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MTHFR GENOTYPE FOR METHOTREXATE TREATMENT MONITORING IN JUVENILE IDIOPATHIC ARTHRITIS

GENOTIP MTHFR PENTRU MONITORIZAREA TRATAMENTULUI METHOTREXAT ÎN ARTRITA IDIOPATICĂ JUVENILĂ

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Summary. The treatment children with juvenile idiopathic arthritis (JIA) includes many non-pharmacological and pharmacological therapies that are interspersed both at the onset and on the escalation stages. Genetic susceptibility to drug efficacy has been shown to be the technological outbreak that could currently guide the practitioner in choosing a personified therapy. For many decades, methotrexate is the world's gold standard for both adults with rheumatoid arthritis and children with JIA. Unfortunately, a considerable number of patients do not achieve an adequate therapeutic response. Folate antagonist, increased plasma clearance, low therapeutic dose - these are key features that induce the need to study its biomolecular effect and predictors of administration outcome. The pharmacogenomic impact of these features is the reduction of early childhood disability due to the inefficiency and subclinical toxicity of methotrexate in children with JIA.

Key words: Methotrexate, arthritis, gene, efficacy.

Rezumat. Tratamentul copiilor cu artrită juvenilă idiopatică (AJI) include multe terapii non-farmacologice şi farmacologice care sunt intercalate atât la debut, cât şi pe etapele de escaladare. Susceptibilitatea genetică asupra eficacităţii medicamentelor reprezintă suportul tehnologic care ar putea ghida în prezent practicianul în alegerea unei terapii personalizate. Timp de multe decenii, metotrexatul este standardul de aur mondial atât pentru adulţii cu artrită reumatoidă, cât şi pentru copiii cu AJI. Din păcate, un număr considerabil de pacienţi nu obţin un răspuns terapeutic adecvat. Antagonist al folatului, clearance-ul plasmatic crescut, doza terapeutică scăzută - acestea sunt caracteristicile cheie care induc necesitatea studierii efectului său biomolecular şi a predictorilor rezultatului administrării. Impactul farmacogenomic al acestor caracteristici este reducerea handicapului în copilăria timpurie datorat ineficienţei şi toxicităţii subclinice a metotrexatului la copiii cu AJI.

Cuvinte cheie: metotrexat, artrită, genă, eficacitate.

INTRODUCTION

Juvenile idiopathic arthritis is a chronic multisystemic inflammatory disease, characterized by clinical and paraclinical signs of joint inflammation of undetermined genesis, lasting at least 6 weeks, onset up to 16 years and requiring specialized support[1-4]. Once the revised classification criteria of the American College of Rheumatology were applied to the Rochester Epidemiology Program Project database, an incidence of 13.9 cases of juvenile idiopathic arthritis per 100,000 children per year was reported[5-6]. A follow-up study, using the same database, noted a decrease in incidence over the next decade.

The pathogenesis and aetiology of idiopathic juvenile arthritis is unclear. As with most autoimmune diseases, interactions between genetic factors, immune mechanisms, and environmental exposures are thought to contribute in most cases to its onset. Most of the genetic predisposition is determined by the loci of the major HLA histocompatibility complex. Multiple sites are associated with the development of juvenile idiopathic arthritis, and in general these associations are significantly different from those seen in rheumatoid arthritis. The spectrum of non-human leukocyte antigen genes that are associated with juvenile idiopathic arthritis is expanding rapidly, but their contribution to the risk of developing the disease appears to be less significant, and their effects can be divided between juvenile idiopathic arthritis and other autoimmune diseases, including rheumatoid arthritis[7-8].

In the progressive evolution of juvenile idiopathic arthritis, monocytes are differentiated into proinflammatory macrophages. Unlike macrophages in synovial tissue that are part of a healthy synovial membrane and are responsible for joint homeostasis, the number of proinflammatory macrophages increases during disease progression and decreases during remission. Hyperplastic synovium results from dysfunction and increased proliferation of fibroblasts such as synoviocytes. These cells produce cytokines and proteinases that allow the accumulation of immune cells in the synovial fluid. Synoviocytes and similar immune cells fibroblasts form an inflammatory cascade with increased production of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-17 [8]. These processes will lead to chondrocyte apoptosis, destruction of the extracellular matrix of cartilage and damage to cartilage. Proinflammatory cytokines are responsible for bone erosion in juvenile idiopathic arthritis by inducing osteoclasts and suppressing osteoblasts. Without proper treatment, in addition to chronic pain, joint damage and bone erosion, the quality of life of patients will permanently decrease. To achieve adequate control of the disease, it is essential to initiate in a timely manner an appropriate therapy to cover the individual effects of the underlying disease.

