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ESTIMATING THE RISK OF DEVELOPING FOLATE-DEPENDENT CONGENITAL MALFORMATIONS OF THE NERVOUS SYSTEM IN ONTOGENESIS

322.01 PAEDIATRICS AND NEONATOLOGY

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The thesis was developed in the Department of *Paediatrics* of the Nicolae Testemitanu State University of Medicine and Pharmacy of the Republic of Moldova

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1. CONCEPTUAL CHARACTERISTICS OF THE RESEARCH

Timeliness and Importance of the Researched Problem. Congenital malformations of the nervous system (CMNS) are the result of neural tube defects (NTDs) that occur due to incomplete or abnormal closure of the neural tube during embryogenesis [1, 2]. Between 2011 and 2022, CMNS accounted for approximately 10–11% of all congenital anomalies in the Republic of Moldova, consistently representing one of the main causes of infant mortality [3, 4]. In the Republic of Moldova, the most common nervous system malformations are cerebral structural anomalies (44.27%), followed by hydrocephalus (29.69%), anencephaly (16.67%), encephalocele (5.21%), and microcephaly (4.17%), with frequencies ranging from 0.39 to 5.27 cases per 10,000 newborns [3, 5]. According to the WHO, the prevalence of NTDs varies significantly globally (6.9–21.9/10,000 births), and is often underreported in many countries [6]. In European countries, according to EUROCAT (2024), the annual incidence is between 10.07 and 10.83/10,000 newborns [7]. The etiology of congenital nervous system malformations is complex and multifactorial [8], including genetic, environmental, and autoimmune factors [2, 3]. The early identification of these determinants remains a priority due to the increased frequency and often unfavorable prognosis associated with these conditions [9].

In recent decades, the concept of *folate-preventable neural tube defects (FP-NTDs)* has emerged a subgroup of malformations whose incidence can be significantly reduced through nutritional interventions (folic acid fortification), especially *spina bifida*, *anencephaly*, and *encephalocele* [10]. Kancherla et al. mention that countries with mandatory fortification programs have registered notable decreases in NTD prevalence. In this context, the Republic of Moldova also implemented a national food fortification policy (Government Decision No. 171 of 19.03.2012) [11, 12]. However, the persistence of some cases of folate-dependent congenital malformations of the nervous system (FCMNS), even with these public health measures in place, suggests that fortification, while effective, is not the sole preventive solution. Thus, there is a need for an extended etiological approach that goes beyond nutritional interventions and analyzes other risk factors involved in the occurrence of FCMNS. Given the lack of systematized data on FCMNS in the Republic of Moldova, it is necessary to investigate relevant etiological directions for this category of malformations. Among these, a central place is occupied by the analysis of polymorphisms of genes involved in folate metabolism [13, 14]. Strengthening the theoretical and methodological framework of the investigation regarding the contribution of maternal genetic *background* and *gene-environment* interactions in the etiopathogenesis of MCSN requires the integration of data from meta-analyses and systematic reviews. The rationale for investigating the genetic background of children's parents is reflected in multiple studies that emphasize the importance of evaluating parental genetic determinants in assessing susceptibility to these congenital anomalies. In this context, critical synthesis of epidemiological evidence is essential for interpreting the role of maternal polymorphisms involved in folate metabolism (*MTHFR C677T, MTHFR A1298C, MTR A2756G* and *MTRR A66G*) considered potential susceptibility factors, whose pathogenic expression is modulated by the interaction with environmental factors.In addition to genetic components, the literature emphasizes the importance of documenting other possible factors [15], such as maternal exposure to toxic agents [16-18] or the use of anti-folate medications during pregnancy, for which there is data on their association with nervous system development anomalies [12, 19]. Moreover, the familial recurrence of NTDs [20], with increased risks in cases with similar family history [21], is considered an important aspect in

assessing population vulnerability [22]. Given these premises, a detailed description of these factors within the population of the Republic of Moldova is essential to strengthen the scientific basis needed to formulate preventive measures that are better adapted to local realities.

Based on the trends observed in specialized literature, we formulated the **hypothesis of this study**: there is a significant association between certain pre- and post-conception risk factors, including environmental ones, as well as polymorphisms of folate cycle genes **(***MTHFR677, MTHFR1298, MTR2756, MTRR66*), and the occurrence of congenital malformations of the nervous system, with marked specificities in the subgroup of forms preventable by folate supplementation (FCMNS). Based on the premises mentioned above, the **purpose of this study** is to evaluate the epidemiological, etiological, and clinical specificities of congenital nervous system malformations in the pediatric population of the Republic of Moldova, especially the forms preventable by folate supplementation, to determine potential risk factors and personalize prophylaxis measures for the occurrence of such pathologies in exposed families. To achieve this purpose, the following **study objectives** were established:

1. To describe the distribution and structure of congenital nervous system malformations in children from the Republic of Moldova, based on data from the National Register of Congenital Malformations for the period 2018–2023, and to compare the frequency in the population with the European EUROCAT network (2018–2021).
2. To evaluate the association between pre- and post-conception, maternal, paternal, and environmental risk factors and the presence of congenital nervous system malformations, highlighting the specificities of forms susceptible to prevention with folate supplementation.
3. To analyze the association variants between different forms of congenital nervous system malformations, including with concomitant neurological pathologies.
4. To evaluate the frequency of maternal single nucleotide polymorphisms of genes involved in folate metabolism (*MTHFR677, MTHFR1298, MTR2756, MTRR66*) and their association with the presence and typology of congenital nervous system malformations, especially folate-dependent forms.

General research methodology. The study was based on an integrated design with a retrospective-descriptive and analytical-observational case-control component, guided by scientifically validated concepts in international literature on the etiology of congenital nervous system malformations (CMNS).

The scientific approach was conceptually structured as follows:

- ***The concept of ethno-geographic variability of congenital malformation determinants***, which highlights the need to investigate the distribution and expression of risk factors (including genetic ones) in the context of regional, ethnic, and sociocultural specificities of the population;
- ***The descriptive epidemiological framework***, based on the analysis of time series regarding the frequency and structure of CMNS in the Republic of Moldova from 2018–2023, in comparison with EUROCAT data;
- ***The multifactorial etiological model of CMNS***, based on the principle of selecting and systematizing relevant predictors (maternal, paternal, and prenatal/intrauterine risk factors);
- ***The concept of folate-preventable forms of CMNS (FCMNS)***, derived from the international term folate-preventable neural tube defects, was used to evaluate the specificities of this distinct subgroup;

- ***Evaluation of maternal genetic background and gene–environment interaction in the risk of MCSN.*** Unlike most studies focused on the genotype of offspring, the current investigation evaluated the distribution of allelic and genotypic variants of genes involved in folate metabolism (MTHFR677, MTHFR1298, MTR2756, MTRR66) in mothers of children with MCSN and those without MCSN, following the approach proposed by Saxena et al. (2018); Chun-Quan et al. (2019);
- ***The concept of co-expression and nosological clustering*** (based on correlation analysis and dimensionality reduction by PCA – *Principal Component Analysis*), was used to identify potential patterns of co-association of different CMNS, as well as with concomitant neurological pathologies.

The study received a favorable opinion (No. 83) from the Research Ethics Committee of the Nicolae Testemițanu State University of Medicine and Pharmacy, during the meeting on January 24.01.2022.

Scientific Novelty. For the first time in the Republic of Moldova, an investigation with a dual design was conducted on congenital malformations of the nervous system (CMNS), combining retrospective analysis of population data with an extended case-control study on the expression of risk factors. The study revealed a statistically significant downward trend in the incidence of CMNS and folate-preventable forms (FCMNS) during the 2018–2023 period, providing the first national evidence regarding their temporal dynamics. Compared to EUROCAT data, the research documented a differentiated profile of the distribution of clinical subtypes, with a greater proportion of cerebral structural anomalies and hydrocephalus in the Republic of Moldova. At the same time, a pronounced link was found between the lack of periconceptional folic acid supplementation and the occurrence of FCMNS (93% of cases). Moreover, a hierarchy of maternal, paternal, and prenatal risk factors was determined, as well as their degree of association with CMNS in the studied population, identifying factors with a potentially major impact: infectious agents (TORCH complex, cytomegalovirus), behavioral vices (smoking, alcohol, or drug use), parental health status (diabetes, arterial hypertension, overweight), medication use, and exposure to toxins during the intrauterine period. The research highlighted relevant specificities regarding the clinical expression of CMNS, trends of association between different forms (clusters), as well as significant correlations between clinical subtypes and associated neurological pathologies, thus strengthening the perspective of an integrative and differentiated approach to these pathological entities. For the first time in the regional context, the existence of significant differences in the distribution of genotypic variants of the *MTHFR677, MTHFR1298, MTR2756, and MTRR66* genes was demonstrated between mothers of children with CMNS and those in the control group, with specific associations between certain genotypes and the risk of FCMNS (OR between 2.18 and 6.78).

The **approval of the results** took place in accordance with the fundamental stages of the study. The main results were presented, discussed, and approved at the meeting of the Department of Pediatrics of the Nicolae Testemițanu State University of Medicine and Pharmacy (IP Universitatea de Stat de Medicină și Farmacie „Nicolae Testemițanu") No. 7 on 05.03.2025 and at the meeting of the Scientific Profile Seminar, Specialty 322.01 Pediatrics and Neonatology, No. 1 on 04.07.2025, as well as at national and international scientific conferences.

The results of the research were communicated at over 20 national and international scientific events, including: European neurology congresses, such as the 4th EAN Congress (2018,

Lisbon), 9th EAN Congress (2022, Budapest), and 20th World Stroke Congress (2022, Singapore); international pediatric-neurological events in Romania: Iasi Pediatrics Days (2021), SNPCAR Congresses, and National Conferences of Child and Adolescent Neurology-Psychiatry (2018–2024); schools and conferences with international participation: Pediatric Medical School (2018, Iasi), MedEspera (2018, Chisinau), Conference "Actualities in Pediatric Practice" (2022, Chisinau), International Congress of the Society of Pediatrics of the Republic of Moldova (2024, Chisinau); national forums of excellence in research: Conference "Research in biomedicine and health: quality, excellence, and performance" (2021, 2023, Chisinau) and the Symposium "Register of rheumatic diseases in children" (2023, Chisinau).

Publications on the thesis topic: The scientific results on the thesis topic were presented in 61 publications, including 1 article in an international SCOPUS-indexed journal, 2 articles in peer-reviewed foreign journals, 4 articles in national scientific conference proceedings with international participation, 11 theses in scientific conference proceedings (6 international; 5 national) ; innovation activity – 1 invention patent, 1 copyright certificate, 5 AGEPI innovator certificates, 5 institutional innovator certificates, 10 institutional implementation acts, 1 gold medal at an international invention forum held abroad; active participation in scientific forums: 11 oral presentations at scientific conferences (international – 8; national with international participation – 2; national – 1), 9 posters at scientific conferences (international – 6; national – 3).

Volume and structure of the thesis

The thesis is written in Romanian as a manuscript. It is presented on 211 pages and contains: introduction, 4 chapters (literature review, research materials and methods, results obtained – in two chapters, discussions), general conclusions and practical recommendations. The bibliography includes 228 titles. The work is illustrated with 11 tables, 37 figures, 1 formula and 18 annexes.

Keywords: central nervous system, nervous system malformations, neural tube defects, anencephaly, spina bifida, infant, folic acid deficiency, sociodemographic factors, risk factors, genetic polymorphism.

2. GENERAL CHARACTERISTICS OF THE STUDY MATERIAL AND RESEARCH METHODS

2.1. General presentation of the study

The study was conducted in specialized departments and clinical bases of the Department of Pediatrics, Nicolae Testemitanu State University of Medicine and Pharmacy, during the period 2017–2022. To achieve the objectives of this study, a dual design was pre-established, involving a retrospective study (based on data from dedicated registries) and an observational, analytical, and case-control study. The study received a favorable opinion (No. 83) from the Research Ethics Committee of the Nicolae Testemitanu State University of Medicine and Pharmacy on January 24, 2022.bFor the retrospective study, data were collected from the National Register of Congenital Malformations of the Republic of Moldova (for the period 2018–2023), investigating the structure and frequency of different types of CMNS in the pediatric population of the Republic of Moldova. Data were collected from the Hereditary Pathologies Prevention Laboratory of the Mother and Child Institute. The data from the National Register for the period 2018–2021 were compared with similar periods from EUROCAT, with the selection of the investigation period based on the complete availability of data in the European database. This allowed for an evaluation of the frequency of different CMNS in the pediatric population of the Republic of Moldova compared to

that of European countries (EUROCAT members). Access to EUROCAT data was achieved through authorized access to the web platform (https://eu-rd-platform.jrc.ec.europa.eu/eurocat_en) with data selection according to the study objectives [23].

For the combined case-control study, a design was developed to investigate two groups: the case group (children with CMNS) and the control group (live newborns without CMNS), including a directed genetic evaluation of their mothers. The ***inclusion criteria*** were as follows: children aged 0 to 18 years with isolated (polygenic) CMNS; live newborns without CMNS (the control group); the mothers of the children studied (for biological sample collection); the signing of the informed consent form by the parents. The ***exclusion criteria*** were: children with monogenic syndromes; children with chromosomal abnormalities; children with mitochondrial diseases; lack of informed consent from participants. Informed consent was signed by each participant before their inclusion in the study. Prior to this, they were informed about the purpose, method of participation, and execution of the study, as well as the potential benefits, disadvantages, and risks of participation. According to the study objectives, the specific characteristics of different indicators were investigated in the subgroup of children with *folate-preventable forms of CMNS* (FCMNS, n=16, consisting of children with a diagnosis of *spina bifida and encephalocele*).

To achieve the study's objectives, two evaluation forms were compiled (a clinical-anamnestic form and a neurological evaluation form), through which data were collected regarding risk factors, life history, disease history, clinical/paraclinical picture, and neurological evaluation data. The rationale for the investigation was based on studies with similar designs conducted in different regions/countries (Bulgaria, Ukraine, Turkey, Tunisia, China), with the goal of assessing local specificities associated with the occurrence of CMNS [24, 25]. The detailed study design is presented in figure 1.

