % of cases), empty sella turcica (16 % of cases) prevailed. Mega cysterna magna (11 %) and Dandy-Walker anomaly (9 % of cases) were less common. In 67 % of cases, several minor brain developmental anomalies occurred in one patient. The obtained data are consistent with the results of a specially designed clinical study by Pavone V. et al. In this study, the authors analyzed MR images of the brain and spinal cord in patients with ASD and psychospeech delay who are carriers of pathogenic polymorphic variants of the folate cycle enzyme gene MTHFR, which showed that small congenital malformations of the brain and spinal cord structures are a typical feature in such children and a characteristic finding during neuroimaging [24].

It is important to clarify the clinical significance of the identified pathological neuroimaging MR signs in children with GDFC associated with ASD. Data on the analysis of associations between certain radiological phenomena and clinical syndromes in SG patients are presented in **Table 7.1**.

Table 7.1. Results of the study of associations (OR; 95 % CI) between pathological radiological phenomena and clinical syndromes in children with GDFC associated with ASD (n = 225)

Sign	Diffuse leukoencephalopathy	Temporal median sclerosis	Hypertrophy of subcortical ganglia	Signs of congenital CMV neuroinfection	Signs of postnatal encephalitis	Minor developmental anomalies
Regressive course of ASD	14,4; 3,0136 – 68,8073*	0,2973; 0,116 – 0,7618	1,6709; 0,5767 – 4,8414	0,8889; 0,3345 – 2,362	1,5235; 0,6101 – 3,8042	0,5365; 0,2122 – 1,3562
Epileptic syndrome	0,5333; 0,1678 – 1.6946	8,7941; 3,0351 – 25,4809*	1,0526; 0,4006 – 2,766	7,5273; 2,7278 – 20,7716*	2,9455; 1,1643 – 7,4515*	0,4068; 0,1614 – 1,0255
Obsessive-compulsive syndrome	0,6327; 0,2095 – 1,9107	1,9685; 0,6853 – 5,6542	13,3333; 4,5013 – 39,4942*	1,4182; 0,5676 – 3,5433	0,5667; 0,2215 – 1,4497	0,4793; 0,192 – 1,1962
Hyperkinesis	0,2605; 0,0678 – 1,0015	1,8158; 0,6296 – 5,2366	10,2222; 3,6044 – 28,9903*	1,5235; 0,6101 – 3,8042	0,6175; 0,2429 – 1,5698	0,5600; 0,226 – 1,3875
Cognitive impairment	0,6636; 0,2589 – 1,7008	18,857; 6,0733 – 58,5495*	0,8889; 0,3345 – 2,362	3,1866; 1,2547 – 8,0929*	7,3981; 2,6492 – 20,6601*	0,7870; 0,3139 – 1,9732
Movement disorders	3,3239; 1,2413 – 8,9007*	0,9502; 0,3316 – 2,7228	0,6545; 0,2536 – 1,689	2,9455; 1,1643 – 7,4515 *	6,4274; 2,3134 – 17,8575*	1,319; 0,5331 – 3,2638

*Note. * – $\alpha = 0,05$*

As can be seen from the data in **Table 7.1**, certain associations were noted between radiological phenomena and clinical syndromes observed in SG children. In general, the identified associations correspond to modern ideas about the functional purpose of various anatomical structures of the human cerebral hemispheres. Thus, the detection of diffuse leukoencephalopathy increased the chance of clinical symptoms of regressive autism in a child by at least 14 times, and motor disorders – by 3 times. Radiological signs of temporal median sclerosis were associated with epileptic syndrome and cognitive disorders, which corresponds to the theory of MTE-HS, and the idea of the location of the center of short-term memory in the hippocampus [36]. Hypertrophy of the subcortical ganglia increased the chance of developing obsessive-compulsive syndrome by 13 times, and hyperkinetic syndrome – by 10 times, which is consistent with the modern concept of autoimmune subcortical encephalitis [6]. Radiological symptoms of congenital CMV neuroinfection, as well as postnatal encephalitis, were associated with epileptic syndrome, cognitive decline and motor disorders, which is consistent with the results of reports on residual phenomena after neuroinfections [5, 9, 25].