Currently, due to the costs of therapy and severe clinical side effects, methotrexate is the therapy of choice for the treatment of idiopathic juvenile arthritis. Unfortunately, almost one third of patients do not have an adequate response to methotrexate therapy due to insufficient relief of disease symptoms or the occurrence of paraclinical or subclinical side effects that lead to discontinuation of treatment [9-11].

These patients lose optimal time to start biologic therapy and experience more severe manifestations of the disease. Numerous pharmacogenetic studies on the genes encoding the proteins involved in its mechanism of action have been performed to predict the response to methotrexate therapy. Their potential for use is high among adult patients, who are limited in children due to the lack of multicentre results on large samples[12]. To date, the results are inconclusive and have not improved clinical practice, as there are several reasons for this [13-14]. The mechanism of action of the low dose of 10 mg / m 2 of methotrexate in RA is not fully determined, but according to current data, the drug achieves its therapeutic effects through several different mechanisms, and it is unclear whether all these mechanisms are included in the response to therapy. a modest contribution from each, or there is a dominant mechanism of its action. Each mechanism includes several proteins encoded by genes that harbour natural polymorphisms that affect the activity of these proteins. Pharmacogenetic studies should also examine the genes encoding the proteins involved in the development and progression of the disease, especially if these genes are directly or indirectly affected by the mechanism of action of the medicinal product. Experts should take this into account when making recommendations for methotrexate therapy in juvenile idiopathic arthritis [13,15,16].

The methodology for the clinical assessment of the efficacy of therapy at various stages for children with idiopathic juvenile arthritis is well known. It consists of calculating disease activity indices and assessing the occurrence of reserved prognostic factors for the disease. To estimate the therapeutic effect, however, the paediatric rheumatology specialist needs some serological biomarkers or genetic profiles. Methotrexate is an antagonist of folic acid. It has an increased plasma clearance at a low therapeutic dose which leads to the need to study its biomolecular effect and predictors of administration.

Two mutations in the regulatory enzyme gene in the metabolic cycle of folates, methylene tetrahydrofolate reductase MTHFR, C667T and A1298C and isolated T677T, are the most common cause of low toxicity and efficacy in high-dose methotrexate therapy, as these mutations lead to delays. of the drug [17].

AIM OF THE STUDY was to investigate the associations between MTHFR gene mutations and low-dose methotrexate intolerance in patients with idiopathic juvenile arthritis.

MATERIAL AND METHODS

Patients hospitalized consecutively or consulted in the Paediatric Rheumatology Department of the Public Health Medical Institution Institute of Mother and Child, from Chisinau, Republic of Moldova, from April 2018 to July 2022, after the written confirmation of informed consent. The study was approved by the research ethics committee Protocol N52, of the State University of Medicine and Pharmacy „Nicolae Testemitanu", Chisinau, Republic of Moldova. The inclusion criteria were children diagnosed with juvenile idiopathic arthritis established according to ILAR criteria, who administered methotrexate therapy for more than 6 months; the age of children between 1 and 18 years; children without any hepatic pathology prior to the therapy onset; children without any cardiac pathology prior to therapy onset. The exclusion criteria were refusal to participate in the clinical trial, children with JIA who do not follow methotrexate and children with acute and chronic liver disease. The following data were extracted from the patients' medical records: age, sex, body weight and height, age at diagnosis, duration of disease, dose and duration of methotrexate administration, route of administration, folic acid supplementation. In addition, liver function tests and treatment with non-steroidal anti-inflammatory drugs, glucocorticosteroids and biologic therapy have been documented.

Methotrexate intolerance was measured using the Methotrexate Intolerance Severity Score (MISS) questionnaire, which was previously validated for use in idiopathic juvenile arthritis. This questionnaire consists of four areas: abdominal pain, nausea, vomiting and behavioural symptoms and records anticipatory symptoms after methotrexate administration, as well as associative symptoms, i.e. symptoms that occur when we think of methotrexate. Symptoms are assessed at the time of response to the questionnaire, without a specific time frame. Behavioural symptoms include restlessness, irritability, and refusal of administration in response to gastrointestinal symptoms. Each of the 12 items on the MISS scale is scored on a psychometric scale (0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms; 3 = severe symptoms) for a maximum score of 36. As previously established, intolerance to methotrexate was defined ≥6 points on the MISS questionnaire, including at least one anticipatory, associative or behavioural symptom.