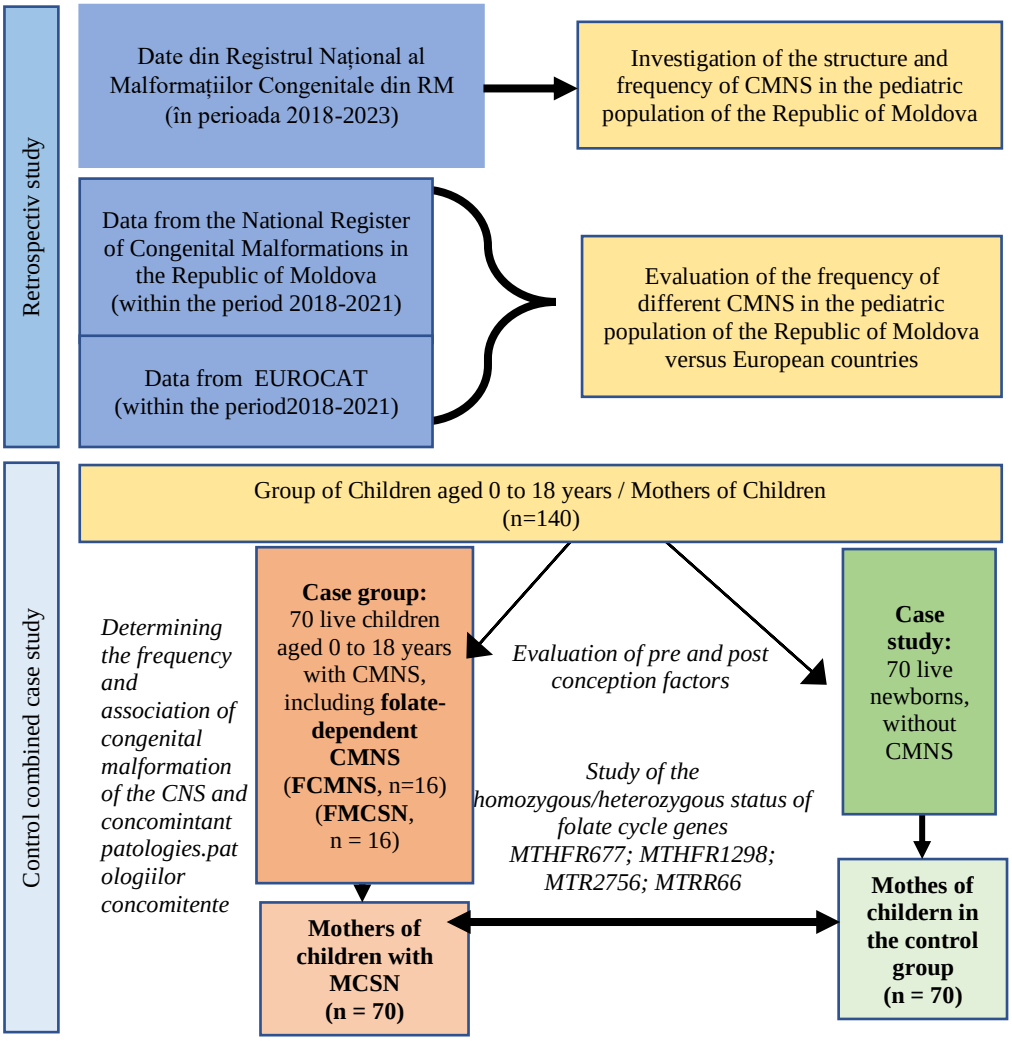


Figure 1. Study design

2.2. Methodology applied during the the study

Clinical and laboratory method: a series of clinical and paraclinical data were also collected from the children's records (according to the diagnostic needs of those with CNSM). The characterization of CNSM included noting neurological symptoms and the presence of concomitant somatic diseases, resulting from complications and congenital malformations of the nervous system in children. The paraclinical examination of hospitalized children with CNSM was performed, as clinically necessary, through laboratory tests (general and biochemical blood analysis), as well as through imaging examination (cerebral ultrasonography, computed tomography, magnetic resonance imaging). The investigations were based on compliance with standardized National Clinical Protocols.

Cerebral ultrasonographic (USG) exploration was performed with the Aplio 300 device (Toshiba, Japan) from the Functional Diagnostic, Department of the IMSP Institute of Mother and Child, according to the institutional protocol. A non-invasive method of functional diagnosis, USG is used as the first imaging step in the evaluation of children with suspected cerebral malformations. *Computed tomography (CT)* and *magnetic resonance imaging (MRI)* were performed according to clinical indications to confirm the diagnosis of CNSM, in compliance with standard protocols. Most of the investigations took place in the Radiology and Imaging,

Department of the IMSP Institute of Mother and Child, using the Aquilion Prime 80 Slice tomograph (Toshiba, Japan), with an effective dose: 621.4 mGy/cm². Cerebral MRI (in selected cases) was performed with the Magnetom Skyra 3T (Siemens, Germany), providing high-resolution images for the early diagnosis of minor cerebral lesions. Other investigations took place at the German Diagnostic Center and Excellence medical centers, using Aquilion Prime TSX-303A/AC (Toshiba) and Somatom 16 (Siemens) equipment. In the study, the *polymorphisms of genes involved in folate metabolism* (*MTHFR677, MTHFR1298, MTR2756, MTRR66*) were investigated in the mothers of children with CNSM (n = 70) and in those in the control group (n = 70). An additional informed consent was signed, approved according to ethical requirements. Molecular-genetic testing was performed by real-time polymerase chain reaction (RT-PCR), in the Invitro Diagnostics laboratory, with funding from the doctoral program.

Mathematical-Statistical Processing of the Material: Data were collected in a tabular database using Microsoft Excel 2021 (Microsoft, USA). For primary statistical analysis (descriptive statistics) post-data collection, SPSS v.27 software package (trial version, IBM Corp., Armonk, NY) was used. For final statistical processing of the data, R-Studio v.2023.12.1 Build 402 software (Posit Software, USA) was used. Descriptive statistics included absolute and relative frequencies (%) for categorical variables, and means, standard deviations, minimum and maximum values for continuous ones (with the Shapiro–Wilk test for normality assessment). For comparing frequencies between groups (e.g., CNSM vs. Control), the χ^2 test was used, with adjustments as needed (Yates' correction, Monte Carlo simulation – 10,000 iterations, Cramer's V coefficient). Temporal trends of CNSM incidence were analyzed with Poisson regression models, with each subtype evaluated individually with the year as a predictor; a generalized Poisson model (GLM) with a year×subtype interaction tested the differences in evolution between malformations. For continuous variables with a non-normal distribution, the Wilcoxon test was applied (with Holm's adjustment). The association of risk factors with CNSM was evaluated by the odds ratio (OR) with 95% confidence intervals (95% CI); additionally, relative risk (RR), attributable risk (AR), and population attributable fraction (PAF) were calculated according to Bruzzi's method. In contingency tables with 0 cells, the Haldane–Anscombe correction (+0.5) was applied. The 95% confidence interval for the OR was estimated by the logarithmic method according to Wald, and for the RR by the logarithmic method according to Katz. Spearman's correlation (rho, p) was used, and principal component analysis (PCA) was applied to identify co-association patterns between variables (based on eigenvectors). Genetic analysis was performed in a tabular format, using the χ^2 test, OR (95% CI), and p, according to current methodologies. Inheritance models were evaluated: multiplicative (allele frequency), general (genotype distribution), additive (Cochran–Armitage test), dominant (association of the allele with the event), and recessive (association of the homozygous genotype with the event). The validity of the genetic models was verified by the Hardy–Weinberg equilibrium test. The results were presented in tables and graphs, highlighting high values in subgroups and statistically significant differences.

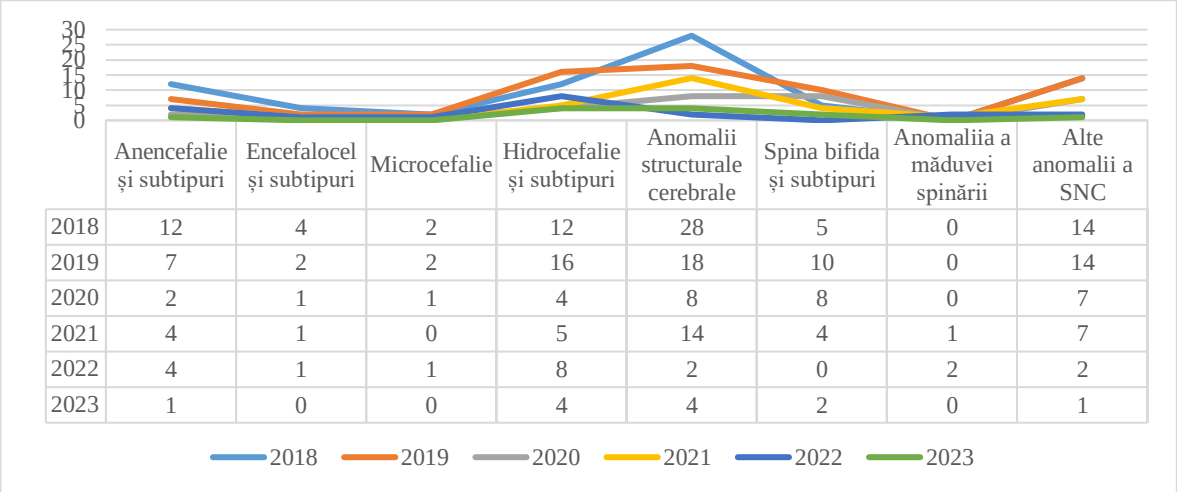
The study's limitations were mainly determined by the low incidence of congenital nervous system malformations in the Republic of Moldova, which reduced the sample size and the possibility of applying complex statistical models, as well as the retrospective case-control nature, which introduced risks of recall bias and social desirability. In addition, not all variables with teratogenic potential could be collected or detailed (e.g., diet, stress, physical activity, minor pollutants), and the lack of data on the fetal genotype limited the differentiated analysis of genetic effects. At the same time, methodological diversity and genetic variations between populations

restricted international comparability, and available resources did not allow for a prospective design or advanced gene-environment interaction models. These limitations were taken into account when interpreting the results and formulating the conclusions.

3. EPIDEMIOLOGICAL, PERICONCEPTIONAL AND CLINICAL ASPECTS OF CONGENITAL MALFORMATIONS OF THE NERVOUS SYSTEM IN CHILDREN FROM THE REPUBLIC OF MOLDOVA

3.1. The distribution of congenital nervous system malformations in the Republic of Moldova: national trends and comparisons with international data

The study of the frequency of CNSM was made possible by analyzing the CNSM cases from 2018-2023 in the *National Registry of Congenital Diseases* of the Republic of Moldova (figure 2).



	Anencefalie și subtipuri	Encefalocel și subtipuri	Microcefalie	Hidrocefalie și subtipuri	Anomalii structurale cerebrale	Spina bifida și subtipuri	Anomaliia a măduvei spinării	Alte anomalii a SNC
2018	12	4	2	12	28	5	0	14
2019	7	2	2	16	18	10	0	14
2020	2	1	1	4	8	8	0	7
2021	4	1	0	5	14	4	1	7
2022	4	1	1	8	2	0	2	2
2023	1	0	0	4	4	2	0	1

Figure 2. Annual evolution of the frequency of CNSM (2018–2023)

Thus, the analysis of the distribution of congenital nervous system malformation cases reported in the Republic of Moldova in the years 2018–2023 showed that during the analyzed period, the total number of CNSM cases was 245, of which 68 were folate-preventable anomalies (27.75%). The total number of cases showed significant annual variations: 2018 – 77 cases; 2019 – 69 cases; 2020 – 31 cases; 2021 – 36 cases; 2022 – 20 cases; 2023 – 12 cases, outlining a general downward trend throughout the analyzed interval. A similar evolution was also observed with regard to folate-preventable anomalies, with decreasing absolute values: 2018 – 21 cases; 2019 – 19; 2020 – 11; 2021 – 9; 2022 – 5; 2023 – 3 cases, possibly due to the increased efficiency of primary prevention measures. The proportion of folate-preventable anomalies in the total number of CNSM cases reported annually remained relatively constant around 25%, with small variations: 2018 – 27.27%; 2019 – 27.53%; 2020 – 35.48%; 2021 – 25%; 2022 – 25%; 2023 – 25%. This shows a relatively stable level of contribution of these anomalies to the global CNSM morbidity, with an isolated increase in 2020, possibly influenced by epidemiological factors (COVID-19) or reporting. Subsequently, the distribution of CNSM cases by year and in the total group was analyzed.

The evaluation of the distribution of different CNSM subtypes per year revealed a non-uniformity of cases. Thus, in 2018, a significantly imbalanced distribution was noted; some subtypes being much more frequent ($\chi2=42.36;df=6;p=1.56e{-}07$). In 2019, significant differences between subtypes were noted and a similar imbalanced profile to the previous year was maintained ($\chi2=25.65;df=6;p=2.58e{-}04$). In 2020, significant differences between subtypes were observed, but more attenuated compared to previous years ($\chi2=13.94;df=6;p=0.030$). In 2021, a significantly imbalanced internal distribution was noted ($\chi2=23.11;df=6;p=7.60e{-}04$). In 2022, a significant difference between the frequencies of CNSM subtypes was maintained ($\chi2=17.60;df=7;p=0.0139$).

In 2023, no significant difference was observed, with the distribution being relatively uniform between subtypes ($\chi2=3.83;df=4;p=0.429$).The total distribution of CNSM cases by subtypes, cumulated during the 2018–2023 period, was analyzed using the $\chi2$ (chi-square) test. The test result was extremely statistically significant ($\chi2=139.29;df=7;p=2.2e-16$), demonstrating that some CNSM subtypes were reported much more frequently than others; thus, the distribution of cases is not random. Overall, for the years 2018–2022, a constant pattern of imbalanced distribution of CNSM subtypes was observed, possibly reflecting the predominance of specific diseases (such as cerebral structural anomalies or hydrocephalus). In contrast to previous years, in 2023, the distribution of cases was relatively homogeneous among subtypes, possibly as a result of the reduced total number of reported cases (n = 12) or a more uniform distribution in clinical practice and reporting.