However, the manifestations of minor anomalies of brain development were not associated with the occurrence of any of the studied clinical syndromes. Most likely, these were clinically insignificant manifestations of dysembryogenesis of the nervous system, which is a characteristic feature of GDFC [24].

Another important task is to find connections between radiological phenotypes and the results of special laboratory tests that reflect the implementation of known pathogenetic mechanisms of CNS damage in children with ASD associated with GDFC. Analysis of associations between neuroimaging studies and the results of a specially designed laboratory paraclinical examination in SG children is given in **Table 7.2**.

● **Table 7.2.** Results of the study of associations (OR; 95 % CI) between pathological radiological phenomena and the results of special laboratory tests in children with GDFC associated with ASD (n = 225)

Sign	Diffuse leukoencephalopathy	Temporal median sclerosis	Hypertrophy of subcortical ganglia	Signs of congenital CMV neuroinfection	Signs of postnatal encephalitis	Minor developmental anomalies
Reactivated HHV-6/HHV-7 infections	5,2662; 2,5064 – 11,0648 *	18,1071; 7,6503 – 42,8568 *	0,7917; 0,3975 – 1,5767	0,8868; 0,4433 – 1,774	1,1485; 0,5744 – 2,2963	0,9787; 0,4919 – 1,9472
Autoantibodies to hippocampal neurons	0,7568; 0,3796 – 1,5088	8,9931; 4,1046 – 19,7036 *	1,1852; 0,5867 – 2,3943	1,0512; 0,525 – 2,1047	0,6686; 0,3352 – 1,3336	1,2536; 0,6276 – 2,5041
Autoantibodies to subcortical ganglia neurons	0,8567; 0,4296 – 1,7085	1,7591; 0,8740 – 3,5407	14,7245; 6,3708 – 34,0318 *	1,9192; 0,9531 – 3,8647	1,3602; 0,6785 – 2,7269	1,200; 0,6005 – 2,398
Signs of autosensitization to myelin	4,4136; 2,1294 – 9,148*	1,4380; 0,7221 – 2,8635	1,5693; 0,7875 – 3,1271	1,1568; 0,5808 – 2,3042	1,040; 0,5246 – 2,0619	0,7653; 0,3849 – 1,5217
Increased serum concentration of tumor necrosis factor alpha	4,6538; 2,2383 – 9,6761*	7,6364; 3,5371 – 16,4866*	7,2100; 3,3498 – 15,4757 *	1,5021; 0,7541 – 2,992	1,6397; 0,8224 – 3,2691	0,9700; 0,4858 – 1,9367
Identification of Streptococcus pyogenes	1,6125; 0,8011 – 3,2457	1,4840; 0,7406 – 2,9737	13,3714; 5,8391 – 30,62*	1,1073; 0,5557 – 2,2065	1,5143; 0,7611 – 3,0128	1,4493; 0.7288 – 2,882

*Note. * – $\alpha = 0,05$*

As shown in **Table 7.2**, MRI features of diffuse leukoencephalopathy were associated with the detection of reactivated HHV-6 and HHV-7 infections and signs of CNS myelin sensitization. It is known that HHV-6 and HHV-7 can infect oligodendrocytes [3] and cause multifocal demyelinating leukoencephalitis, which resembles autoimmune demyelinating diseases of the nervous system on neuroimaging [26], which may explain the observed association. In addition, the role of HHV-6 and HHV-7 as triggers of autoimmune

reactions in demyelinating diseases of the CNS, which resemble leukoencephalopathy on neuroimaging in children with ASD associated with GDFC, is now well known and studied [29].

