Influence of Methylenetetrahydrofolate Reductase Polymorphisms on Efficacy and Toxicity of Methotrexate in Patients with Juvenile Idiopathic Arthritis

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ABSTRACT. ***Objective.*** To study the relationship of C677T and A1298C polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene to toxicity and efficacy of methotrexate (MTX) in patients with juvenile idiopathic arthritis (JIA).

Methods. Single nucleotide polymorphisms of the MTHFR gene were investigated by polymerase chain reaction and restriction enzyme analysis of DNA extracted from peripheral blood cells. The fasting plasma homocysteine concentration was analyzed by enzyme immunoassay. Clinical data of 58 patients with JIA treated with MTX were analyzed retrospectively.

Results. The 1298A/A genotype was present in 31 patients, 1298C/C in 4 patients, and 21 patients were heterozygous. The 677C/C genotype was present in 29 patients, 677 T/T in 3 patients, and 26 patients were heterozygous. 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, chi-square test). There was no relationship between the C677T polymorphism and the efficacy of MTX treatment. Forty-two adverse events were noted in 26 patients; gastrointestinal symptoms were most common (n = 20), followed by elevated serum levels of transaminases (n = 19) and hair loss (n = 3). There was no cytopenia. Patients with the heterozygous genotype 677C/T exhibited adverse events more frequently than patients with the homozygous C/C genotype (65% vs 31%; $p < 0.05$, chi-square test). The A1298C polymorphism, however, was not associated with occurrence of adverse events. Plasma homocysteine was elevated in 6 patients with up to 16.9 mmol/l. No association was found to a specific genotype or to adverse events.

Conclusion. These preliminary data suggest 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. (J Rheumatol 2005;32:1832–6)

Key Indexing Terms:
METHYLENETETRAHYDROFOLATE REDUCTASE METHOTREXATE
JUVENILE IDIOPATHIC ARTHRITIS

Methotrexate (MTX) is the most common disease modifying antirheumatic drug used for treatment of juvenile idiopathic arthritis (JIA) in Germany[1]. The mechanism of action of MTX in the treatment of JIA remains controversial[2]. MTX acts as a folate antagonist, but it also exerts its action on other related metabolism pathways, such as purine and pyrimidine metabolic and the homocysteine-methionine pathway. MTX inhibits dihydrofolate reductase, resulting in a depletion of tetrahydrofolates (THF). Reduced availability of 5-methyl-THF will reduce homocysteine remethylation to methionine, leading to accumulation of homocysteine and decreased intracellular transmethylation capacity. Failure to synthesize active folate compounds may also lead to the inhibition of de-novo purine biosynthesis, which may then result in the intracellular accumulation of 5-aminoimidazole-4-carpoxamide ribonucleotide and induce adenosine release at sites of inflammation. The resultant antiinflammatory effect was shown to be mediated via adenosine A_2 receptors[3,4]. Several single nucleotide polymorphisms (SNP) in the enzyme methylenetetrahydrofolate reductase (MTHFR) gene have effects on folate metabolism, as MTHFR is one of the regulating enzymes in the remethylation of homocysteine to methionine. More than a dozen SNP have been described in the MTHFR gene[5]. Of these, the C677T and A1298C polymorphisms have been associated with altered phenotypes and adverse drug reactions[6].

The C677T SNP, first described in the mid-1990s, results

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in an alanine to valine substitution in the amino acid sequence of the MTHFR protein. It leads to a thermolabile variant of MTHFR with decreased enzyme activity and subsequently increased plasma homocysteine levels[6]. The homozygous 677T/T variant, with about 30% of wild-type activity, is present in about 8%–10% of the general population. Heterozygotes have about 60% activity and form roughly 40% of the population.

In 1998, another polymorphism in the MTHFR gene, A1298C, causing a glutamine to alanine substitution, was described[7]. Both the homozygous and the heterozygous polymorphisms lead to reduced activity of the MTHFR enzyme, although not to a thermolabile variant[8]. The homozygous genotype, with approximately 60% of enzyme activity in lymphocytes, has been found in about 10% of the Canadian population. The A1298C SNP by itself does not result in increased plasma homocysteine levels.