Figure 3. Percentage structure of congenital malformations of the central nervous system in the period 2018-2023

Over the years, the following frequencies of CNS congenital malformation (MCSN) subtypes have been observed (arranged hierarchically in descending order): 2018: *cerebral structural anomalies* (36.4%, n = 28) > *other CNS anomalies* (18.2%, n = 14) > *anencephaly* (15.6%, n = 12) and *hydrocephalus* (15.6%, n = 12) > *spina bifida* (6.5%, n = 5) > *encephalocele* (5.2%, n = 4) > *microcephaly* (2.6%, n = 2). 2019: *cerebral structural anomalies* (26.1%, n = 18) > *hydrocephalus* (23.2%, n = 16) > *other CNS anomalies* (20.3%, n = 14) > *spina bifida* (14.5%, n = 10) > *anencephaly* (10.1%, n = 7) > *encephalocele* (2.9%, n = 2) and *microcephaly* (2.9%, n = 2). 2020: *cerebral structural anomalies* (25.8%, n = 8) and *spina bifida* (25.8%, n = 8) > *other CNS anomalies* (22.6%, n = 7) > *hydrocephalus* (12.9%, n = 4) > *anencephaly* (6.5%, n = 2) > *encephalocele* (3.2%, n = 1) and *microcephaly* (3.2%, n = 1). 2021: *cerebral structural anomalies* (38.9%, n = 14) > *other CNS anomalies* (19.4%, n = 7) > *hydrocephalus* (13.9%, n = 5) > *anencephaly* (11.1%, n = 4) and *spina bifida* (11.1%, n = 4) > *encephalocele* (2.8%, n = 1) and *spinal cord anomalies* (2.8%, n = 1). 2022: *hydrocephalus* (40.0%, n = 8) > *anencephaly* (20.0%, n = 4) > *cerebral structural anomalies* (10.0%, n = 2) and *spinal cord anomalies* (10.0%, n = 2) and *other CNS anomalies* (10.0%, n = 2) > *encephalocele* (5.0%, n = 1) and *microcephaly* (5.0%, n = 1).2023: *hydrocephalus* (33.3%, n = 4) and *cerebral structural anomalies* (33.3%, n = 4) > *spina bifida* (16.7%, n = 2) > *anencephaly* (8.3%, n = 1) and *other CNS anomalies* (8.3%, n = 1). Overall, during the evaluated period (2018–2023), the following hierarchy was observed: *cerebral structural anomalies* (30.2%, n = 74) > *hydrocephalus* (20.0%, n = 49) > *other CNS anomalies* (18.4%, n = 45) > *anencephaly* (12.2%, n =

30) > *spina bifida* (11.8%, n = 29) > *encephalocele* (3.7%, n = 9) > *microcephaly* (2.4%, n = 6) > *spinal cord anomalies* (1.2%, n = 3) (figure 3).

During the 2018–2023 period, a total of 205 patients were diagnosed with congenital nervous system malformations, corresponding to 245 total cases (as some children had multiple malformations).There was a clear decrease from 63 patients in 2018 to 11 patients in 2023(figure 6).The case structure showed a predominance of patients with a single diagnosis (one type of anomaly per patient) at 168 cases (81.95%), followed by double diagnoses (two subtypes per patient) at 33 cases (16.1%), and triple diagnoses (three subtypes per patient) at 4 cases (1.95%).

Assessment of MCSN frequency in the pediatric population of the Republic of Moldova versus EUROCAT (in 2018-2021)

From 2018 to 2021, 154,066 children were born in the Republic of Moldova. Of these, 1,978 had congenital malformations, with 202 having CNS malformations, which accounted for 10.21% of all anomalies (figure 4).

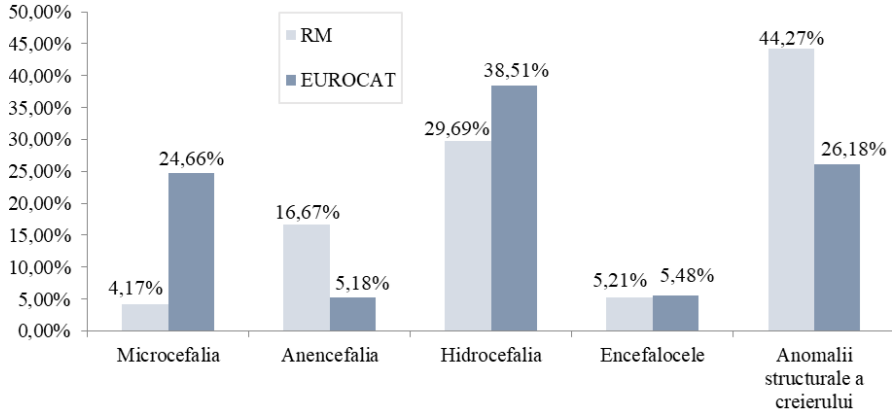


Figure 4. Comparison of the frequency of MCSN in the Republic of Moldova and in the EUROCAT registry (in the period 2018-2021)

A comparative analysis of the incidence of cerebral anomalies was conducted for the Republic of Moldova (RM) and countries within the EUROCAT (European Registration of Congenital Anomalies and Twins) registry. In contrast to Moldova, the structure of cerebral congenital anomalies in EUROCAT countries is dominated by hydrocephalus, which accounts for 38.51% of cases (2.53 per 10,000 live births, or 1:3953). In Moldova, during the same study period, the frequency of hydrocephalus was 1:2703 live births. Cerebral congenital anomalies were observed in EUROCAT countries in 26.18% of live births (1.72 per 10,000, or 1:5814), whereas in Moldova, this malformation ranked first in the structure of cerebral anomalies (1:1898 live births). In EUROCAT, microcephaly was the third most frequent anomaly, at 24.66% of cases (1.62 per 10,000, or 1:6173 live births).

Table 1. Structure of Congenital Cerebral Malformations in the Republic of Moldova and in EUROCAT, 2018-2021

Malformations	Republic of Moldova		EUROCAT	
	at 10.000 n.n	Abs Frequency	at 10.000 n.n	Abs. Frequency
Microcephaly	0,51	1:19607	1,62	1:6173
Encephalocele	0,39	1:25641	0,36	1:27777
Anencephaly	2,01	1:4975	0,34	1:29412
Hydrocephalus	3,70	1:2703	2,53	1:3953
Cerebral congenital anomalies	5,27	1:1898	1,72	1:5814

The frequency of encephalocele in EUROCAT countries (0.36 per 10,000, or 1:27,777 live births) was comparable to the frequency of this cerebral anomaly in Moldova (0.39 per 10,000, or 1:25,641 live births) (table 1).Anencephaly in newborns was 6 times less frequent in EUROCAT countries than in Moldova during the study period, at 0.34 and 2.01 per 10,000 live births, respectively (or 1:29,412 vs. 1:4975 live births).This significant difference can be explained by a higher level of prenatal diagnosis of these pathologies in European countries.

Taking into account that EUROCAT's website provided data up to and including 2021, the dynamic frequency of cerebral anomalies in newborns in Moldova (RM) and EUROCAT countries was studied by comparing data from the National Registry of Congenital Malformations in RM with EUROCAT data for the 2018-2021 period [13]. The dynamic of CNS malformation cases in RM shows significant fluctuations, whereas in European countries, the frequency of different forms of cerebral congenital anomalies remains practically unchanged, with the exception of anencephaly, where the trends were similar in both RM and EUROCAT.

3.2. Analysis of the influence of preconceptional and postconceptional factors on the occurrence of congenital malformations of the nervous system

Paternal Factors: *Infections:* Fathers of children with MCSN and FMCSN had a higher frequency of TORCH complex infections compared to the control group (62,5%). *Lifestyle:* Fathers of children with MCSN more frequently reported harmful vices, particularly tobacco use and alcohol consumption (>50% vs. 2,9-4,3%), a trend that was even more pronounced in the FMCSN subgroup (tobacco use: 68,8%; alcohol use: 68,8%). Although overall drug use was low, it was more frequent in the FMCSN group (12,5%), reflected in a significant OR of 24,31 (95% CI: 1,11-533,51). *General Health:* Fathers of children with MCSN, especially those with FMCSN, more frequently reported health issues like *diabetes mellitus* and *hypertension* (*HTA*). This was confirmed by a significant OR for FMCSN of 36,56 (95% CI: 1,78-749,00). Other factors like epilepsy, heart defects, and being overweight were also noted, though with less consistent significance.

Maternal Factors: *Infections:* Mothers of children with MCSN and FMCSN had a higher frequency of TORCH complex infections (>50% and 68.8% respectively) compared to the control group. *Lifestyle:* While not widespread (up to 25% of cases), mothers of children with MCSN more frequently reported vices, especially alcohol and drug use, a trend that was also present in the FMCSN subgroup. *General Health:* The evaluation of maternal health revealed varied frequencies of issues such as being overweight, HTA, pyelonephritis, anemia, and use of medication. These health problems, as well as being under the care of various medical specialists, were more common in mothers of children with MCSN. Notably, the differences in frequencies and OR values for FMCSN were similar to those for the total MCSN group.

Comparison of Maternal vs. Paternal Factors Some risk factors were common to both parents of children with MCSN (e.g., TORCH infections, alcohol use, medication use, HTA), while others were more specifically associated with either fathers (tobacco use, heart defects, diabetes) or mothers (anemia, pyelonephritis, epilepsy, genetic diseases). Obstetric-Gynecological Anamnesis (Prenatal Factors) The study found a generally low frequency (<25%) of investigated prenatal factors. However, two factors were associated with an increased risk: a family history of genetic diseases and a history of stagnant pregnancies. While there were slight frequency differences among the MCSN subtypes, they were not statistically significant, and the odds ratios were similar to the control group.

Also, based on the anamnesis data, we compared the groups regarding the administration of folic acid during pregnancy (pre- and post-conception). Thus, in the control group, the majority of mothers (88,6%, n = 62) were administered this supplement during the mentioned periods according to the indications, while among the mothers with children with MCSN, the mothers who did not receive folic acid predominated (78,6%, n = 55), the differences between these two groups being statistically significant (p <0.001). Examination of the subgroup of mothers with children with FMCSN reveals that in 93,8% of cases folic acid was not administered, with statistically significant differences compared to the control group (p <0.001), data shown in figure 5.

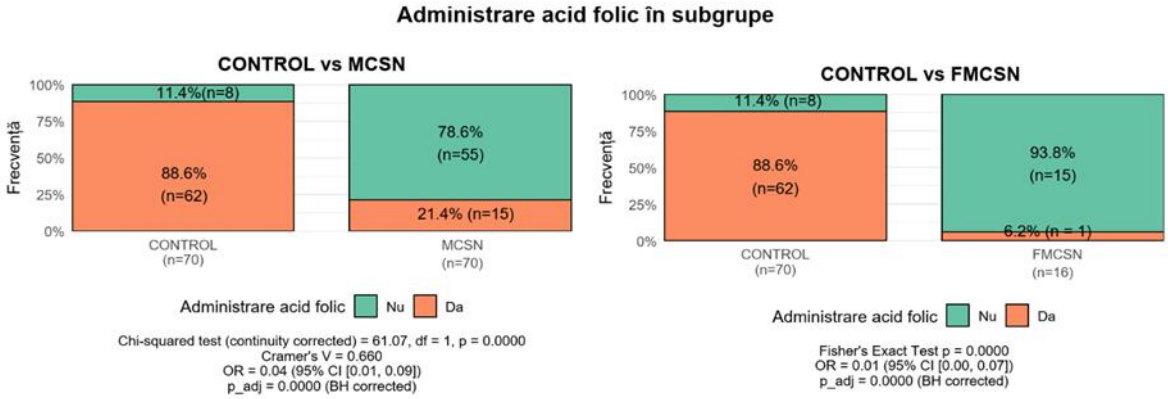


Figure 5. Folic acid administration in the subgroups investigated

The results obtained would indicate a series of potential risk factors for the occurrence of MCSN, which requires the expansion of studies on larger samples of patients, to evaluate the involvement and role of these factors in the pathogenesis of the pathologies investigated. Also, the trends observed regarding certain factors and FMCSN require further studies to confirm those observed in our study.

3.3. Exploring nosological and clinical relationships within congenital malformations of the nervous system: association patterns and associated neurological comorbidities

In the study group, the following frequencies (arranged hierarchically) of the forms of MCSN were attested (according to ICD-10): *congenital hydrocephalus* (75,71%) > *cerebral cyst* (24,29%) > *craniostenosis* (17,14%) > *spina bifida* and *congenital melingomyelocele* (11,43%) > *microcephaly* (10%) > *hypogenesis hypoplasia corpus callosum* and *agenesis corpus callosum* (8,57%) > *Arnold-Chiari syndrome* (2,86%) > *cerebellar atrophy* (1,43%). No cases with Dandy Walker syndrome were recorded. In the investigated group, isolated MCSN represented 38,6% (n = 27), and clinical cases with co-presence of several types of MCSN were: 2 types of MCSN – 51,4% (n = 36), 3 types of MCSN – 10% (n = 7), data shown in figure 6.

In the case of a single form of ***one forms of MCSN*** (n = 27), the following frequency hierarchy was observed that the results reveal different trends for certain MCSNs, with *hydrocephalus* and *meningomyelocele* occurring as monopathologies more frequently than other MCSNs (*microcephaly, agenesis, craniostenosis*).In the case of the presence of ***two forms of MCSN*** (bipathology, n = 36), the results obtained demonstrate that most frequently bipathology or double pathology is based on one of the pathological entities *hydrocephalus* (28 cases), followed by *cerebral cyst* (15 cases), *craniostenosis* (8 cases), *spina bifida* (6 cases), *microcephaly/meningomyelocele/agenesis of the corpus callosum* (4 cases), *hypogenesis of the*

corpus callosum (3 cases). It is necessary to mention that, in the studied sample, spina bifida as an element of bipathology was identified only in combination with hydrocephalus.

