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ASSOCIATION OF THE MTHFR GENE POLYMORPHISMS WITH EFFICACY AND TOXICITY OF METHOTREXATE IN PATIENTS WITH JUVENILE IDIOPATHIC ARTHRITIS

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Abstract

The aim of this study was to investigate whether the C677T and A1298C polymorphisms of methylenetetrahydrofolate reductase (MTHFR) gene might be related to the prediction of toxicity and efficacy of methotrexate (MTX) in juvenile idiopathic arthritis (JIA) in clinical practice.

Sixty-three patients (50 girls and 13 boys) fulfilling the International League of Associations for Rheumatology (ILAR) criteria for JIA were included in the study. Patients were divided into two groups – those undergoing treatment with MTX monotherapy and putative optimal response ($n = 28$), and those with poor response to therapy with MTX and therefore shifted to treatment with MTX and a biological agent ($n = 35$). DNA for SNP analysis was automatically isolated from whole blood through chemagic Magnetic Separation Module I instrument (PerkinElmer chemagen Technologie GmbH, Baesweiler, Germany). The following SNPs in MTHFR gene – 677C>T (rs1801133) and 1298A>C (rs1801131) – were analyzed using High Resolution Melt (HRM) analysis. A real-time PCR (RotorGene 6000, Qiagen, USA) was performed for amplification of DNA prior to HRM analysis.

We did not find any significant difference in the distribution of genotype ($\chi 2 = 1.04$; df $= 2/\chi 2 = 0.60$; df $= 2$) and allele ($\chi 2 = 0.11$; df $= 1/\chi 2 = 0.03$; $df = 1$) frequencies of polymorphisms C677T and A1298C between the two

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groups. Adverse events (nausea, dizziness, headache, hepatotoxicity) due to MTX were noted among four of the patients. It was found that all of the patients who experienced side effects of the treatment carry the allelic variant C677T (three CT heterozygotes and one TT homozygote). The variant allele A1298C was found in one of these four patients.

We did not find any significant association between the C677T and A1298C polymorphisms of MTHFR and the efficacy and toxicity of methotrexate.

Key words: juvenile idiopathic arthritis, *MTHFR* polymorphisms, methotrexate toxicity and efficacy

Introduction. Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease in childhood. JIA encompasses a clinically heterogeneous group of arthritides of unknown etiology, with a duration of at least 6 weeks, and onset before 16 years of age [1]. According to a systematic review its prevalence and incidence rates in the European population in 2010 range between 3.8 to 400 and 1.6 to 23 per 100 000, respectively [2]. The therapeutic approach in JIA includes initial treatment with nonsteroidal antiinflammatory drugs and/or intra-articular administration of glucocorticosteroids, which is sometimes sufficient to achieve disease control in patients with oligoarthritis. In those in whom the desired effect is not achieved, methotrexate (MTX) is administered. In patients with polyarthritis or systemic JIA, the biological agents, which target the cytokine mediators (TNF-α, IL-1 and IL-6) of inflammation, take an essential part in the treatment. Despite the availability of new drugs, MTX still plays a key role in the treatment of JIA [3]. JIA is a disease with a relapsing remitting course and may lead to short- and long-term disability.

Methotrexate (MTX) is the main disease-modifying anti-rheumatic drug used in the treatment of JIA [3]. Since 1992 when the efficacy of MTX in the treatment of JIA was discovered, its efficacy and acceptable safety profile in JIA have been confirmed [4, 5]. Nevertheless, it is known that MTX might be ineffective in some of JIA patients [5, 6]. Poor or insufficient response to MTX might be observed in up to 50% of JIA patients [7]. In those cases biological agents are added to the treatment plan. The goal is to achieve an early disease control and prevent irreversible joint damage. Recently, it has been proven that biological agents, and TNF blockers in particular, are more effective if they are added early in the disease course [8]. The implementation of early and successful treatment strategy leads to avoidance of the long-term sequelae of JIA.

It is known that in about 8% of the children with JIA the disease continues in adulthood. JIA is associated with a significant burden of morbidity in adults in whom the manifestation of arthritis was in childhood and particularly in those patients where joint inflammation was not fully controlled [9, 10]. A number of studies have shown that uncontrolled joint inflammation can lead to irreversible changes in the joints. With advances in therapy the pediatrician adapts the so-called "zero tolerance" to joint inflammation. The aim is to achieve complete

remission early in each child [11].

