

The impact of single nucleotide polymorphisms in *MTHFR* and *MTRR* genes on disease activity and the presence of methotrexate treatment side effects in children with juvenile idiopathic arthritis

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Abstract

Introduction: To determine the impact of single nucleotide polymorphisms (SNPs) in *MTHFR* and *MTRR* genes on disease activity and the presence of MTX therapy adverse events in Polish children with juvenile idiopathic arthritis (JIA).

Methods: SNP genotyping was performed using genomic DNA isolated from peripheral blood samples.

Results: Patients with *MTHFR* rs1801133 CT/TT variant had higher values of inflammatory markers, number of joints with active arthritis, and JADAS-71 value at the baseline of MTX treatment. Children with *MTRR* rs1801394 AG/AA variant presented higher inflammatory marker values at JIA diagnosis.

Conclusions: *MTHFR* rs1801133 and *MTRR* rs1801394 polymorphisms are associated with higher disease activity at the moment of JIA diagnosis.

Key words: methotrexate, pharmacogenomics, single nucleotide polymorphism, juvenile idiopathic arthritis.

Methotrexate (MTX) administered at the dose of 10–15 mg/m² is currently recommended as the first-line disease-modifying agent in most juvenile idiopathic arthritis (JIA) subtypes [1]. Despite the prediction of early MTX treatment response and development of drug adverse effects is crucial to individualize JIA therapy and in consequence prevent long-term effects of inadequate treatment, up to date we are not able to predict whether the patient benefits from MTX therapy [2]. Single nucleotide polymorphisms (SNPs) in the genes involved in the MTX cellular pathway are candidate biomarkers of MTX toxicity and treatment response, but currently available studies failed to establish a definite link [3, 4].

Folate metabolism is perceived as the major target of MTX anti-inflammatory action. As a competitive inhibitor of dihydrofolate reductase, MTX prevents the regeneration of tetrahydrofolate, which is essential for the generation of folate cofactors required for de novo purine and pyrimidine synthesis. Moreover, MTX may influence several other steps in folate metabolism, leading to cellular folate depletion and inhibition

of methylenetetrahydrofolate reductase (*MTHFR*), methionine synthase (*MTR*), and methionine synthase reductase (*MTRR*), which play pivotal roles in both folate and homocysteine metabolism [5]. Genetic variability within folate metabolism genes has been associated with MTX treatment efficacy and the presence of therapy side effects both in rheumatoid arthritis (RA) and JIA [6, 7], although, due to small sample sizes and differences in study design, currently available data are inconclusive.

The aim of our study was to identify the association between genetic variability within *MTHFR* rs1801133 (C677T) and *MTRR* rs1801394 (A66G) genes and the disease activity, MTX treatment efficacy, and the presence of therapy side effects in Polish JIA patients.

Methods. Patients and study design. The inclusion criteria were the fulfilment of International League Against Rheumatism (ILAR) JIA diagnostic criteria [8] and current or past MTX treatment. MTX was administered once a week at the recommended dose (10–15 mg/m² of body surface area – BSA) by either oral or subcutaneous route. All patients were receiving folic acid supplementation. Demographic, clinical (number of joints with active arthritis and limited range of motion, Child-

hood Health Assessment Questionnaire (CHAQ) value, Juvenile Arthritis Disease Activity Scale (JADAS) [9] value), and laboratory parameters (C-reactive protein [CRP], erythrocyte sedimentation rate (ESR)) were collected at the moment of JIA diagnosis and on a control visit 4–6 months (median: 5.09 months) after MTX treatment initiation (Table I). Therapy adverse effects (AEs) were evaluated at the control visit. AEs were classified as gastrointestinal (nausea, vomiting, abdominal pain), hepatotoxicity (defined as alanine aminotransferase and/or aspartate aminotransferase activity exceeding normal range upper limit), dermatological, neurotoxicity, recurrent infections, and other AEs.

Genotyping. Genomic DNA was isolated with the QIAamp DNA Mini Kit and assessed by ultraviolet spectrophotometry. Genotyping was performed with TaqMan GTXpress Master Mix and dedicated TaqMan SNP Genotyping Assay (*MTHFR* rs1801133, *MTRR* rs1801394). For allelic discrimination, SDS Software 2.0 was used.