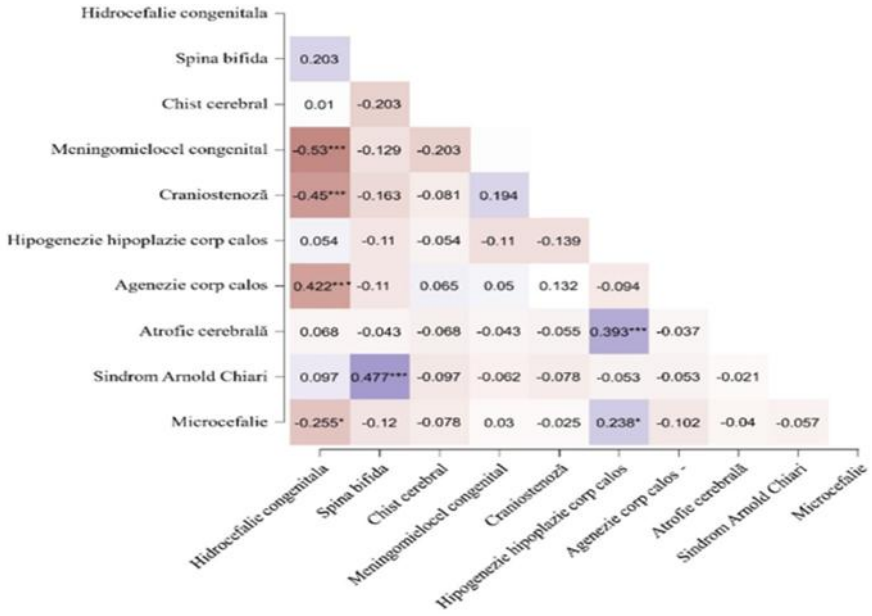


Figura 6. The matrix of Spearman correlations between MCSN within the studied group. *Note: interpretation of the statistical significance level of correlations: *** – p < 0.001; ** p – < 0.01; * – p < 0.05.*

In the case of the presence of ***three forms of MCSN*** (tripathology, n = 7), the results show that the most frequent tripathology contains one of the pathological entities *hydrocephalus* (6 cases), followed by *hypogenesis of the corpus callosum* (3 cases), *spina bifida/Arnaud Chiari syndrome/microcephaly/cerebral cyst* (2 cases), *atrophy/meningomyelocele/agenesis of the corpus callosum/craniosynostosis* (1 case).We can mention that, in the studied sample, *Arnaud Chiari syndrome* was identified only in association with *hydrocephalus* and *spina bifida* as part of the tripathology, and *cerebellar atrophy* – only in association with *hydrocephalus* and *hypogenesis of the corpus callosum*. We can also mention that *spina bifida* was associated only with *hydrocephalus*, in most cases as a bipathology (75%) and less often as a tripathology with *Arnold Chiari syndrome* (25%). Also, the combination of *hydrocephalus + hypogenesis of the corpus callosum* was identified as a bipathology, as well as a tripathology with the association of various other MCSN (*microcephaly, cerebellar atrophy, cerebellar cyst*).

Another aspect that we studied was the broad analysis of the structure of concomitant morbidity in children with MCSN (figure 7). from the studied sample (n = 70). The results reveal that the most frequent concomitant multipathology contains as one of the pathological entities: *epilepsy* (7 cases), followed by *tetraplegia/strabismus* (4 cases), *cerebral edema/retardation/West syndrome/tetraparesis* (3 cases), *hemiparesis/bulbar syndrome* (1 case). We mention that within the concomitant multipathology, no cases of *paraplegia* and *hemiplegia* were recorded. Based on the previously observed trends regarding the association of different MCSN, we performed a correlational analysis to establish the probability of theseinterrelationships. Thus, among a series of MCSN pathologies and concomitant pathologies, statistically significant associations of varying strength were observed; strong correlations: *spina bifida – flaccid paraplegia* (rho = 0,671; p <0,001); moderate correlations: *cerebral atrophy – congenital strabismus* (rho = 0,361; p = 0,003);

Arnold Chiari syndrome – flaccid paraplegia (rho = 0,377; p = 0,002); *microcephaly – tetraplegia* (rho = 0,332; p = 0,005); weak correlations: *hypogenesis/hypoplasia of the corpus callosum – bulbar syndrome* (rho = 0,254; p = 0,034); *hypogenesis/hypoplasia of the corpus callosum* – congenital strabismus (rho = 0,238; p = 0,046).

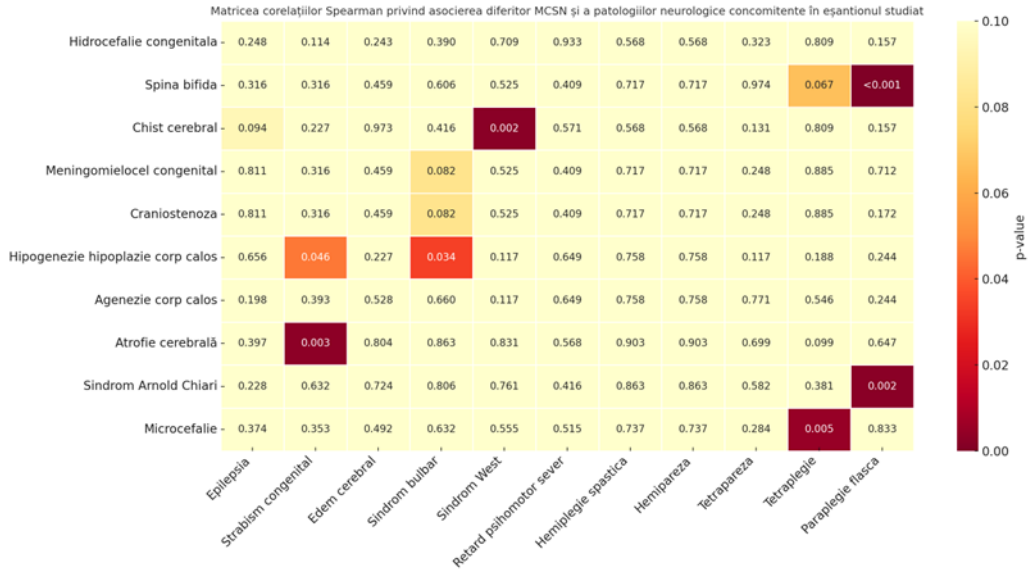


Figure 7. Matrix of Spearman correlations regarding the association of different MCSN and concomitant neurological pathologies in the studied sample

Generally, there has been a significant decrease in the incidence of MCSN in Moldova, including folate-dependent forms, which demonstrates the effectiveness of preventive interventions. The results obtained outline a complex profile of sociodemographic, prenatal, maternal/paternal factors associated with the risk of MCSN. For certain pathologies, the data from Moldova exceeded the prevalence of those from European countries in EUROCAT. Thus, the results obtained require further research on larger samples, including other components (such as genetic evaluation).

4. ANALYSIS OF FOLATE CYCLE GENES POLYMORPHISMS IN MOTHERS OF CHILDREN WITH CONGENITAL MALFORMATIONS OF THE NERVOUS SYSTEM

The identification of the genotypes of *MTHFR, MTR and MTRR* polymorphisms (genes involved in folate metabolism and in the methylation cycle – fundamental processes for DNA synthesis and repair, as well as epigenetic methylation) constitutes a precise method for molecular-genetic evaluation of their relationships with the status of the children included in the study (absence/presence of MCSN and its subtypes). Thus, to investigate this aspect, we performed statistical analysis in the following subgroups: mothers of children without MCSN (control, n = 70); mothers of children with MCSN (MCSN, n = 70). In the MCSN group, the FMCSN subgroup was highlighted, consisting of mothers of children with folate-dependent MCSN (FMCSN, n = 16). Two comparisons were made regarding the frequency and association of genetic polymorphisms of folate cycle genes (*MTHFR677, MTHFR1298, MTR2756* and *MTRR66*) and subgroup membership: mothers of children with MCSN versus mothers of children without MCSN (MCSN vs control); mothers of children with folate-dependent MCSN versus mothers of children

without MCSN (FMCSN vs control). The results of data processing were presented per gene studied.

4.1. Distribution and association of MTHFR677 gene variants in mothers of children with MCSN

Initially, we investigated the distribution of genotype variants of the *MTHFR677* gene in the mentioned groups, observing a series of differences (figure 8). In the control group, consisting of mothers of children without MCSN, the CC genotype variant was the most frequent (77,1%), followed by the CT variant (20,0%) and the TT variant, observed in only 2,9% of cases. In contrast, in the MCSN group (mothers of children with MCSN), the distribution of genotypes was different; thus, the CT variant was predominant (45,7%), followed by the CC variant (38,6%) and TT, identified in 15,7% of cases. These distribution differences would indicate an association between the presence of MCSN and the frequency of certain genotypes, given with statistical significance ($\chi2 = 22.27$; gl = 2; p = 1.46e-05). Also, when evaluating the differences in proportions within the groups, it was observed that they differ statistically significantly from the expected proportions in the control group ($\chi2 = 63.5$; gl = 2; p = 1.591636e-14), as well as in MCSN ($\chi2 = 10.3$; gl = 2; p = 5.758128e-03). In the FMCSN subgroup (mothers of children with folate-dependent MCSN) the following hierarchy of genotype variants was observed: CT (56,2%) > CC (37,5%) > TT (6,2%), with significant differences in proportions within the group ($\chi2 = 6.12$; gl = 2; p = 4.677062e-02). Compared to the control group, where statistically significant differences were observed in the distribution of genotype variants (Monte Carlo simulation – 10,000 iterations, $\chi2 = 9.76$; p = 8.80e-03), which would indicate an association between the presence of FMCSN and the frequency of certain genotypes. These results would indicate a possible association between certain *MTHFR677* genotypic variants in mothers and the risk of giving birth to children with MCSN. Based on the differences in proportions regarding different genotype variants of the genes examined in the studied groups, we performed their analysis based on allele frequency variations and different inheritance models (multiplicative, general, additive, dominant, recessive), with the Hardy Weinberg equilibrium test.

For the *MTHFR677* gene, in the studied groups (MCSN vs control) it was observed that both in the group of mothers with children with MCSN and in the control group the C allele predominates, but with a higher frequency in the control group (n = 86 vs n = 122), while the T allele occurs with a higher frequency in the MCSN group (n = 54 vs n = 18).

For the *MTHFR677* gene, when evaluating the distribution of allele and genotype frequencies and inheritance models in mothers of children with folate-dependent CMNS (FMCSN) versus those without CMNS (control), it was observed that the C allele predominated in both groups. However, it had a higher frequency in the control group (n = 122; 87.1%) compared to the FMCSN group (n = 21; 65.6%).

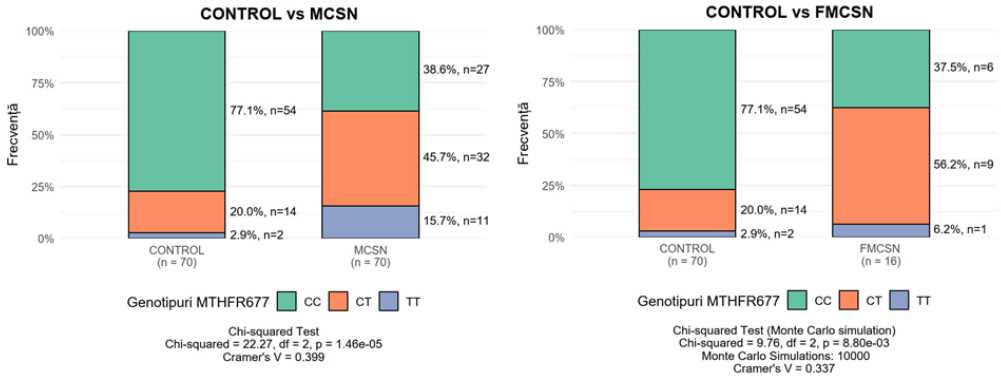


Figure 8. Distribution of *MTHFR677* genotype variants in mothers of children included in the study

Conversely, the T allele was more frequent in the FMCSN group (n = 11; 34.4%) compared to the control group (n = 18; 12.9%). These differences were significant according to the multiplicative inheritance model ($\chi2$=8,60, p = 0,003), suggesting an association between the presence of FMCSN and the frequency of the T allele (significant odds ratio – OR = 3,55; 95% CI: 1,47–8,57). According to the general inheritance model, statistically significant differences were observed for the CC, CT, and TT genotypes ($\chi2$=9,76, p = 0,008). The CC genotype was predominant in the control group (77,1%) compared to the FMCSN group (37,5%), with a non-significant odds ratio (OR = 0,18; 95% CI: 0,06–0,56). The CT genotype was more frequent in the FMCSN group (56,3%) compared to the control group (20,0%), with a significant odds ratio (OR = 5,14; 95% CI: 1,63–16,21). The TT genotype was generally rare but more frequent in the FMCSN group (6.3%) compared to the control group (2.9%), with a non-significant odds ratio (OR = 2,27; 95% CI: 0,19–26,66). The analysis of linear trends via the additive inheritance model confirmed the statistical significance of these differences ($\chi2$=8,22, p = 0,004). Hardy-Weinberg equilibrium (HWE) analysis reveals that the genotype distribution was in equilibrium in both groups (control: p = 0.49; FMCSN: p = 0.42).

4.2. Distribution and association of MTHFR1298 gene variants in mothers of children with MCSN

Initially, we investigated the distribution of genotype variants of the *MTHFR1298* gene in the mentioned groups, observing a series of differences (figure 9). In the *control* group, consisting of mothers of children without MCSN, the AA genotype variant was the most frequent (84,3%), followed by the AC variant (12,9%) and the CC variant, observed in only 2,9% of cases. In contrast, in the MCSN group (mothers of children with MCSN), the hierarchy of genotype distribution was similar, with the predominance of AA (54,3%), followed by the AC variant (42,9%) and CC, identified in 2,9% of cases. The frequency differences indicate an association between the presence of MCSN and the frequency of certain genotypes, which were statistically significant (Monte Carlo simulation – 10,000 iterations, $\chi2$ = 15,85; p = 1,00e-04). Also, when evaluating the differences in proportions within groups, it was observed that they differed statistically significantly from the expected proportions in the control group ($\chi2$ = 82,8; gl = 2; p = 1,032771e-18), as well as in MCSN ($\chi2$ = 30,6; gl = 2; p = 2,234036e-07).