The activity of the disease was assessed according to the DAS28 score, which consists of the evaluation of 4 variables: the number of painful and swollen joints, the global evaluation of the disease and the value of the erythrocyte sedimentation rate. These parameters are entered into the electronic application that calculates the DAS according

to the default formula. Depending on the value obtained, a low, moderate or high disease activity is established.

Subjects were primarily distributed in batches according to the detected genetic polymorphism. DNA was prepared from peripheral mononuclear cells using standardized protocols. The presence of the two most common mutations in the MTHFR gene (C677T and A1298C, as well as isolated T677T) was tested using real-time chain polymerization reaction.

Demographic and clinical data were analysed using descriptive statistics. Other statistical methods used were Mann-Whitney U Test, Chi-Square Test and ROC Analysis.

RESULTS

The demographic data of the 68 patients included in the study are presented in the figures below. More than half of the patients enrolled in the study are teenagers and only a quarter represent the young age group. This is since the Rheumatology Clinic performs remote follow-up on all children who have previously initiated background treatment. Thus, if we refer to the age groups in figure 1 and analyse the study groups with the distribution by sex in figure 2, we observe a tangent with the literature data on the onset of the disease.

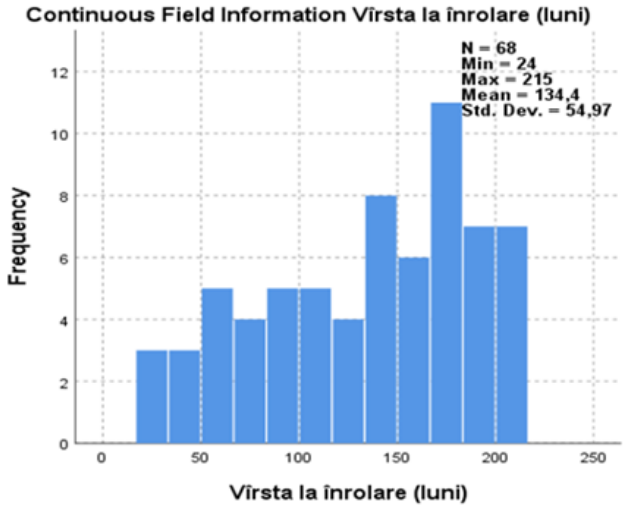

Figure 1. Analysis of the distribution of age groups included in the study (months)

A female prevalence was found for the study sample. It predominates in the age groups up to the age of 5 and in the school groups. It is necessary to consider the form of the disease that each patient in the study manifests to be able to explain this gender distribution.

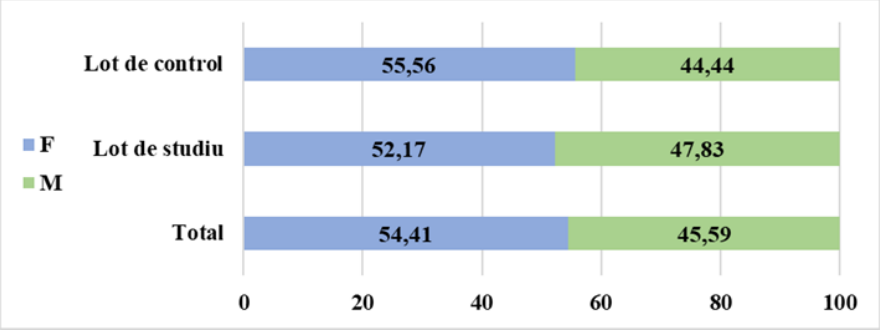

Figure 2. Analysis of the sex distribution of the patients included in the study (%)

According to figure 3, the highest rate is represented by the seronegative polyarticular form, which occupies approximately half of the volume of the disease, followed by the oligoarticular form. An imposing value of 14% expresses the systemic form of the disease.

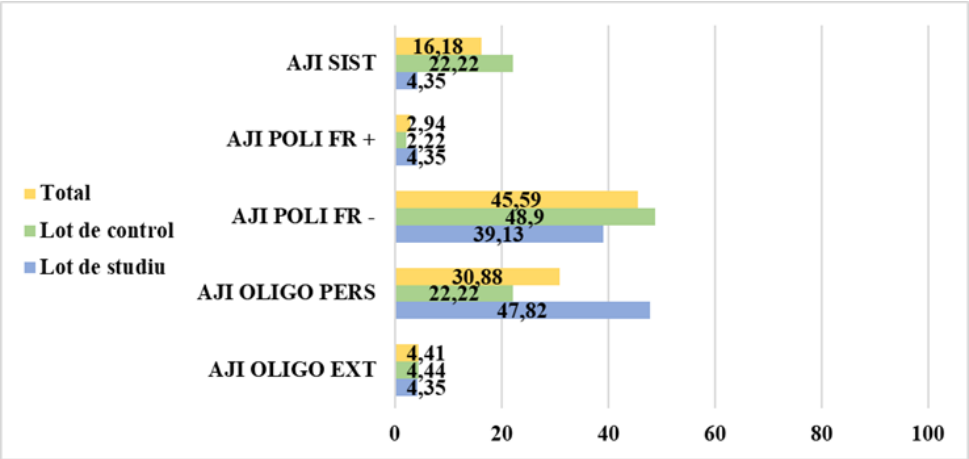

Figure 3. Analysis of the distribution of the disease form regarding genetic variants (%)