Statistical analysis. Continuous variables are presented as the median with the interquartile range and assessed using the Mann-Whitney *U* test. Categorical variables are presented as the number with an appropriate percentage, and com-

Table I. The influence of *MTHFR*rs1801133 and *MTRR* rs1801394 polymorphism on JIA activity

Characteristic	<i>MTHFR</i> rs1801133			<i>MTRR</i> rs1801394		
	CC (N = 51)	CT/TT (N = 49)	P-value	GG (N = 33)	AG/AA (N = 67)	P-value
	Me (25–75%)	Me (25–75%)		Me (25–75%)	Me (25–75%)	
Age at disease diagnosis [years]	8.42 (4.29–10.92)	8.10 (4.01–11.82)	0.92	7.51 (4.01–9.98)	9.20 (4.29–12.61)	0.15
Dose of MTX [mg/m ² BSA]	10.71 (9.89–11.80)	10.71 (9.53–12.15)	0.95	10.40 (9.81–12.15)	10.76 (9.78–12.06)	0.69
Before MTX: treatment:						
PGA	4.00 (3.00–5.00)	4.00 (3.00–6.00)	0.41	4.00 (3.00–4.00)	4.00 (3.00–6.00)	0.21
PGE	6.00 (3.00–7.00)	6.00 (5.00–7.00)	0.54	6.00 (4.00–7.00)	6.00 (3.00–7.00)	0.68
CHAQ	0.25 (0.25–0.63)	0.63 (0.25–0.63)	0.28	0.25 (0.25–0.63)	0.63 (0.25–0.63)	0.20
Joints with active arthritis	1.00 (1.00–3.00)	2.00 (1.00–5.00)	0.04	2.00 (1.00–4.00)	2.00 (1.00–4.00)	0.85
Joints with limited ROM	2.00 (1.00–3.00)	2.00 (1.00–4.00)	0.06	2.00 (1.00–3.00)	2.00 (1.00–4.00)	0.50
ESR [mm/h]	16.00 (7.00–30.00)	34.00 (10.00–53.00)	0.02	8.00 (5.00–30.00)	26.00 (12.00–51.00)	< 0.01
CRP [mg/l]	3.20 (0.30–12.10)	8.00 (0.90–36.90)	0.02	0.70 (0.30–6.70)	8.00 (1.00–26.40)	< 0.01
JADAS-71	11.40 (9.00–14.00)	14.50 (11.00–18.10)	0.02	11.00 (9.00–14.50)	13.00 (10.50–18.20)	0.15

MTX – methotrexate, BSA – body surface area, PGA – Physician’s Global Assessment of Disease Activity, PGE – parent’s general evaluation of well-being, CHAQ – Childhood Health Assessment Questionnaire, ROM – range of motion, ESR – erythrocyte sedimentation rate, CRP – C-reactive protein, JADAS-71 – Juvenile Arthritis Disease Activity Score including 71 joints.

pared using the χ^2 , χ^2 with Yates correction, and Fisher exact tests on an individual basis. The logistic regression model was used to identify associations between SNPs and the occurrence of the side effects when adjusted to the age at disease diagnosis, sex, dose, and route of MTX administration. A p -value < 0.05 was considered statistically significant. Both *MTHFR* rs1801133 and *MTRR* rs1801394 were in Hardy-Weinberg equilibrium.

Results. The study group consisted of 100 JIA patients: 77 girls and 23 boys with a median age of 8.26 (4.15–11.10) years. 64% of patients were diagnosed with oligoarthritis, 17% with polyarticular seronegative JIA subtype, and 2% with polyarticular seropositive JIA subtype. Enthesitis-related arthritis was present in 8% of the study population, followed by systemic arthritis (6%) and psoriatic arthritis (3%). The median dose of MTX was 10.71 (9.80–12.07) mg/m² BSA.

At the moment of JIA diagnosis patients with *MTHFR* rs1801133 CT/TT variant had significantly higher values of inflammatory markers (ESR 34.00 mm/h vs. 16.00 mm/h, $p = 0.02$; CRP 8.00 mg/l vs. 3.20 mg/l, $p = 0.02$), a higher number of joints with active arthritis (2.00 vs. 1.00, $p = 0.04$), and higher Juvenile Arthritis Disease Activity Score (JADAS) 71 value (14.50 vs. 11.40, $p = 0.02$) in comparison with CC individuals. Children with *MTRR* rs1801394 AG/AA genotype presented significantly higher inflammatory markers values at the baseline of MTX therapy than GG individuals (ESR 26.00 mm/h vs. 8.00 mm/h, $p < 0.01$; CRP 8.00 mg/l vs. 0.70 mg/l, $p < 0.01$) (Table I). We did not observe any association between the studied SNPs and the efficacy of MTX treatment assessed at the control visit. There was no statistically significant association between the occurrence rate of MTX therapy side effects and the frequency of the investigated SNPs, although, after adjustment for sex, dose, and route of MTX administration,

patients with *MTHFR* rs1801133 CT/TT variant had 3.3-fold greater likelihood of hepatotoxicity development, with the p -value at the border of statistical significance (OR = 3.3, 95% CI: 0.95–11.53, $p = 0.06$).