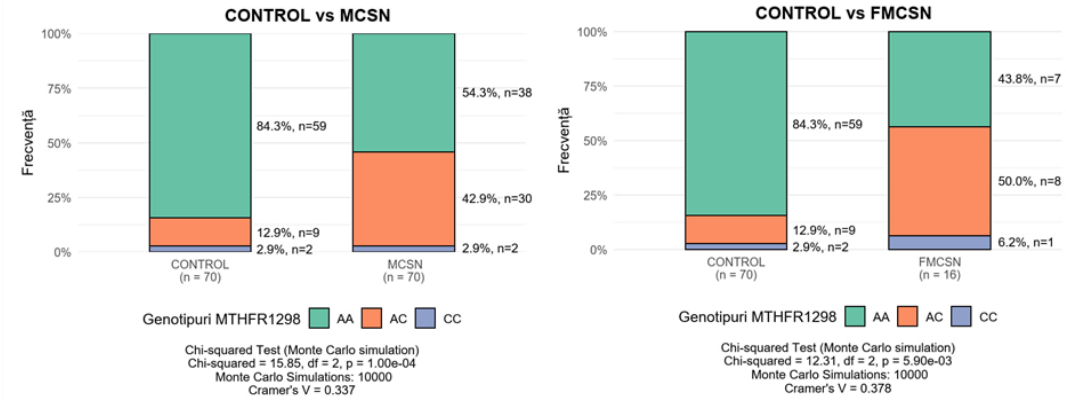


Figure 9. Distribution of *MTHFR1298* genotype variants in mothers of children included in the study

In the FMCSN subgroup (mothers of children with folate-dependent MCSN), the following hierarchy of genotype variants was observed: AC (50%) > AA (43,8%) > CC (6,2%), with insignificant differences in the proportions within the group ($\chi2 = 5,38$; gl = 2; p = 6,805085e-02). Compared to the *control* group, statistically significant differences in the distribution of genotype variants are observed (Monte Carlo simulation – 10,000 iterations, $\chi2 = 12,31$; p = 5,90e-03), which reveals an association between the presence of FMCSN and the frequency of certain genotypes of *MTHFR1298*.Based on the differences in proportions regarding different genotype variants of the genes examined in the studied groups, we performed their analysis based on allele frequency variations and different inheritance models (*multiplicative, general, additive, dominant, recessive*), including performing the Hardy-Weinberg equilibrium test.

For the MTHFR1298 gene, in the studied groups (MCSN vs control) it was observed that in both the group of mothers with children with MCSN and in the control group the A allele predominates, but with a higher frequency in the *control* group (n = 127 vs n = 106), while the C allele occurs with a higher frequency in the MCSN group (n = 34 vs n = 13). The analysis confirmed the association between the genotype variant of the MTHFR1298 gene and group membership (absence/presence of MCSN), this relationship being statistically significant based on the *multiplicative/additive/general/dominant inheritance models*, but not the recessive one. It should be noted that for the *MTHFR1298* gene, Hardy-Weinberg equilibrium was respected in both groups (control – p = 0,38; MCSN – p = 0,32).

4.3. Distribution and association of MTR2756 gene variants in mothers of children with MCSN

First, we investigated the distribution of genotype variants of the *MTR2756* gene in the mentioned groups, observing a series of differences (figure 10). In the *control* group, consisting of mothers of children without MCSN, the AA genotype variant was the most frequent (81.4%), followed by the AG variant (17,1%) and the GG variant, observed in only 1,4% of cases. In the MCSN group (mothers of children with MCSN), the hierarchy of genotype distribution was similar, with the predominance of AA (55,7%), followed by the AG variant (41,4%) and GG, identified in 2.9% of cases. The frequency differences reveal a statistically significant association between the presence of MCSN and the frequency of certain genotypes (Monte Carlo simulation – 10,000 iterations, $\chi2 = 10,76$; p = 2,50e-03). Also, when evaluating the differences in proportions within groups, it was observed that they differ statistically significantly from the expected proportions in the control group ($\chi2 = 75,5$; gl = 2; p = 4,118031e-17), as well as in MCSN ($\chi2 = 31,4$; gl = 2; p = 1,519066e-07).In the FMCSN subgroup (mothers of children with folate-

dependent MCSN), the following hierarchy of genotype variants was observed: AA (56,2%) > AG (43,8%) and the absence of GG (0%), with significant differences in proportions within the group ($\chi 2$ = 8,38; gl = 2; p = 1,51842e-02). Compared to the *control* group, statistically insignificant differences were observed in the distribution of genotype variants (Monte Carlo simulation – 10,000 iterations, $\chi 2$ = 5,48; p = 8,99e-02), which reveals the lack of association between the presence of FMCSN and the frequency of certain genotypes of MTR2756. Based on the differences in proportions regarding the different genotype variants of the genes examined in the studied groups, we performed their analysis based on allele frequency variations and different inheritance models (*multiplicative, general, additive, dominant, recessive*), including performing the Hardy-Weinberg equilibrium test for the *MTR2756* gene, in the studied groups (MCSN vs *control*) it was observed that both in the group of mothers with children with MCSN and in the control group the A allele predominates, but with a higher frequency in the *contro*l group (n = 126 vs. n = 107), while the G allele presents a higher frequency in the MCSN group (n = 33 vs n = 14).

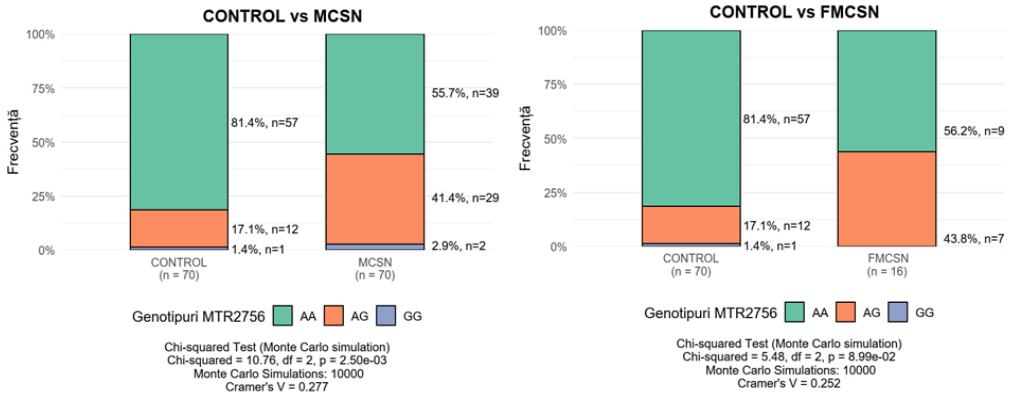


Figure 10. Distribution of genotype variants of *MTR2756* in mothers of children included in the study

The analysis confirmed the association between the genotype variant of the *MTR2756* gene and group membership (absence/presence of MCSN), this relationship being statistically significant based on the *multiplicative/additive/general/dominant inheritance models*, but not the recessive one. It should be noted that for the MTR2756 gene, Hardy-Weinberg equilibrium was respected in both groups (control – p = 0,86; MCSN – p = 0,31).For the MTR2756 gene, when evaluating the distribution of allele frequencies, genotypes and inheritance patterns in mothers of children with folate-dependent MCSN versus mothers of children without MCSN (*FMCSN vs control*), it was established that the A allele predominates in both groups, but with an increased frequency in the *control* group (n = 126, 90%), compared to FMCSN (n = 25; 78,1%), and the G allele was more frequent in the FMCSN group (n = 7; 21,9%), compared to the control group (n = 14; 10%). However, these differences were not statistically significant according to the *multiplicative inheritance model* ($\chi 2$ = 3,43; p = 0,06), which would not indicate an association between the presence of FMCSN and the increased frequency of the G allele in mothers, despite the higher chance of its presence (odds ratio not significant). – OR = 2,52; 95% CI: 0,92–6,88). It should be noted that for the MTR2756 gene, Hardy-Weinberg equilibrium was respected in both groups (control – p = 0.86; MCSN – p = 0.31).

4.4. Distribution and association of MTRR66 gene variants in mothers of children with MCSN

We investigated the distribution of genotype variants of the *MTRR66* gene in the mentioned groups, observing a series of differences (figure 11). In the *control* group, consisting of mothers of children without MCSN, the AA genotype variant was the most frequent (40,0%), followed by the AG variant (38.6%) and the GG variant (21.4%). In contrast, in the MCSN group (mothers of children with MCSN), the hierarchy of genotype distribution was different, with the predominance of AG (48,6%), followed by GG (35,7%) and AA identified in only 15,7% of cases. The frequency differences reveal an association between the presence of MCSN and the frequency of certain genotypes, which is statistically significant ($\chi2$ = 10,71; gl = 2; p = 4,72e-03). When evaluating the differences in proportions within the groups, it was observed that they did not differ statistically significantly from the expected proportions in the *control* group ($\chi2$ = 4,49; gl = 2; p = 1,061548e-01), but were statistically significantly different in MCSN ($\chi2$ = 11,5; gl = 2; p = 3,160128e-03).

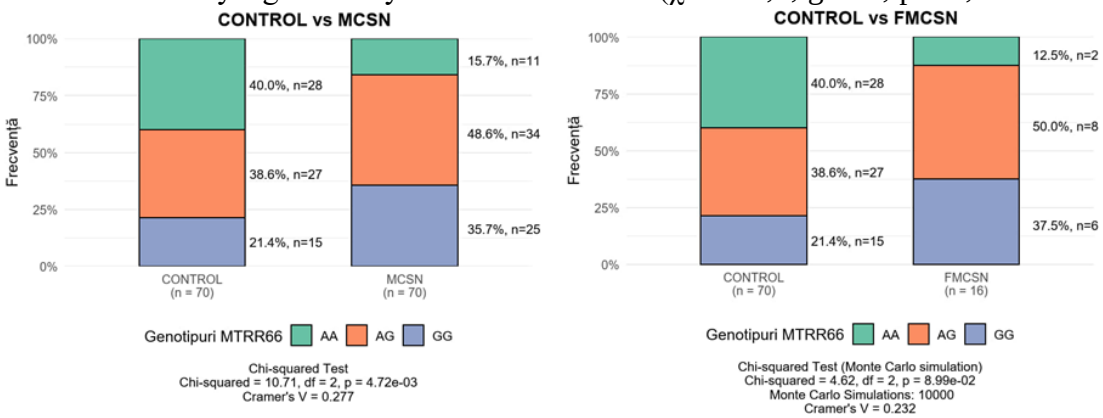


Figure 11. Distribution of *MTRR66* genotype variants in mothers of children included in the study

In the FMCSN subgroup (mothers of children with folate-dependent MCSN), the following hierarchy of genotype variants was observed: AG (50%) > GG (37,5%) > AA (12,5%), with insignificant differences in the proportions within the group ($\chi2$ = 3,5; gl = 2; p = 1,737739e-01). Compared to the control group, statistically insignificant differences in the distribution of genotype variants are observed (Monte Carlo simulation – 10,000 iterations, $\chi2$ = 4,62; p = 8,99e-02), which reveals a lack of association between the presence of FMCSN and the frequency of certain genotypes of MTRR66. These results would indicate a possible association between certain genotypic variants of MTRR66 in mothers and the risk of giving birth to children with MCSN, which would require further investigation in other studies. Based on the differences in proportions of the different genotype variants of the genes examined in the studied groups, we performed their analysis based on allele frequency variations and different inheritance models (*multiplicative, general, additive, dominant, recessive*), including performing the Hardy-Weinberg equilibrium test. The analysis confirmed the association between the genotype variant of the MTRR66 gene and group membership (absence/presence of MCSN), this relationship being statistically significant based on most inheritance models (*multiplicative/additive/general/dominant*). It should be noted that for the MTRR66 gene, Hardy-Weinberg equilibrium was respected in the MCSN group (p = 1) and in the control group (p = 0,25).

For the MTRR66 gene, when evaluating the distribution of allele frequencies, genotypes and inheritance patterns in mothers of children with folate-dependent MCSN versus those of children

without MCSN (*FMCSN vs control*), it was observed that the A allele predominates in the *control* group (n = 83; 59,3%), compared to the FMCSN group (n = 12; 37,5%), while the G allele has an increased frequency in the FMCSN group (n = 20; 62,5%), compared to the *control* (n = 57; 40.7%), these differences were statistically significant according to the *multiplicative inheritance model* ($\chi 2 = 5,00$; $p = 0,03$), which would indicate an association between the presence of FMCSN and the increased frequency of the G allele in mothers (significant odds ratio – OR = 2.43; 95% CI: 1,10–5,35). Hardy-Weinberg equilibrium (HWE) analysis shows that the genotype distribution was in equilibrium in the FMCSN group (p = 1) and the *control* group (p = 0,25).

4.5. Evaluation of the distribution of folate cycle gene polymorphisms in different forms of MCSN

Based on the results obtained, we subsequently investigated the particularities of the folate cycle gene genotypes in different forms of MCSN, the statistical processing being carried out by determining the degree of association between the MCSN subtype and the particularities of the maternal genotype, as well as by calculating the odds ratio (versus mothers in the control group) and confidence intervals, the inheritance model studied being the general one. In the mothers of children in the control group, it is attested that the *MTHFR677* gene presents the following distribution of genotype variants: CC (77,1%, n = 54); CT (20,0%, n = 14); TT (2,9%, n = 2), thus, the "pathological" (risk) genotypes CT and TT represent 22,9% of the cases. Subsequently, we analyzed the distribution of maternal genotype variants for the *MTHFR677* gene, evaluating the cumulative share of "pathological" (risk) variants, as well as the risks presented by their presence (attributable risk and relative risk) per subtype of MCSN.