From the group of nosologically forms of juvenile idiopathic arthritis, the seronegative and oligoarticular polyarticular form is presented with an increased rate of MTHFR genetic polymorphism in the children in the study. Table 3 also shows these genetic variations and their relationship to the form of the disease. Thus, it was found that in the study group, subjects with the oligoarticular form, with persistent evolution, predominated and constituted 47.82% (11 cases) of the total number of the group, followed by those with the polyarticular, seronegative form, which represented 39.13% (9 subjects) of the total number of the group. In the control group, however, the clinical forms mirrored each other with values of 48.9% (22 cases) for the polyarticular, seronegative form and 22.22% (10 cases) each for the oligoarticular forms with persistent evolution and the systemic form with active arthritis (χ^2 = 6.789; gl = 4; p = 0.147). The overall genotype variation is expressed in figure 4.

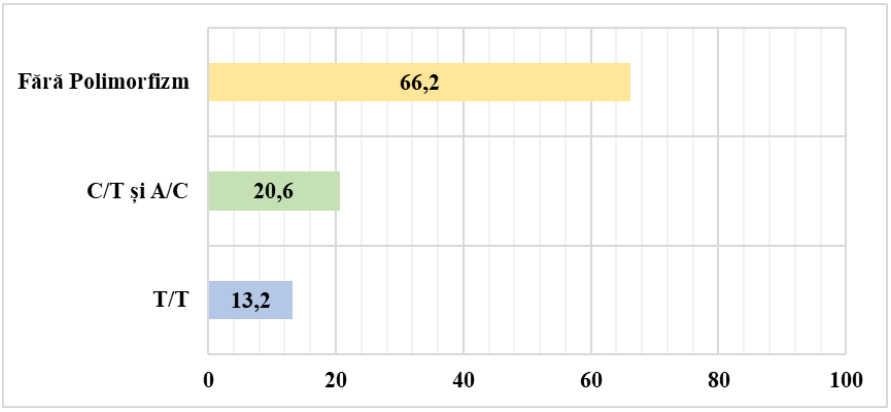

Figure 4. Analysis of the distribution of the genetic variants in the subgroups (%)

We used the Hardy-Weinberg formula to calculate, with an acceptable error, allele frequencies from the phenotypes observed in the population for all gene variants assessed in the case and control groups. Thus, it was noted that the analysis of the genotype frequency distribution did not yield differences from this equilibrium. The allele frequencies in children with JIA enrolled in the research were as follows: C = 0.67, T = 0.33 and A = 0.71, C = 0.29, respectively.

The study also analysed the duration of disease in these patients. Thus, the mean duration of illness from diagnosis for the study group was 48.5 months (SD = 33.75; 95% CI: 31.72 - 65.28), while for the control group this value was 55.66 months (SD = 48.97; 95% CI: 38.00 - 73.31). For the study subgroups, a mean of 53.09 months (SD = 34.57; 95% CI: 29.87 - 76.32) was recorded for subjects with compound MTHFR polymorphism, and - 41.29 months (SD = 33.71; 95% CI: 10.11 - 72.47) for those with homozygous polymorphism by pathologic allele, respectively.

The duration of methotrexate administration was also assessed in both groups. After analysing the data obtained, it was found that the subjects in the study group had a mean duration of administration of 38.78 months (SD = 35.94; 95% CI: 20.9 - 56.65), while in the control group the mean duration of administration was 35.03 months (SD = 39.65; 95% CI: 20.73 - 49.33). The range includes the minimum of 6 months and the extreme of 159 months.

The analysis determined that the DAS28 value ≥ 3.245 is a threshold value that can be used to identify potential cases with polymorphic variant. ROC analysis revealed the sensitivity of the assay to be 87.0%, the specificity to be 93.3% (χ^2 = 43.83; p<0.001).

The ROC curve, constructed based on the sensitivity-specificity relationship, has relevant values AUC=0.949 (95% CI: 0.89 - 1.00, p<0.001), which confirms the correctness of the established relationship. Therefore, it is recommended to test the genetic polymorphism of the MTHFR gene in the case of continuation of MTX therapy and persistence of high degree of disease activity in patients with JIA, figure 5.