In order to achieve an early disease control and improve the long-term prognosis of the disease, a tailor-made treatment is needed. It would be of great value if clinicians could predict the individual prognosis and response to treatment at the onset of the disease. It is still not possible to differentiate which patients will have a good response to MTX and which will not and will require addition of biological agents. Meanwhile, MTX can lead to intolerance and adverse events (mainly gastrointestinal symptoms, bone marrow suppression and hepatotoxicity) in some JIA patients [12–15]. A variety of studies concerning different prognostic factors have been performed. The desired prognostic factor should be an objective predictor of the treatment response. Genetic polymorphisms fulfil this requirement in some disease conditions. Several single nucleotide polymorphisms (SNPs) in genes encoding enzymes and transporters involved in the MTX metabolic pathway are known. Some of the SNPs could influence MTX efficacy and toxicity. The C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) gene have been reported to be associated with the toxicity and efficacy of MTX in JIA [16–19]. Both these SNPs are associated with reduced activity of the MTHFR enzyme. The homozygous 677T/T variant is present in about 8–10% of the general population. The activity of the MTHFR enzyme in the carriers of the wild type allele is about 30%. Heterozygotes 677C/T have about 60% activity of the enzyme and constitute approximately 40% of the population [18]. A reduced activity of the MTHFR enzyme is observed in the carriers of A1298C polymorphism in a homozygous or heterozygous state, as well. The reduced activity of the enzyme leads to low levels of the enzyme product – 5-methyltetrahydrofolate. This in turn is associated with accumulation of homocysteine and decreased intracellular transmethylation capacity and attenuated methylation of DNA, proteins and lipids [19].

There are few studies in patients with JIA and the data are conflicting [16-20]. Differences in the frequencies of the SNPs in different populations have been reported. To our knowledge this is the first study which investigates the association of carriage of C677T and A1298C polymorphisms of *MTHFR* in Bulgarian children with JIA and the prediction of toxicity and efficacy of methotrexate in clinical practice.

Methods. The study included 63 patients (50 girls and 13 boys) fulfilling the International League of Associations for Rheumatology (ILAR) criteria for JIA. Informed consent was obtained from the parents of all individual participants included in the study. All the patients were on MTX therapy for JIA. Patients were divided into two groups – those undergoing treatment with MTX monotherapy and putative optimal response ($n = 28$), and those with poor response to therapy with MTX and therefore shifted to treatment with MTX and a biological agent ($n = 35$). DNA for SNP analysis was automatically isolated from whole blood through chemagic Magnetic Separation Module I instrument (PerkinElmer chema-

gen Technologie GmbH, Baesweiler, Germany). The following SNPs in *MTHFR* gene – 677C>T (rs1801133) and 1298A>C (rs1801131) – were analyzed using High Resolution Melt (HRM) analysis. A real-time PCR (RotorGene 6000, Qiagen, USA) was performed for amplification of DNA prior to HRM analysis. Allele and genotype frequencies in both groups were compared by χ^2 test.

Results. The mean age of the patients included in the study was 9.43 years (2.1–17.75 years), and the mean age of disease onset was 4.99 years (0.83–16.25 years). The baseline characteristics of the patients can be seen in Table 1.

T a b l e 1

Baseline characteristics of the patients

JIA subtype	*N*, (%)
• Oligoarthritis	34/63 (53.9%)
• Rheumatoid factor negative polyarthritis	21/63 (33.3%)
• Rheumatoid factor positive polyarthritis	3/63 (4.8%)
• Systemic arthrithis	3/63 (4.8%)
• Enthesitis-related arthritis	2/63 (3.2%)
Female sex	50/63 (79.4%)
Mean age at disease onset, yrs	4.99 ± 3.48 (0.83–16.25)
Mean age at MTX start, yrs	5.81 ± 3.53 (0.9–17.33)

The average age of initiation of therapy with MTX was 5.81 years (0.9–17.33 years). All patients were on methotrexate therapy in a mean dose 7.81 mg/m^2 (2.5–12.5). The therapy with MTX was considered ineffective if a biological agent was added to the treatment strategy during follow-up. Accompanying biological treatment was used in 62.5% of polyarthritis JIA and 44% of the oligoarthritis JIA patients. Forty of the 63 patients had additional treatment with corticosteroids. Uveitis was observed in 5 of the 63 patients with four of them being girls. The distribution of the different genotypes is shown in Table 2.

T a b l e 2

MTHFR polymorphisms distribution

MTHFR polymorphisms			
C677T		A1298C*	
Patients without BA**	Patients with BA	Patients without BA	Patients with BA
14 CC	18 CC	13 AA	17 AA
13 CT	14 CT	10 AC	15 AC
1 TT	3 TT	4 CC	2 CC

*Due to technical reasons two of the samples could not be genotyped for A1298C polymorphism. **BA – biological agent

We did not find any significant difference in the distribution of genotype ($\chi^2 = 1.04$; df = 2) and allele ($\chi^2 = 011$; df = 1) frequencies of polymorphisms C677T between the two groups. Similar results in the distribution of genotype ($\chi^2 = 0.60$; df = 2) and allele ($\chi^2 = 0.03$; df = 1) frequencies of polymorphism A1298C between the two groups were observed. Carriers of the variant T-allele in homo- or heterozygous state did not show statistically significant association with the need for treatment with biological agents (C677T OR 0.64; A1298C OR 1.21). Thirty-two out of thirty-five of the patients treated with biological agent carried the A allele of A1298C polymorphism – 17 homozygotes AA and 15 heterozygotes AC.