In general, the previously observed trend of a higher proportion of genotypes containing the T allele in mothers of children with different forms of MCSN, compared to mothers of children in the control group, is confirmed, especially the proportion of the heterozygous CT variant, which in most subtypes of MCSN in the study sample was predominant in mothers of these children. To elucidate this phenomenon, we explored the associations between maternal genotype variants of the gene with the subtype of MCSN, and the cases with an increased chance (significant OR) are presented below. Thus, in the case of mothers of children with congenital *hydrocephalus*, a different distribution is observed compared to mothers of those in the control group, with a higher frequency of variants containing the T allele, such as heterozygous CT (significant odds ratio – OR = 2,84; 95% CI: 1,27-6,32) and homozygous TT (significant odds ratio – OR = 6,04; 95% CI: 1,23-29,78), the differences being statistically significant ($\chi 2 = 15,81$; $p = 0,0004$). In mothers of children with *meningomyelocele*, a different distribution is observed compared to mothers of children in the control group, with a higher frequency of variants containing the T allele, especially heterozygous CT (significant odds ratio – OR = 6,67; 95% CI: 1,42-31,30), the differences being statistically significant ($\chi 2 = 9,78$; $p = 0,008$). In mothers of children with cerebral cyst, a different distribution is observed compared to those of children in the control group, with a higher frequency of variants containing the T allele, especially heterozygous CT (significant odds ratio – OR = 3,56; 95% CI: 1,16-10,88), the differences being statistically significant ($\chi 2 = 8,84$; $p = 0,01$). In mothers of children with *craniostenosis*, a different distribution is attested, compared to mothers of those in the control group, with a higher frequency of variants containing the T allele, especially heterozygous CT (significant odds ratio – OR = 8,00; 95% CI: 2,10-30,42), the differences being statistically significant ($\chi 2 = 17,81$; $p = 0,0001$). In mothers of children with agenesis of the corpus callosum, a different distribution is attested versus mothers of children in the control group, with

a higher frequency of variants containing the T allele, especially heterozygous CT (significant odds ratio – OR = 8,00; 95% CI: 1,33-48,18), the differences being statistically significant ($\chi 2$ = 10,54; p = 0,005). For the other comparisons, despite some trends of distribution differences, no statistical significance of differences was observed between MCSNs, as well as when comparing with mothers of children in the control group.

In the mothers of children in the control group, for the *MTHFR1298* gene the following distribution of genotype variants is observed: AA (84,3%, n = 59); AC (12,9%, n = 9); CC (2,9%, n = 2), and the "pathological" (risk) genotypes AC and CC represent 15.8% of the cases. Subsequently, we analyzed the distribution of maternal genotype variants for the MTHFR1298 gene, evaluating the cumulative share of "pathological" (risk) variants, as well as the risks presented by their presence (attributable risk and relative risk) per subtype of MCSN.

In general, the previously observed trend of a higher proportion of genotypes containing the C allele in mothers of children with different forms of MCSN, compared to mothers of children in the *control* group, is confirmed, especially of the heterozygous AC variant, which in most subtypes of MCSN in the study sample showed a predominance in mothers of these children. To elucidate this phenomenon, we explored the associations between maternal genotype variants of the gene with the subtype of MCSN, and the cases with an increased chance (significant OR) are presented below. Thus, in the case of mothers of children with *congenital hydrocephalus*, a different distribution is observed compared to those of children in the *control* group, with a higher frequency of the heterozygous AC variant (significant odds ratio – OR = 4,45; 95% CI: 1,83-6,32), the differences being statistically significant ($\chi 2$ = 11,72; p = 0,003). In the case of mothers of children with *spina bifida*, a different distribution is observed compared to mothers of children in the control group, with a higher frequency of the heterozygous AC variant (significant odds ratio – OR = 11,30; 95% CI: 2,30-55,58), the differences being statistically significant ($\chi 2$ = 12,06; p = 0,002). Mothers of children with *cerebral cysts* show a different distribution compared to mothers of children in the *contro*l group, with a higher frequency of the heterozygous AC variant (significant odds ratio – OR = 9,68; 95% CI: 2,94-31,92), the differences being statistically significant ($\chi 2$ = 17,07; p = 0,0002). Mothers of children with *craniostenosis* show a different distribution compared to those of children in the control group, with a higher frequency of the heterozygous AC variant (significant odds ratio – OR = 6,78; 95% CI: 1,79-25,64), and the differences are statistically significant ($\chi 2$ = 9,59; p = 0,008). In mothers of children with *hypogenesis/hypoplasia* of the *corpus callosum*, a different distribution is observed compared to mothers of children in the *control* group, with a higher frequency of the heterozygous AC variant (significant odds ratio – OR = 6,78; 95% CI: 1,18-38,87), the differences reach statistical significance ($\chi 2$ = 9,29; p = 0,01). In mothers of children with *agenesis of the corpus callosum*, a different distribution is observed compared to mothers of those in the control group, with a higher frequency of the heterozygous AC variant (significant odds ratio – OR = 6,78; 95% CI: 1,18-38,87), the differences do not have statistical significance ($\chi 2$ = 5,80; p = 0,06). In mothers of children with *Arnold-Chiari syndrome*, a different distribution is observed compared to mothers of children in the *contro*l group, with a higher frequency of the heterozygous AC variant (significant odds ratio – OR = 32,37; 95% CI: 1,44-727,44), the differences being statistically significant ($\chi 2$ = 11,41; p = 0,003). In mothers of children with *microcephaly*, a different distribution was observed compared to those of children in the *control* group, with a higher frequency of variants containing the C allele – heterozygous AC and homozygous CC, the

differences being statistically significant ($\chi 2 = 7,16$; $p = 0,03$), but no significant OR values were observed. For the other comparisons, despite some trends in distribution differences, no statistical significance was observed in the differences between MCSN or in the comparison with mothers of children in the *control* group.

In the mothers of children in the control group, for the *MTR2756* gene the following distribution of genotype variants is observed: AA (81,4%, n = 57), AG (17,1%, n = 12); GG (1,4%, n = 1), and the "pathological" (risk) genotypes AG and GG represent a total of 18,6% of cases. Subsequently, we analyzed the distribution of maternal genotype variants for the MTR2756 gene, evaluating the cumulative share of "pathological" (risk) variants, as well as the risks determined by their presence (attributable and relative risk) per subtype of MCSN.

In general, it is found that, in different subtypes of MCSN, in most cases the normal homozygous AA variant predominates, as in the mothers of children in the *control* group, but a tendency is observed to increase the frequency of genotypes containing the G allele in mothers of children with different forms of MCSN compared to mothers of children in the control group, especially the heterozygous AG variant. To elucidate this phenomenon, we explored the associations between maternal genotype variants of the gene with the MCSN subtype, and the cases with an increased chance (significant OR) are presented below. Thus, in the case of mothers of children with *congenital hydrocephalus*, a different distribution is observed compared to those of children in the control group, with a higher frequency of the heterozygous AG variant (significant odds ratio – OR = 3,71; 95% CI: 1,62-8,46), the differences being statistically significant ($\chi 2 = 10,42$; $p = 0,005$). In the case of mothers of children with *congenital meningomyelocele*, a different distribution is observed compared to mothers of children in the *control* group, with a higher frequency of the heterozygous AG variant (significant odds ratio – OR = 4,83; 95% CI: 1,06-22,08), but the differences do not reach statistical significance ($p = 0,09$). In mothers of children with cerebral cyst, a different distribution is observed compared to mothers of those in the control group ($\chi 2 = 10,02$; $p = 0,0007$), with a higher frequency of the heterozygous AG variant (significant odds ratio – OR = 3,38; 95% CI: 1,07-10,67). Also, this form of MCSN differs statistically significantly regarding the distribution of MTR2756 gene genotypes in mothers with children with other forms of MCSN ($\chi 2 = 6,54$; $p = 0,004$), but without significant OR values.

In mothers of children with *agenesis of the corpus callosum*, there is a different distribution compared to mothers of children in the *control* group, with a higher frequency of the heterozygous AG variant, the differences being statistically significant ($\chi 2 = 6,35$; $p = 0,04$), but this was not reflected in significant OR values. For the other comparisons, despite some trends of differences in the distribution, no statistical significance of the differences between different MCSNs was observed, nor when compared with mothers of children in the control group.

In the mothers of children in the control group, for the *MTRR66* gene the following distribution of genotype variants is observed: AA (40%, n = 28), AG (38,6%, n = 27) and GG (21,4%, n = 15), thus, the "pathological" (risk) genotypes AG and GG represent in total 60% of the cases. We subsequently analyzed the distribution of maternal genotype variants for the MTRR66 gene, evaluating both the cumulative share of "pathological" (risk) variants and the risks presented by their presence (attributable and relative risk) per subtype of MCSN.

In general, it is found that in different subtypes of MCSN, the gene variants containing the G allele predominate in most cases, in contrast to the pattern observed in the mothers of children in the *control* group in which the normal homozygous AA variant predominates. However, a

different dynamic is observed for this gene, with a much lower attributable risk presented by pathological variants of the gene in mothers, as well as cases in which children with MCSN pathology had mothers with a predominantly normal type. Possibly, this phenomenon can be attributed to the low number of cases, as well as the influence of other factors (both genetic and epigenetic), which cannot be fully explored due to the limitations related to the sample size. Subsequently, we explored the associations between maternal genotype variants of the gene and the MCSN subtype, and the cases with an increased chance (significant OR) are presented below. In children with *congenital hydrocephalus* and *cerebral cyst*, statistically significant differences were observed regarding the distribution of maternal genotype variants for the *MTRR66* gene ($p = 0,005$, $p = 0,03$, respectively), but this was not reflected in significant ORs. It is worth noting that for this gene, no other significant differences regarding maternal genotype were identified in children with different types of MCSN versus mothers of those in the control group. The results obtained in the study highlight a significant association between certain genetic variants involved in folic acid metabolism and the risk of congenital malformations of the nervous system, especially in folate-dependent forms. Comparative analyses between subgroups found an increased prevalence of risk alleles and heterozygous or homozygous genotypes in mothers of affected children, supporting the hypothesis of the involvement of *MTHFR*, *MTR* and *MTRR* polymorphisms in the etiopathogenesis of these conditions. Observations regarding the distribution of variants according to clinical subtypes of MCSN suggest the existence of common genetic mechanisms, but also of distinct particularities associated with certain phenotypes. The validity of the data was supported by the respect of Hardy-Weinberg equilibrium, and the extension of research to larger and heterogeneous groups will allow for the deepening of these findings and the development of personalized preventive directions.

Risk assessment of epigenetic and genetic factors in the development of MCSN/FMCSN

A comparative analysis was performed between the control group (healthy children) and two study groups: children with congenital malformations of the nervous system (CMNS) and their subgroup with folate-dependent malformations (FMCNS). For each risk factor, the relative risk (RR) and the attributable risk (AR) were calculated, expressed as a percentage, highlighting the factors with the greatest impact on the development of these malformations. ***Paternal health factors:*** It was observed that the father's presence in the records of specialist doctors was a protective factor ($RR < 1$) against the occurrence of MCSN in the offspring, but this was not as evident in the case of the comparison FMCSN versus control ($RR = 1,55$), which would show that there are other, stronger factors that would increase the risk of developing this subtype of MCSN in the population. In general, the other indicators collected presented an increased risk for the development of both MCSN and FMCSN. A general trend observed is that for most paternal health indicators, the relative risk had higher values in the case of FMCSN. Separate analysis of attributable risk values reveals that, in the case of MCSN, a series of factors are attributable to the occurrence of the disease in 4-20% of all cases of the disease, but it should be noted that, in the case of FMCSN, attributable risk values increase, especially noting factors related to addictions (smoking, alcohol abuse) and infections in the TORCH complex ($RA > 50\%$). ***Maternal factors:*** It was found that the relative risk values are varied, especially noting a more marked impact of infectious factors (cytomegalovirus, viral hepatitis), exposure to noxious substances (chemical, physical) and certain vices (smoking). A similar trend with paternal health factors is the fact that, for most indicators, the relative risk had higher values in case of FMCSN. The assessment of

attributable risk reveals the following key factors (AR >50%) common to both MCSN and FMCSN: TORCH infections, being in the records of specialist doctors, cytomegalovirus infection, pathological pregnancy. Also, a strong influence of the anemia factor during pregnancy on the occurrence of MCSN (AR = 50,84%), as well as of the smoking factor during pregnancy on the occurrence of folate-dependent congenital malformations of the SN (AR = 61,95%). Folic acid administration during pregnancy revealed a significant protective effect against the occurrence of FMCS, while its lack increased the relative risk (RR = 19,09), which reconfirms the usefulness of this preventive measure. *Sociodemographic factors* reconfirmed the risk presented by male gender, rural location, and poor socioeconomic status in the occurrence of FMSCN, which have also been reported in other studies as potential factors associated with this subtype of congenital malformations of SN. When assessing the attributable risk, economic vulnerability status was noted to be associated with the occurrence of FMSCN (RA >50%). Regarding the folate cycle gene polymorphisms analyzed in the study, their different impact on the relative and attributable risk values was observed. Thus, in general, a strong association is observed especially with folate-dependent forms of MCSN for maternal SNP variants (CT for *MTHFR677,* AC for *MTHFR1298*, AG for *MTR2756*), with a more marked attributable risk (RA >25%). Also, the presence of homozygous variants of these genes (*MTHFR677CC*, *MTHFR 1298AA*, *MTR2756AA* and *MTRR66AA*) in mothers revealed a protective effect (RR <1), confirmed by a significant decrease in attributable risk. Thus, these data, including those from the previous statistical evaluation, demonstrate that the development of new health policies, oriented towards highlighting and controlling these factors, could have a beneficial effect on public health by reducing the incidence of cases of congenital malformations of the SN, especially folate-dependent ones. At the same time, a limitation of these estimates that must be taken into account is the fact that the low incidence of the disease in the general population of the Republic of Moldova, as well as the difficulty of sampling, did not allow the development of genetic-epigenetic interaction models.

GENERAL CONCLUSIONS

1. In the Republic of Moldova, a statistically significant downward trend in the incidence of Congenital Nervous System Malformations (MCSN) was observed between 2018 and 2023, including cases of folate-preventable NTDs. This likely reflects the impact of primary prevention measures and increased public access to prenatal diagnosis. The evaluation of MCSN frequency in the pediatric population of the Republic of Moldova (2018–2021) identified a higher frequency of *structural brain anomalies* and *hydrocephalus*, with *anencephaly*, *encephalocele*, and *microcephaly* being predominant. In the EUROCAT region, cases of microcephaly are more frequent compared to RM, while the incidence of encephalocele is relatively similar in RM and EUROCAT.

2. The analysis of risk factors for MCSN highlighted significant differences between the studied groups, particularly for factors during pregnancy (13 associations), followed by maternal factors (10 associations), and paternal factors (7 associations). For factors during pregnancy, significant odds ratio values were observed for cytomegalovirus (CMV), smoking, and viral hepatitis. Among maternal factors, strong associations were found for overweight/obesity, genetic diseases, and infections. Among paternal factors, smoking, alcohol consumption, and TORCH infections predominated.