Our aim was to test whether the C677T and A1298C polymorphisms are related to toxicity and/or efficacy of MTX. We retrospectively recorded efficacy and the occurrence of adverse events of treatment with MTX and then analyzed both SNP in 58 patients with JIA.

MATERIALS AND METHODS

Patients. A total of 58 JIA patients (43 girls, 15 boys; 21 with seronegative polyarticular onset, 8 seropositive polyarticular onset, 14 oligoarticular onset, 7 with enthesitis related arthritis, 4 with psoriatic arthritis, 2 systemic onset, and 2 unclassified arthritis) who had been administered MTX orally for at least 3 months at a mean dosage of 12.8 ± 2.6 mg/m^2 (range 8.4–20.5 mg/m^2) were included in this retrospective, single center study. All patients were diagnosed according to the International League of Associations for Rheumatology classification criteria for JIA[9]. The mean age at disease onset was 6.9 years (range 1.1–14.5), the mean age at start of MTX therapy was 9.6 years (range 2.2–17), and the mean duration of MTX therapy was 192 weeks (range 12–532). Folic acid supplementation was not performed at that time. Clinical data were collected from medical records without knowledge of SNP status.

Clinical efficacy and adverse effects of MTX. To evaluate the efficacy of MTX, the numbers of tender and swollen joints and serum concentrations of CRP (normal value < 6 mg/l) and ESR (normal value < 15 mm/h) were recorded before institution of MTX therapy and at the last visit upon MTX therapy. Adverse effects of MTX therapy were recorded during treatment. Alanine aminotransferase and aspartate aminotransferase levels were defined as increased if they were at least 2 times the upper normal serum levels. Patients with elevated serum levels of transaminases were routinely advised to interrupt MTX therapy until transaminases normalized.

Genotype analysis. Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Genotypes resulting from MTHFR gene polymorphisms were determined using a polymerase chain reaction (PCR) restriction fragment length polymorphism method. The C677T genotype was determined as described[10]: a 198 base pair fragment was amplified by PCR and subjected to Hinfl digestion. The 677T allele contains a Hinfl site, resulting in 175 bp and 23 bp fragments, whereas a C at position 677 (677C allele) does not. A1298C genotypes were determined as described[11]: a second PCR amplified a 256 bp fragment. DNA from a patient homozygous for the 1298A allele appears as a fluorescent band of 176 bp length relative to the size marker, with 3 smaller fragments of 30, 28, and 22 bp. Presence of the A1298C polymorphism abolishes an MboI cut site; thus, DNA from a patient homozygous for the 1298C allele appears as a fluorescent band of 204 bp with smaller fragments of 30 and 22 bp.

Homocysteine. The fasting plasma homocysteine level was analyzed by enzyme immunoassay (Axis-Shield, Dundee, UK; normal value < 9 mmol/l).

Statistical analysis. A statistical analysis to evaluate the differences between groups was carried out by chi-square test (available from: http://schnoodles.com/cgi-bin/web_chi_form.cgi). Odds ratios and 95% confidence intervals were calculated when possible.

RESULTS

The 677C/C genotype was present in 29 patients and the 677T/T genotype in 3 patients; 26 patients were heterozygous. Thus, the T allele frequency was 28%. The 1298A/A genotype was present in 31 patients and the 1298C/C in 4 patients; 21 patients were heterozygous. The C allele frequency was 26%.

The efficacy of MTX was evaluated in 58 patients, and only if duration of treatment with MTX exceeded 3 months. The mean duration of treatment was 48 months (range 3–133 mo). Two patients were treated for 13 and 12 weeks, respectively; treatment was judged to be effective in one patient, and ineffective in the other. The latter patient experienced elevated serum levels of transaminases and stomach ache.

Patients with seronegative polyarticular JIA (10 of 21), patients with oligoarticular onset JIA (7 of 14), and patients with psoriatic arthritis (2 of 4) responded more frequently to treatment compared to patients with systemic onset (0 of 2), seropositive polyarticular JIA (one of 8), or enthesitis related arthritis (2 of 7) as well as unclassified JIA (0 of 2).