Adverse events (nausea, dizziness, headache, hepatotoxicity) due to MTX were noted in four of the patients. It was found that all of the patients who experienced side effects of the treatment carry the allelic variant C677T (3 CT heterozygotes and one TT homozygote). Due to this finding we may suppose that carriage of this allele could provide additional risk for development of adverse drug reactions. The variant allele A1298C was found in one of these four patients. It can be assumed that its role is less significant in terms of appearance of side effects from treatment with MTX. We did not find any statistically significant association between the C677T and A1298C polymorphisms of *MTHFR* and the efficacy and toxicity of methotrexate.

Discussion. One of the most common studied genes in the MTX metabolism is the *MTHFR* gene. Most of the studies that are examining the relation between toxicity and efficacy of MTX treatment and *MTHFR* single nucleotide polymorphisms are performed in adult patients with rheumatoid arthritis. There are few studies in patients with JIA and the data are conflicting. Schmeling et al. [16] performed the first study concerning the influence of *MTHFR* polymorphisms on efficacy and toxicity of MTX in JIA. The team reported an association of the *MTHFR* 677C/C polymorphism to a higher tolerability of MTX, and of the 1298A/A to lower clinical efficacy of MTX therapy in JIA. Adverse events were noted in 34% of the patients – mainly gastrointestinal symptoms, followed by elevated serum levels of liver enzymes and hair loss. Adverse events were observed more often in patients with the heterozygous genotype 677C/T than in patients with the homozygous C/C genotype (65% vs. 31%; $p < 0.05$, χ^2 test). In patients who presented the C allele of the A1298C polymorphism, improvement with respect to the number of swollen joints, the number of tender joints, and a decrease in erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels occurred more frequently than in 1298 A/A homozygous patients ($p < 0.05$ for ESR, $p < 0.01$ for CRP, χ^2 test). In a study of 69 patients with JIA performed by Tukova et al. [17] it was concluded that genotyping may have a predictive value for the risk of MTX-associated toxicity. The frequency of 677T allele was higher in patients with adverse effects (52.4% vs. 20.9%; OR 3.88, 95% CI 1.8–8.6, $p < 0.002$). The probability of any adverse effect was significantly higher in pa-

tients with 677TT compared to the 677CC genotype (OR 55.5, 95% CI 2.9–1080, $p < 0.001$). They reported a 30% frequency of any adverse events. The median dose of MTX that was used in the study was 14.9 mg/m^2/week which is almost twice higher than the dose that we use. This might be one of the reasons why our team did not observe so much adverse events due to MTX treatment and could not find a statistically significant association between C677T polymorphisms and occurrence of side effects of MTX treatment.

However, in the study performed of Yanagimachi et al. [18] in an Asian population, no association of *MTHFR* polymorphisms and the toxicity of MTX was observed. Regarding the association between the efficacy of MTX and genetic predictors, they did not find any of the investigated gene polymorphisms, including *MTHFR*, to be significantly associated with efficacy of treatment.

Albers et al. [19] studied a cohort of 128 JIA patients and supported the idea that different SNPs can influence MTX efficacy. They investigated six SNPs situated in five MTX-related genes and found out that the *MTHFR* 1298A–677C haplotype had a significantly lower copy number in MTX nonresponders when compared with responders.

In the latest study concerning MTX efficacy and toxicity, performed by van Dijkhuizen et al. [20], a prospective JIA cohort was investigated. The scientists determined the clinical variables and single nucleotide polymorphisms at MTX start. They did not find also statistically significant association between SNPs of the *MTHFR* gene and prediction of MTX intolerance. In their study, only 4 of the 27 investigated SNPs were moderately associated with MTX intolerance.

So far, the results for different SNPs are still conflicting. Taken together with our current study they show that it is still difficult to predict reliably the efficacy and toxicity of MTX treatment. The differences may reflect different treatment regimens, different genotypic and allelic frequencies in the studied populations. Still there is no established genetic marker associated with response to therapy with MTX in JIA. The validation of a marker that will differentiate patients who could achieve a good response to monotherapy with MTX will support the development of rational and personalized approach to the treatment of children with JIA.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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