3. The study identified the most frequent form of MCSN as congenital hydrocephalus (75,71%), followed by cerebral cysts (24,29%), craniosynostosis (17,14%), spina bifida/congenital

myelomeningocele (each 11,43%), and microcephaly (10%). Other pathologies (hypogenesis/agenesis of the corpus callosum, cerebral atrophy, Arnold-Chiari syndrome) were rarely found (<10% of cases). Various combinations of NTD forms were observed, identifying two MCSN clusters: 1) agenesis of the corpus callosum/congenital myelomeningocele/craniosynostosis; 2) spina bifida/Arnold-Chiari syndrome. Different associations between the NTD form and concomitant pathologies (single/multiple) were identified in the investigated sample, with strong correlations observed especially between *spina bifida* and *flaccid paraplegia* (ρ=0,671), moderate correlations between *cerebral atrophy* and *congenital strabismus* (ρ=0,361), and between *Arnold-Chiari* syndrome and *flaccid paraplegia* (ρ=0,377).

4. The performed statistical analyses demonstrate that mothers of children with MCSN, more pronounced in the folate-dependent forms (FMCSN), show an overrepresentation of risk alleles in folate cycle genes (*MTHFR677-T*, *MTHFR1298-C*, *MTR2756-G*, *MTRR66-G*), most frequently in heterozygotes (CT/AC/AG). Homozygous "wild-type" genotypes (e.g., *MTHFR677-CC*, *MTHFR1298-AA*, *MTR2756-AA*, *MTRR66-AA*) had a protective effect (RR<1;PAF<0). From the non-genetic domain, TORCH/CMV infections, maternal vices (smoking/alcohol), exposure to harmful agents, and socioeconomic vulnerability contributed substantially to the disease burden, with Attributable Risk Percent (RA%) often exceeding 50% in the exposed FMCSN group. Folic acid supplementation was protective, while the lack of it was associated with a marked RR (≈19). Although formal *gene-environment/gene-gene* interaction models were not possible due to small subtype samples, the directional convergence-risk alleles + unfavorable exposures-suggests at least additive, possibly synergistic effects. Overall, the data support the existence of a composite risk profile (genetic + epigenetic/environmental) and indicate prevention targets with high potential impact: periconceptional folic acid, infection control, smoking/alcohol cessation, and socioeconomic interventions.

PRACTICAL RECOMMENDATIONS

1. For public health policies, it is recommended to maintain and continuously update the National Registry of Congenital Malformations, with complete standardization according to international networks (EUROCAT, GBD), to ensure effective epidemiological surveillance and support national maternal and child health intervention policies.

2. In this context, it would be useful to conduct periodic comparative analyses between national and European data to identify possible trend deviations or shortcomings in the implemented preventive measures, allowing national programs to be adjusted according to the identified risk level.

3. For medical practice, it is advisable to include the systematic evaluation of periconceptional risk factors in family planning consultations, focusing on educating both parents about the role of environmental, nutritional, and behavioral factors in MCSN prevention, as well as recommending periconceptional folic acid supplementation, personalized according to the risk profile.

4. Future research needs to focus on developing clinico-statistical models for correlating different forms of MCSN with associated neurological comorbidities, in order to identify useful phenotypic patterns for early diagnosis, prognosis, and the personalization of therapeutic interventions.

5. With the translation of evidence from genetic research into medical practice, it would be advisable to gradually implement genetic testing for *MTHFR* and related gene polymorphisms in reproductive medicine centers. The goal is to identify women at increased risk for folate-dependent MCSN and to develop management guidelines adapted to the individual genetic profile.

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LIST OF PUBLICATIONS AND PARTICIPATION IN SCIENTIFIC FORUMS

by Ms. **Tihai Olga**, completed for her doctoral thesis in medical sciences,
on the topic "**Estimating the risk of developing folate-dependent congenital malformations of the nervous system during ontogenesis**",
Doctoral program: 322.01 - Pediatrics and Neonatology,
Nicolae Testemitanu State University of Medicine and Pharmacy of the Republic of Moldova,
Doctoral supervisor: Sprincean, Mariana, Dr. Hab. Med., Associate Professor

- **Articles in foreign scientific journals:**

✓ **articles in ISI, SCOPUS, and other international databases recognized by ANACEC**

1. **Tihai O**, Sprincean M, Anton-Paduraru D, Gorduza E, Calcii C, Lupusor N, Racovita S, Marga S, Feghiu L, Cuznet L, Revenco N, Hadjiu S. The genetics of folate metabolism and maternal risks of birth of a child with congenital brain malformations. Archives of the Balkan Medical Union. 2023;58(2):187-194. ISSN: print 1584-9244, ISSN electronic 2558-815X, DOI:10.31688/ABMU.2023.58.2.12 (IF Scopus: 0,5)

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✓ **Articles in scientific conference proceedings**

✓ **national with international participation**

4. **Tihai O**, Sprincean M, Racoviță S, Barbova N, Halabudenco E, Egorov V, Călcâi C, Revenco N, Hadjiu S. Aspecte ale diagnosticului prenatal la fetușii cu malformații congenitale cerebrale: prezentare de caz clinic. In: Materialele simpozionului național cu participare internațională „Registrul maladiilor reumatice la copii: experiența Republicii Moldova". 2023. p.69-73. ISBN 978-9975-58-285-8. https://ibn.idsi.md /ro/vizualizare_articol/196920

✓ **proceedings of international conferences**

5. Sprincean M, Hadjiu S, **Tihai O**, Barbova N. Prenatal diagnosis of cerebral congenital malformations folate dependent in pregnancies with genetic risk. In: Middle East International Conference on Contemporary Scientific Studies, Ed. 5, 27-28 martie 2021, Ankara. Ankara: Farabi Publishing House; 2021, vol. 2, pp. 394-401. ISBN 978-625-7898-38-6. https://ibn.idsi.md/ro/vizualizare_articol/219355

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6. Sprincean M, Hadjiu S, Călcîi C, Galbur V, **Tihai O**, Revenco N. Bolile genetice și epilepsia (reviu de literatură). In: Actualități în pediatrie și impactul imunizării asupra morbidității și mortalității copiilor în Republica Moldova; 22-23 septembrie 2023, Chișinău. Chișinău: Taicom (Ridgeone Group), 2023, pp. 31-36. ISBN 978-9975-58-293-3 https://ibn.idsi.md/ro/ vizualizare_articol/197770
7. Sprincean M, Sprincean S, Hadjiu S, Sidorenko , Calcii C, Racoviță S, **Tihai O**, Popa C, Dumitraș A, Galbur V, Ababii M, Balica N, Revenco N, Groppa S. Bioethical principles in prenatal diagnostics and genetic counseling. In: NANO: - 2024: "Quo Vadis – Ethics of the Scientific Research", Chișinău. Chișinău, Republica Moldova, 2024, pp. 44-47. ISBN 978-9975-64-422-8. https://ibn.idsi.md/ro/vizualizare_articol/204131

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8. **Tihai O**, Hadjiu S, Sprincean M, Călcîi C, Lupuşor N, Bejan N, Revenco N. Malformatiile congenitale cerebrale la copii: cosideratii clinico-genetice. Supliment la Revista de Neurologie si Psihiatrie a Copilului si Adolescentului din România. Predeal, România: Editura ArtPress, 2018;3(24S):73-74. ISSN 2068-8040. https://ibn.idsi.md /ro/vizualizare_articol/143090
9. **Tihai O**, Hadjiu S, Sprincean M, Barbova N, Egorov V, Halabudenco E, Revenco N Congenital cerebral malformations in the pregnancies with genetic risc. In: International Congress of Geneticists and Breeders from the Republic of Moldova, Ed. 11, 15-16 iunie 2021, Chişinău. Chișinău, Republica Moldova: Centrul Editorial-Poligrafic al Universității de Stat din Moldova, 2021, Ediția 11, p. 64. ISBN 978-9975-933-56-8. DOI: https://doi.org/10.53040/cga11.2021
10. **Tihai O**, Hadjiu S, Barbova N, Revenco N, Sprincean M. Factorii de risc al malformatiilor congenitale cerebrale folat-dependente. Supliment la Revista de Neurologie si Psihiatrie a Copilului si Adolescentului din Romania. București, România, 2021;27(3R):44-45. ISSN 2068-8040. https://ibn.idsi.md/ro/vizualizare_articol/200487
11. **Tihai O**, Hadjiu S, Sprincean M, Calcii C, Lupusor N, Griu C, Feghiu L, Revenco N. Risk of cerebral vascular accident in children with congenital cerebral malformations. In: European Stroke Journal. Lyon, France, 2022;7(1S):121. DOI:10.1177/23969873221087559
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13. **Tihai O**, Sprincean M, Racoviță S, Călcâi C, Lupuşor N, Feghiu L, Cuzneț L, Grîu C, Revenco N, Hadjiu S. Evoluția și prognosticul malformațiilor congenitale ale sistemului nervos central. Caz clinic. În: Revista Română a SNPCAR. Cel de-al XXIV-lea Congres SNPCAR şi a 46-a Conferinţă Naţională de Neurologie-Psihiatrie a Copilului şi Adolescentului şi Profesiuni Asociate din România cu participare internaţională. Craiova, România, 2023;29(3R):33. ISSN 2068-8040. https://ibn.idsi.md/ro/vizualizare_articol/200185

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15. **Tihai O**, Sprincean M, Barbova N, Revenco N, Hadjiu S. Identification of genes involved in folic acid synthesis in mothers of children with congenital cerebral malformations. In: Revista de Ştiinţe ale Sănătăţii din Moldova. 2022;3,1(29):391. ISSN 2345-1467. https://ibn.idsi.md/ro/vizualizare_articol/ 169111
16. **Tihai O**, Sprincean M, Barbova N, Egorov V, Revenco N, Hadjiu S. Diagnosticul genetic al malformațiilor congenitale cerebrale folat-dependente la copii. În: Materialele Conferinței Chișinău-Sibiu Managementul interdisciplinar al copilului. Chișinău: Tipografia „Taicom", 2022;156-157. ISBN 978-9975-58-274-2. https://ibn.idsi.md/ro/vizualizare_articol/171790
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- **Invention patents, patents, registration certificates, inventions**
- **Patent for inventions**

19. **Tihai O**, Sprincean M, Hadjiu S, Revenco N. Metodă de stabilire a riscului dezvoltării malformaţiilor congenitale cerebrale folat-dependente la copii. Patent for inventions MD 1731 Y. BOPI. 2024 Dec. 2023.

- **Registration certificates**

20. **Tihai O**, Sprincean M, Hadjiu S, Revenco N. Metodă de stabilire a riscului dezvoltării malformaţiilor congenitale cerebrale folat-dependente la copii. Intellectual Property Rights OȘ 7704 2023 Oct. 30.

- **Inventions certificates**

21. **Tihai O**, Sprincean M, Hadjiu S, Racovița S, Litovcenco A, Barbova N, Revenco N. Estimarea riscului dezvoltării în ontogeneză a malformațiilor congenitale cerebrale folat-dependente. Innovation Certificate Nr. 5985. 21 Feb. 2023.
22. **Tihai O**, Sprincean M, Hadjiu S, Racovița S, Litovcenco A, Barbova N, Revenco N. Metodă de diagnostic al polimorfismului în gena MTHFR677 la mamele copiilor cu malformații congenitale cerebrale. Innovation Certificate Nr. 5986. 21 Feb. 2023.
23. **Tihai O**, Sprincean M, Hadjiu S, Racovița S, Litovcenco A, Barbova N, Revenco N. Metodă de diagnostic al polimorfismului în gena MTHFR1298 la mamele copiilor cu malformații congenitale cerebrale. Innovation Certificate Nr. 5987. 21 Feb. 2023.
24. **Tihai O**, Sprincean M, Hadjiu S, Racovița S, Litovcenco A, Barbova N, Revenco N. Metodă de diagnostic al polimorfismului în gena MTR2756 la mamele copiilor cu malformații congenitale cerebrale. Innovation Certificate Nr. 5988. 21 Feb. 2023.
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26. **Tihai O**, Sprincean M, Hadjiu S, Racovița S, Litovcenco A, Barbova N, Revenco N. Estimarea riscului dezvoltării în ontogeneză a malformațiilor congenitale cerebrale folat-dependente. Innovation Certificate Nr. 511. 21 Feb. 2023.
27. **Tihai O**, Sprincean M, Hadjiu S, Racovița S, Litovcenco A, Barbova N, Revenco N. Metodă de diagnostic al polimorfismului în gena MTHFR677 la mamele copiilor cu malformații congenitale cerebrale. Innovation Certificate Nr. 512. 21 Feb. 2023.
28. **Tihai O**, Sprincean M, Hadjiu S, Racovița S, Litovcenco A, Barbova N, Revenco N. Metodă de diagnostic al polimorfismului în gena MTHFR1298 la mamele copiilor cu malformații congenitale cerebrale. Innovation Certificate Nr. 513. 21 Feb. 2023.
29. **Tihai O**, Sprincean M, Hadjiu S, Racovița S, Litovcenco A, Barbova N, Revenco N. Metodă de diagnostic al polimorfismului în gena MTR2756 la mamele copiilor cu malformații congenitale cerebrale. Innovation Certificate Nr. 514. 21 Feb. 2023.
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- **Implementations acts**

31. **Tihai O**, Sprincean M, Hadjiu S, Racovița S, Litovcenco A, Barbova N, Revenco N. Estimarea riscului dezvoltării în ontogeneză a malformațiilor congenitale cerebrale folat-dependente. Act de implementare Nr. 16. 21 Feb. 2023.
32. **Tihai O**, Sprincean M, Hadjiu S, Racovița S, Litovcenco A, Barbova N, Revenco N. Estimarea riscului dezvoltării în ontogeneză a malformațiilor congenitale cerebrale folat-dependente. Act de implementare Nr. 51. 21 Feb. 2023.

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- **Materials at invention salons**

41. **Tihai O**, Sprincean M, Hadjiu S, Revenco N. Method for determining the risk of developing folate-dependent cerebral congenital malformations in children. Salonul internațional de invenții și inovații „Traian Vuia". Timișoara, România, 13-15 iunie 2024.