In patients with the 1298 A/A genotype, the number of swollen joints decreased from a mean of 9.9 to 8.1 at last observation; the number of tender joints decreased from a mean of 7.9 to 4.5 at last observation. The mean ESR decreased from 45.3 to 28.2 at last observation, and the mean serum level of CRP decreased from 48.8 to 32.3 at last observation. In patients with the 1298 A/C and C/C genotype, the number of swollen joints decreased from a mean of 7.2 to 5.1, and the number of tender joints decreased from a mean of 5.2 to 2.0. The mean ESR decreased from 37.2 to 14.0 mm/h at last observation, and the mean serum level of CRP decreased from 41.9 to 10.4 mg/dl at last observation. Eleven of 31 patients with 1298A/A reached an ESR of up to 15 mm/h at last observation, but 18 of 25 patients with either 1298A/C or 1298C/C reached a normal ESR ($p = 0.014$, chi-square test). Seven of 31 patients with 1298A/A reached a CRP level < 6 mm/h at last observation, but 16 of 25 patients with either 1298A/C or 1298C/C achieved this result ($p = 0.004$, chi-square test). In addition, the clinical response rate was higher in patients who possessed the C allele (1298 A/C or C/C) than in 1298 A/A homozygous patients with regard to the improvement of swollen and tender joints ($p < 0.05$, chi-square test; Figure 1). There was no

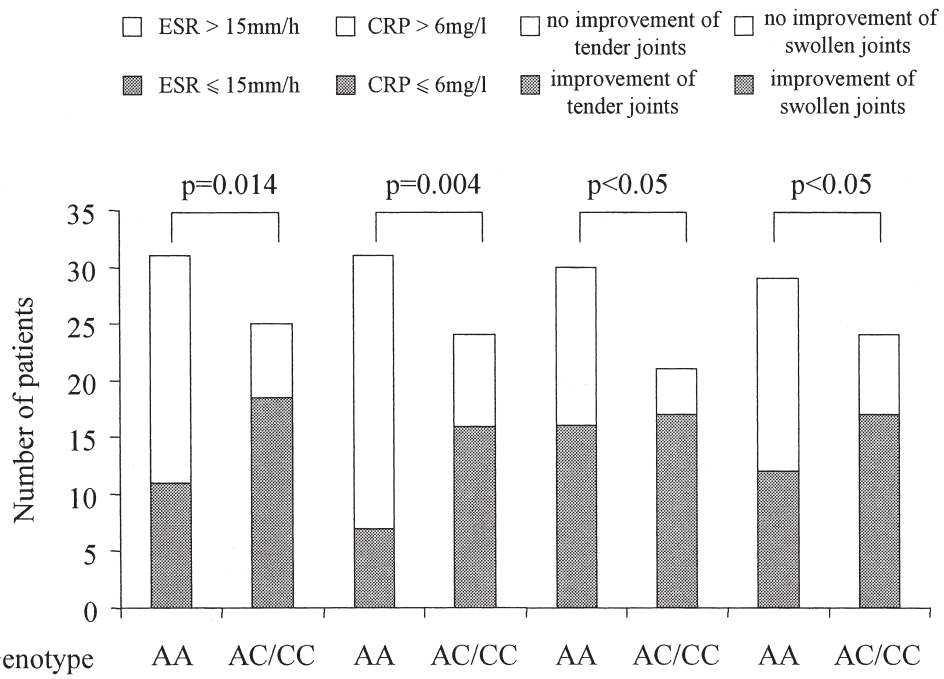

Figure 1. MTHFR A1298C polymorphism and clinical efficacy: Number of patients reaching normal values of ESR, CRP, and clinical improvement in relation to MTHFR genotype.

relation between the C677T polymorphism and the efficacy of treatment with MTX. No differences could be found between patient groups with a specific genotype with regard to the mean weekly dosage of MTX.

A total of 42 adverse events related to MTX therapy occurred in 26 patients, including gastrointestinal (GI) symptoms (n = 20; nausea n = 12, vomiting n = 5, and stomach ache n = 3), elevated serum levels of transaminases (n = 19), and hair loss (n = 3). There was no cytopenia.