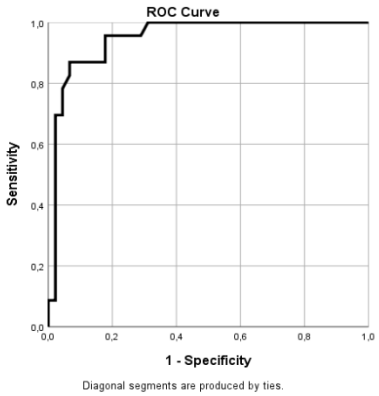

Figure 5. ROC curve for diagnostic assessment of DAS28

At the same time, paradoxical results were obtained, according to the qualitative indicator of the degree of disease activity expressed by DAS28, which were observed only in the subjects who fall into the category of the compound polymorphic variant, in whom the Pearson index is -.388** (p=0.001), compared to the subjects homozygous for the pathologic allele, who recorded a Pearson index of -0.192 (p=0.117)

This finding, regrettably, has no statistical support as it does not have a statistically significant trend. This can be explained by the fact that the number of subjects is not large enough to make a more general statistical conclusion. According to the Mann-Whitney U-test in the independent subgroups U = 61,500, p=0,925, and χ^2 =3.665, p = 0.300.

The MISS Questionnaire revealed that patients may develop anticipatory symptoms, which occur before MTX intake, and associative symptoms, when patients think about the drug, as well as behavioural symptoms. These adverse effects occur in response to previous symptoms experienced by MTX-treated patients and are often not clinically evident; therefore, they are often inadequately managed. Although folic acid supplementation during MTX therapy may reduce such effects, many patients discontinue treatment, which adversely affects disease control and quality of life.

The predominant factor that has been found to drive MTX intolerance is behavioural factors, as shown in the radar diagram in figure 6.

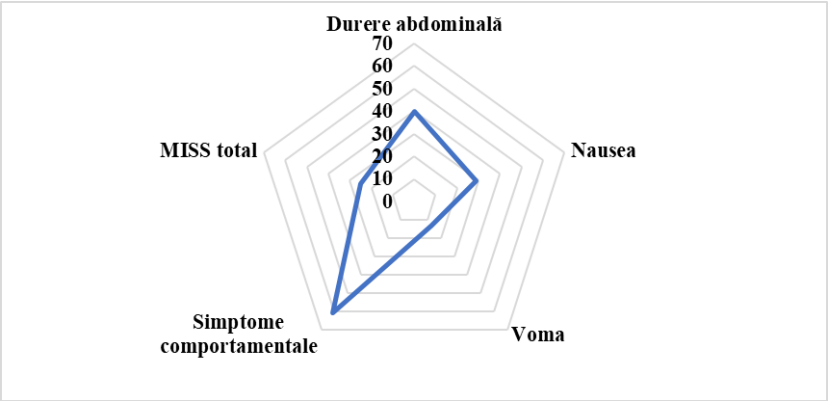

Figure 6. Radar plot of the Methotrexate Intolerance Severity Score (MISS) and related components

CONCLUSIONS

This study demonstrates an interaction between two common mutations in the MTHFR gene and methotrexate tolerance in a small cohort of patients with idiopathic juvenile arthritis. Mutations were found more frequently in patients with clinical and paraclinical intolerance to methotrexate. The duration of the disease was significantly longer in patients with methotrexate intolerance, and correlation with age and duration of methotrexate treatment was also shown in various forms of the disease, which is not surprising.

An insignificant trend toward identical intolerance to methotrexate with higher doses of methotrexate can be explained by a clear inverse correlation between dose and age. The observation that advanced age at treatment is not correlated with methodology is interesting in itself: we might expect older children to be more easily affected by a purely psychological effect. This is the data of the MISS questionnaire used in the study.

The results of the study demonstrated the importance of the DAS28 clinical score for estimating the risk of polymorphic variants in the MTHFR genetic confirmation of non-responders to methotrexate therapy. Using the sensitivity - specificity relationship, the cut-off values for MTHFR polymorphism testing (DAS28 ≥ 3.245) were determined.

The importance of MTHFR polymorphism in alleles 677 and 1298 for the prognosis of non-response rate and side effects of methotrexate therapy in juvenile idiopathic arthritis has been demonstrated clinically and through laboratory data. Validation of at least nationally, in an independent cohort, of genotype screening for the MTHFR in juvenile idiopathic arthritis would help clinicians identify patients at risk of developing therapeutic intolerance.

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