Discussion. Our study demonstrates an association between SNPs rs1801133 in *MTHFR* and rs1801394 in *MTRR* genes and the JIA inflammatory activity at the moment of disease diagnosis. To the best of our knowledge, this is the first study to reveal this relationship. We hypothesise that this association is the effect of increased plasma concentration of homocysteine in individuals with studied SNPs.

As the key enzyme in the folic acid cycle, *MTHFR* is essential for the conversion of homocysteine to methionine. SNP rs1801133, a missense variation from cytosine to thymine, attenuates its catalytic activity, which leads to hyperhomocysteinaemia. Furthermore, an increase in the plasma level of homocysteine is also the result of *MTRR* polymorphism. SNP rs1801394 alters isoleucine into methionine in the protein chain and subsequently disrupts the methionine/homocysteine cycle [10, 11]. The impact of the homocysteine concentration on the degree of inflammation in the course of autoimmune diseases has been demonstrated in several studies (Figure 1).

The most convincing data come from the research conducted on patients with RA, where the positive relationship between the concentration of homocysteine and proinflammatory markers (human soluble interleukin 2 receptor – IL-2sR α , soluble tumour necrosis factor α receptor – sTNF-R75), adhesion molecules (intercellular adhesion molecule 1 – sICAM-1), and CRP, along with clinical features of RA (disease activity, number of joints with active inflammation), was observed [12]. Moreover, the concentration of homocysteine in the synovial fluid of RA patients is significantly

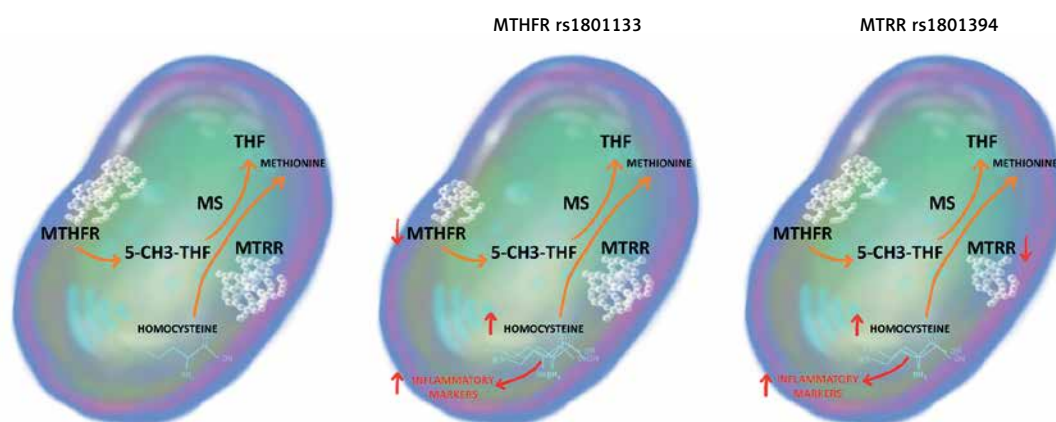


Figure 1. The impact of *MTHFR* rs1801133 and *MTRR* rs1801394 on the methionine/homocysteine cycle
 5-CH3-THF – 5-methyltetrahydrofolate, MS – methioninesynthase, *MTHFR* – methylenetetrahydrofolatereductase, *MTRR* – 5-methyltetrahydrofolate-homocysteine methyltransferase reductase, THF – tetrahydrofolate.

higher than in healthy individuals, leading to enhanced interleukin-6 and interleukin-8 production in synoviocytes and nuclear factor κ B (NF- κ B) activation in rheumatoid cells [13]. Meanwhile, studies conducted in children with JIA revealed comparable results, indicating an increase in the serum concentration of homocysteine in comparison to healthy controls [13] and in patients with a polyarticular subtype of JIA in comparison to children with oligoarthritis [14]. No correlation between the homocysteine concentration and administration of MTX was established [15]. Genotyping of *MTHFR* rs1801133 and *MTRR* rs1801394 may become a useful tool in everyday practice of the paediatric rheumatologist, leading to early recognition of patients at risk of greater disease activity and therefore personalisation of JIA treatment in order to achieve early disease remission, as advised in the treat-to-target strategy.

Because JIA is a rare disease, the sample size was relatively small, and the lack of positive associations between clinical response to MTX and investigated genetic variants may be the effect of low study power. Furthermore, because the primary aim of our research was the evaluation of SNPs within the MTX cellular pathway genes' influence on MTX early efficacy, the measurement of plasma homocysteine concentration in our study group was not performed.

In conclusion, JIA patients with *MTHFR* rs1801133 and *MTRR* rs1801394 genotypes present higher disease activity than children with wild-type alleles, and hence they require a more intensive treatment and follow-up strategy at the moment of JIA diagnosis. Due to the relatively small study group and lack of homocysteine concentration measurement, our results should be interpreted as preliminary.

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Conflict of interest

The authors declare no conflict of interest.

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