All but 5 patients were simultaneously treated with nonsteroidal antiinflammatory drugs. Three of them exhibited adverse events with MTX, 2 had elevated serum levels of transaminases, one nausea, and one stomach ache. All but 10 patients were treated with oral corticosteroids in parallel at a maximum dose of 10 mg/day. While 6 patients showed good toleration of MTX, one exhibited elevated serum levels of transaminases and 3 had abdominal symptoms and nausea.

In 21 patients, combination therapy with sulfasalazine (n = 16), chloroquine (n = 5), cyclosporin A (n = 1) or gold salts (n = 1) was performed. Thirty-seven patients received monotherapy with MTX. In summary, 16 patients taking MTX monotherapy exhibited 20 adverse events (elevated serum levels of transaminases n = 12, nausea n = 3, abdominal pain n = 2, hair loss n = 2, vomitus n = 1). Twenty-one patients on MTX monotherapy had no adverse events. Of 21 patients with combination therapy, 10 had event-free toleration of treatment and 11 had at least one adverse event. Thus, no correlation of combination pharmacotherapy to the occurrence of adverse events could be noted.

Seventeen of 26 patients (65%) with the 677C/T genotype exhibited a total number of 30 adverse events, and 9 of 29 patients (31%) with the 677C/C genotype had a total of 12 adverse events. Thus, patients with the heterozygous genotype 677C/T exhibited adverse events more frequently than patients with the homozygous C/C genotype ($p < 0.05$, chi-square test, OR 4.198, 95% CI 1.359 to 12.964). Three patients with the homozygous 677T/T did not show any adverse event. The presence of the T allele therefore was associated with a higher frequency of adverse events (59% vs 31%; $p < 0.05$, chi-square test). Fourteen patients with the T allele had elevated serum levels of transaminases, compared to 5 patients without T allele (48% vs 17%; $p < 0.05$, chi-square test). Fifteen GI adverse events were observed in 10 patients with the T allele compared to 5 GI adverse events in 4 patients without T allele (48% vs 17%; $p = 0.055$, chi-square test; Figure 2). The A1298C polymorphism, however, was not associated with the occurrence of adverse events.

Plasma homocysteine levels were elevated in 6 patients, with up to 16.9 mmol/l. No association was found between the homocysteine levels and the MTHFR SNP or to adverse events in our study.

DISCUSSION

We observed that the 2 SNP C677T and A1298C seemed to have different effects on the toxicity and the efficacy of methotrexate in patients with JIA, with the 677T allele being associated with greater toxicity and the 1298C allele with better efficacy. The risk of having at least one adverse event