- **Participation with presentations at scientific forums:**
- ✓ **Internaţional**

42. **Tihai O**, Hadjiu S, Călcîi C, Lupușor N, Bejan N, Revenco N, Sprincean M. Unele aspecte ale malformațiilor congenitale cerebrale la copii. Comunicarea prezentată la: Școala medicală pediatrică cu participare internațională, 15-17 Mai 2018, Iași, România.
43. **Tihai O**, Hadjiu S, Sprincean M, Călcîi C, Lupușor N, Bejan N, Revenco N. Malformațiile congenitale cerebrale la copii: considerații clinico-genetice. Comunicarea prezentată la: A 40-a Conferinţă Naţională de Neurologie-Psihiatrie şi Profesiuni Asociate a Copilului şi Adolescentului din România cu participare internaţională, 21 Sept 2018, Predeal, România.
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45. **Tihai O**, Hadjiu S, Barbova N, Revenco N, Sprincean M. Factorii de risc al malformațiilor congenitale cerebrale folat-dependente. Comunicarea prezentată la: Al XXI-lea Congres și a 43-a Conferință națională de Neurologie-Psihiatrie a copilului si adolescentului și profesiuni asociate din România cu participare internaţională, 23 Sept. 2021.
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49. **Tihai O**, Sprincean M, Galbur V, Dumitraș A, Revenco N, Hadjiu S. Particularități genetice în malformațiile congenitale ale sistemului nervos central. Prezentare de caz clinic. Comunicarea prezentată la: A 46-a Conferinţă Naţională de Neurologie-Psihiatrie a Copilului şi Adolescentului şi Profesiuni Asociate din România cu participare internaţională, 25-28 Sept 2024, Craiova, România.
    - ✓ **naţional with international participation**
50. **Tihai O**, Hadjiu S, Sprincean M, Natalia B, Racoviță S, Egorov V, Revenco N. Impactul metabolismului acidul folic asupra dezvoltării malformațiilor congenitale cerebrale la copii: efectul genotipului matern. Comunicarea prezentată la: Conferința națională cu participare internațională „Actualități în practica pediatrică: provocări și succese", 16 Sept 2022, Chișinău, Moldova.
51. **Tihai O**, Sprincean M, Barbova N, Egorov V, Halabudenco E, Călcîi C, Lupușor N, Revenco, Groppa S, Hadjiu S. Evaluarea clinico-genetică postnatală a copiilor cu defecte ale tubului neural folat-dependente: caz clinic. Comunicarea prezentată la: Al VIII-a Congres Internațional al Societății de Pediatrie din Republica Moldova „Pediatria – specialitate multidisciplinară", 06-08 iunie 2024, Chișinău, Moldova.
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52. **Tihai O**, Sprincean M, Racoviță S, Barbova N, Halabudenco E, Egorov V, Călcâi C, Revenco N, Hadjiu S. Aspecte ale diagnosticului prenatal la fetușii cu malformații congenitale cerebrale: prezentare de caz clinic. Comunicarea prezentată la: Simpozionul „Registrul maladiilor reumatice la copii: experiența Republicii Moldova", 31 martie 2023, Chișinău, Moldova. https://ibn.idsi.md/ro/vizualizare_articol/196920
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    - ✓ **internaţional**
53. **Tihai O**, Sprincean M, Revenco N, Lupusor N, Bejan N, Calcîi C, Hadjiu S. The clinical-genetic manifestation in tuberous sclerosis: case report. In: The 4th EAN Congress Neurology, 7-10 Oct 2018, Lisbon, Portugal.
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55. **Tihai O**, Sprincean M, Hadjiu S, Revenco N. Identification of genes involved in folic acid synthesic in mothers of children with congenital cerebral malformations. In: The 8th European Academy of Neurology congress, 25-28 June 2022, Vienna, Austria.
56. **Tihai O**, Mariana S, Hadjiu S, Barbova N, Egorov V, Revenco N. Identification of genes involved in folic acid synthesis in mothers of children with congenital cerebral malformations. In: The 14th World Stroke Congress, 26-29 Oct 2022, Singapore, Asia.
57. **Tihai O**, Hadjiu S, Sprincean M, Călcîi C, Lupușor N, Grîu C, Cuznet L, Feghiu L, Revenco N. Identification of genes involved in folic acid synthesis in mothers of children with congenital cerebral malformations. In: The 9th EAN Congress, 1-4 iulie 2023, Budapest, Hungary.
58. **Tihai O**, Sprîncean M, Galbur V, Dumitraș A, Revenco N, Hadjiu S. Particuarități genetice în

malformațiile congenital ale sistemului nervos central. Prezentare de caz. In: A 46-a Conferinţă Naţională de Neurologie, Psihiatrie şi Profesiuni Asociate Copii şi Adolescenţi din România, 25-28 Sept 2024, Craiova, România.

- ✓ **naţional**

59. **Tihai O**, Sprincean M. Prenatal diagnosis of congenital malformations of the brain in pregnancies with genetic risc. Comunicarea prezentată la: The 7th International Medical Congress for Students and Young Doctors MedEspera, 3-5 Mai 2018, Chișinău, Moldova. https://ibn.idsi.md/ro/vizualizare_articol/122997
60. Barbova N, Egorov V, **Tihai O**, Sprincean M, Halabudenco E. Frecvența malformațiilor congenitale ale sistemului nervos central în Moldova, comparativ cu datele Registrului Internațional EUROCAT. Comunicarea prezentată la: Conferința Științifică Anuală „Cercetarea în Biomedicină și Sănătate: Calitate, Excelență și Performanță”, 10-22 Oct 2021, Chișinău, Moldova. https://ibn.idsi.md/ro/vizualizare_articol/206455
61. **Tihai O**, Hadjiu S, Sprincean M, Călcîi C, Lupușor N, Racoviță S, Revenco N. Malformațiile congenitale cerebrale la copiii cu accident vascular cerebral în Republica Moldova. Comunicarea prezentată la: Conferința Științifică Anuală „Cercetarea în Biomedicină și Sănătate: Calitate, Excelență și Performanță”, 18-20 Oct 2023, Chișinău, Moldova. https://ibn.idsi.md/ro/vizualizare_articol/195012

ADNOTARE

Tihai Olga

„Estimarea riscului dezvoltării în ontogeneză a malformațiilor congenitale ale sistemului nervos folat-dependente”

Teză de doctor în științe medicale, Chișinău, 2025

Structura tezei. Teza este scrisă în limba română cu titlu de manuscris. Este expusă pe 211 de pagini și conține: introducere, 4 capitole (revista literaturii, materiale și metode de cercetare, rezultate obținute - în două capitole, discuții), concluzii generale și recomandări practice. Bibliografia include 228 de titluri. Lucrarea este ilustrată cu 11 tabele, 37 de figuri, 1 formulă și 18 anexe.

Cuvinte-cheie: sistem nervos central, malformații ale sistemului nervos, defecte ale tubului neural, anencefalie, spină bifidă, sugar, deficit de acid folic, factori sociodemografici, factori de risc, polimorfism genetic

Domeniul de studiu: 322.01 – Pediatrie și neonatologie

Scopul lucrării. Evaluarea particularităților epidemiologice, etiologice și clinice ale malformațiilor congenitale ale sistemului nervos la populația pediatrică din Republica Moldova, în special ale formelor prevenibile prin suplimentare cu folat.

Obiectivele cercetării. Descrierea distribuției și a structurii malformațiilor congenitale ale sistemului nervos la copiii din Republica Moldova, pe baza datelor din Registrul Național al Malformațiilor Congenitale pentru perioada 2018-2023. Determinarea frecvenței malformațiilor congenitale ale sistemului nervos în Republica Moldova și compararea acesteia cu datele din rețeaua europeană EUROCAT pentru perioada 2018-2021. Evaluarea asocierii dintre factorii de risc pre- și postconcepționali, materni, paterni și de mediu și prezența malformațiilor congenitale ale sistemului nervos, cu evidențierea particularităților formelor susceptibile de prevenție prin suplimentare cu folat. Analiza variantelor de asociere între diferite forme ale malformațiilor congenitale ale sistemului nervos, inclusiv cu patologiile neurologice concomitente. Evaluarea frecvenței polimorfismelor uninucleotidice materne ale genelor implicate în metabolismul folatului (*MTHFR677*, *MTHFR1298*, *MTR2756*, MTRR66) și a asocierii acestora cu prezența și tipologia malformațiilor congenitale ale sistemului nervos, în special a formelor folat-dependente.

Noutatea și originalitatea științifică. Pentru prima dată în Republica Moldova a fost realizată o investigație cu design dual asupra malformațiilor congenitale ale sistemului nervos (MCSN), combinând analiza retrospectivă a datelor populaționale cu un studiu caz-control extins privind expresia factorilor de risc. Studiul a evidențiat o tendință semnificativă statistic de scădere a incidenței MCSN și a formelor folat-prevenibile (FMCSN) în perioada 2018-2023, oferind primele dovezi naționale privind dinamica temporală a acestora. În comparație cu datele registrelor EUROCAT, cercetarea a documentat un profil diferențiat al distribuției subtipurilor clinice, cu o pondere mai mare a anomaliilor structurale cerebrale și a hidrocefaliei în Republica Moldova. Totodată, s-a constatat o asociere pronunțată între lipsa suplimentării periconcepționale cu acid folic și apariția FMCSN (93% din cazuri). De asemenea, a fost determinată o ierarhie a factorilor de risc materni, paterni și prenatali, precum și gradul de asociere a acestora cu MCSN în populația studiată, fiind identificați factori cu impact potențial major: agenți infecțioși (complexul TORCH, citomegalovirus), vicii comportamentale (tabagism, consum de alcool și/sau droguri), statusul de sănătate parental (diabet, hipertensiune arterială, supraponderalitate), utilizarea medicației și expunerea la noxe în perioada intrauterină. Cercetarea a evidențiat particularități relevante privind expresia clinică a MCSN, tendințele de coasociere între diferite forme (clustere), precum și corelații semnificative între subtipurile clinice și patologiile neurologice asociate, consolidând astfel perspectiva unei abordări integrative și diferențiate a acestor entități patologice. Pentru prima dată în context regional au fost demonstrate diferențe semnificative în distribuția variantelor genotipice ale genelor *MTHFR677*, *MTHFR1298*, *MTR2756* și MTRR66 între mamele copiilor cu MCSN și cele din lotul de control, cu asocieri specifice între anumite genotipuri și riscul de FMCSN (OR între 2,18 și 6,78).

Implementarea rezultatelor științifice. Rezultatele cercetărilor științifice au fost implementate în procesul de cercetare, în activitatea metodologică și clinică în Departamentul *Pediatrie* al IP USMF „Nicolae Testemițanu”, în Clinica de Neurologie Pediatrică a Departamentului *Pediatrie* IP „Nicolae Testemițanu” din IMSP Institutul Mamei și Copilului.

ANNOTATION

Tihai Olga

"Risk Assessment of the Development of Folate-Dependent Congenital Malformations of the Nervous System During Ontogenesis"

Doctoral thesis in medical sciences, Chisinau, 2025

Thesis structure. The thesis is written in Romanian as a manuscript. It is presented on 211 pages and contains: introduction, 4 chapters (literature review, research materials and methods, results obtained – in two chapters, discussions), general conclusions and practical recommendations. The bibliography includes 228 titles. The work is illustrated with 11 tables, 37 figures, 1 formula and 18 annexes.**Keywords:** central nervous system, congenital nervous system malformations, neural tube defects, anencephaly, spina bifida, infant, folate deficiency, sociodemographic factors, risk factors, genetic polymorphism.

Field of study: 322.01 – Pediatrics and Neonatology

The purpose of the work: To evaluate the epidemiological, etiological, and clinical characteristics of congenital nervous system malformations (CNSM) in the pediatric population of the Republic of Moldova, with a particular focus on folate-preventable forms.

Research objectives. To describe the distribution and structure of congenital nervous system malformations in children in the Republic of Moldova based on data from the National Registry of Congenital Malformations for the period 2018–2023. To determine the frequency of congenital nervous system malformations in the Republic of Moldova and compare it with data from the European EUROCAT network for the period 2018–2021. To assess the association between pre- and postconceptional, maternal, paternal, and environmental risk factors and the presence of congenital nervous system malformations, particularly for the folate-preventable forms. To analyze the patterns of association between various types of nervous system malformations, as well with comorbid neurological pathologies. To evaluate the frequency of maternal single-nucleotide polymorphisms in genes involved in folate metabolism (*MTHFR677*, *MTHFR1298*, *MTR2756*, MTRR66) and their association with the presence and type of congenital nervous system malformations, especially folate-dependent forms.

Scientific novelty and originality. For the first time in the Republic of Moldova, a dual-design study was conducted on congenital malformations of the nervous system (CNSM), combining retrospective population-based analysis with an extended case-control study focused on risk factor expression. The research revealed a statistically significant downward trend in the incidence of both overall CNSM and folate-preventable forms (FCNSM) during 2018–2023, offering the first national data on their temporal dynamics. Compared to EUROCAT data, the study documented a distinct distribution profile of clinical subtypes, with a higher proportion of cerebral structural anomalies and hydrocephalus in Moldova. A strong association was found between the absence of periconceptional folic acid supplementation and the occurrence of FCNSM (in 93% of cases). The study also established a risk hierarchy of maternal, paternal, and prenatal factors and quantified their associations with CNSM in the study population. Major contributing factors identified include infectious agents (TORCH complex, cytomegalovirus), behavioral risk factors (smoking, alcohol, drug use), parental health status (diabetes, hypertension, overweight), medication use, and toxic exposure during gestation. The study highlighted clinically relevant patterns in the expression of CNSM, co-association trends (clusters), and statistically significant correlations between clinical subtypes and associated neurological disorders – supporting a differentiated, integrative clinical approach. For the first time in a regional context, significant differences were demonstrated in the distribution of genotypic variants of the *MTHFR677*, *MTHFR1298*, *MTR2756*, and MTRR66 genes between mothers of affected children and control subjects, with specific associations between certain genotypes and increased risk of FCNSM (OR range: 2.18–6.78).

Implementation of scientific results. The results of this research have been implemented in scientific, methodological, and clinical activities at the Department of Pediatrics, Nicolae Testemițanu State University of Medicine and Pharmacy, and in the Pediatric Neurology Clinic of the Department of Pediatrics at the IMSP Mother and Child Institute.