MRI features of temporal median sclerosis have been associated with the identification of reactivated HHV-6 and HHV-7 infections and autoantibodies to hippocampal neurons. These findings are consistent with the notion that HHV-6 and HHV-7 are involved in the pathogenesis of MTE-HS, as suggested by a recent meta-analysis and systematic review of randomized controlled trials [36] and the current concept of autoimmune limbic encephalitis in humans [22]. Furthermore, there are reports that HHV-6 and HHV-7 may be triggers for the development of autoimmunity in autoimmune limbic encephalitis [35], and there have been rare cases of sudden onset of ASD symptoms in humans [9, 14] and animals [32] with autoimmune limbic encephalitis.

MRI signs of subcortical ganglion hypertrophy have been associated with serum autoantibodies to basal ganglia neurons and cases of group A beta-hemolytic streptococcus in the oropharynx. These findings are consistent with the current concept of PANDAS as an autoimmune subcortical encephalitis caused by the production of autoantibodies to subcortical ganglion neurons, with Streptococcus pyogenes being the typical trigger for autoimmunity [6].

Elevated serum TNF-alpha concentrations have been associated with three radiological phenomena: diffuse leukoencephalopathy, temporal median sclerosis, and subcortical ganglion hypertrophy, which may reflect the well-known inflammatory nature of such CNS lesions [6, 29, 36]. However, an inverse relationship is also possible, since the neurotoxic properties of TNF-alpha with the induction of damage to both neurons and myelin in the CNS have been described and studied [16], and the overproduction of this cytokine may not be secondary, but primary to some of the aforementioned neuroimaging signs, since systemic inflammation, being a consequence of immune dysregulation in GDFC, is considered by some researchers as an independent damaging factor in the formation of encephalopathy in children with ASD [18].

Radiological signs of congenital CMV neuroinfection, postnatal encephalitis, and minor brain anomalies were not associated with any of the laboratory test results that characterize the known mechanisms of CNS damage in ASD associated with GDFC in children. This may be due to the fact that these radiological phenomena do not reflect a current pathological process that is being implemented in real time, but are signs of residual phenomena of cerebral damage that occurred in the past.

The generalization of the results of **Tabs. 7.1** and **7.2** allows us to speak about the detection of certain close associations between laboratory signs of known damaging factors of the CNS, radiological signs of nervous system damage and clinical manifestations of cerebral dysfunction in children with ASD associated with GDFC. These associations allow us to distinguish typical laboratory-radiological-clinical complexes, or diagnostic patterns, such as virus-induced temporal median sclerosis, autoimmune limbic encephalitis, autoimmune subcortical encephalitis, autoimmune or virus-induced demyelinating damage to the hemispheres, the consequences of previous neuroinfections and small anomalies of brain development. Encephalopathy in SG children was the result of a combination of these complexes in different ways in different patients. A feature of SG children was the possibility of combining several of these complexes in one patient, which created a large number of heterogeneous combinations and determined the heterogeneity of clinical symptoms of the disease, while in the available scientific literature these complexes were described mostly as isolated phenomena [4, 29, 36].

Conclusions. In children with ASD associated with GDFC, 5 main groups of neuroimaging features are noted, characteristic of leukoencephalopathy, temporal median sclerosis, PANS/PITANDS/PANDAS, congenital CMV neuroinfection and postnatal encephalitis, and minor CNS developmental anomalies.

The identified neuroimaging features are closely associated with the results of special laboratory tests characterizing known immune-dependent mechanisms of CNS damage and with the appearance of corresponding clinical syndromes, consistent with modern concepts of the main infectious or autoimmune lesions of the human nervous system in patients with immunosuppression.

It is possible to distinguish certain laboratory-radiological-clinical complexes in children with ASD associated with GDFC (virus-induced temporal median sclerosis, autoimmune limbic encephalitis, autoimmune subcortical encephalitis, autoimmune or virus-induced demyelinating lesion of the cerebral hemispheres, consequences of previous neuroinfections and small anomalies of brain development), which, combining in different ways in different patients, form a specific encephalopathy with heterogeneous clinical and radiological signs and, most likely, a complex pathogenesis.

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