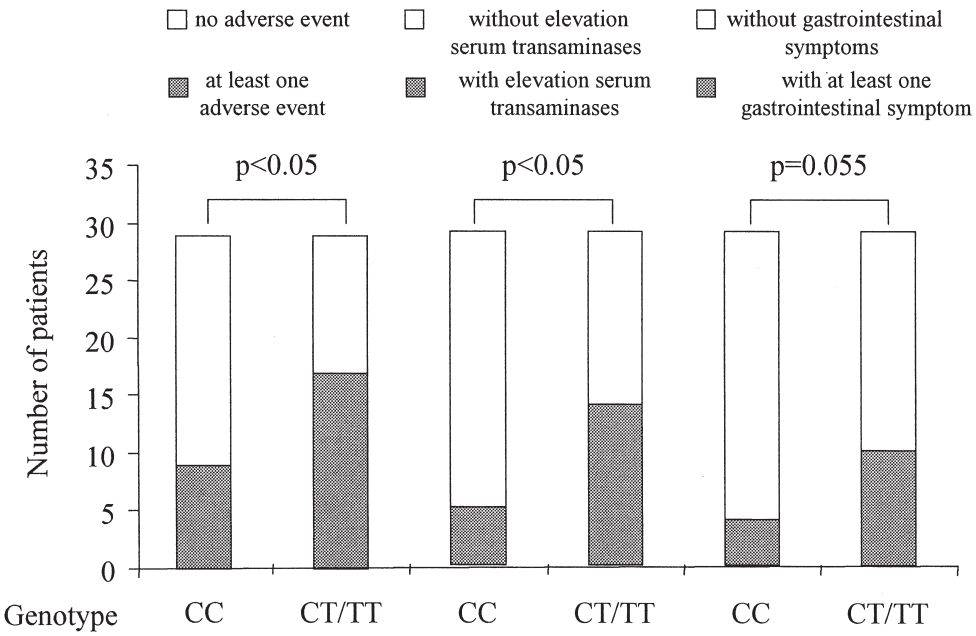

Figure 2. Association between MTHFR C677T polymorphism and adverse events: Number of patients with at least one adverse event, those with elevated serum transaminases, and those with GI adverse events are shown in relation to MTHFR C677T genotype.

while undergoing MTX therapy increased by a factor of 4 in patients with the MTHFR 677C/T genotype compared to patients with the 677C/C genotype. This was not related to a higher dosage of MTX or to concomitant therapy.

Elevated levels of plasma homocysteine were noted in 6 patients only. No association was found with specific genotypes of C677T or A1298C. Measurements of plasma MTX levels or the polyglutamate form of MTX were not done, but these may influence the frequency of adverse events.

To date there have been no studies in patients with JIA about the relation between toxicity and efficacy of treatment with MTX and MTHFR single nucleotide polymorphisms. Thus, a comparison of our data is only possible with adult studies.

Haagsma, *et al*[12] reported that the increase of homocysteine plasma level induced by MTX treatment was greater in patients with rheumatoid arthritis (RA) carrying the MTHFR 677T allele than in those who did not (total patient number = 105). An increased frequency of GI adverse events was found to be related to this[12]. Increased plasma levels of homocysteine were found to be associated with liver enzyme elevations during treatment with MTX[13]. Further, the increase of plasma homocysteine level with MTX treatment can be prevented by supplementation with folic acid or folinic acid. In another study, no relationship was found between the change in homocysteine concentration and the presence or absence of the 677T allele in the MTHFR gene, and no relationship was found between homocysteine metabolism and the efficacy or toxicity of MTX therapy (total patient number = 113)[14].

In another study of 236 patients with RA, the presence of the 677T allele was associated with an increased risk of discontinuation of MTX treatment because of adverse events. No relationship was found between the polymorphism and the efficacy of MTX[15]. These studies show that the 677T allele is associated with a higher toxicity of MTX in patients with RA. Our findings in patients with JIA are in agreement with these studies. In a study of 167 Japanese patients with RA, no association was found between MTHFR C677T and A1298C polymorphisms and MTX related toxicity or efficacy[16].

The efficacy and toxicity of MTX were assessed in 106 patients with RA in a retrospective analysis[17]. Patients with the 1298C allele received significantly lower doses of MTX compared to patients without. However, these patients did show a better improvement upon treatment with MTX. The authors concluded that in patients without 1298C, higher dosages had been tried to achieve a clinical response, but this was not sufficient. This observation in adult patients is in agreement with our findings in JIA patients. Our patients with 1298C also showed a better clinical and laboratory improvement than those with the 1298A/A genotype (Figure 1). A higher rate of overall MTX toxicity was observed in patients with the 677T allele[17]. This result from a study in adults is also in accord with our observations in JIA patients. JIA patients with the 677C/C genotype exhibited adverse events less frequently than those with the 677T allele. However, there were only 3 patients with a 677T/T genotype and none of them had intolerance to MTX.

The 1298C SNP affects MTHFR function and intracellu-

lar folate to a greater extent in the target tissue with respect to immunosuppression or inflammation, without lowering systemic folate concentrations (i.e., no association with toxicity). Because the 1298C SNP alone is not consistently associated with decreased plasma folate levels, but has been associated with lower MTHFR activity in lymphocytes, it is plausible that these patients have better disease control with lower toxicity[18]. This may explain why therapeutic efficacy of MTX in patients with the 1298C allele has been found to exceed the efficacy in those without the allele.

Polymorphisms within the MTHFR gene seem to be associated with both the efficacy and toxicity of methotrexate by both single locuses. It may be speculated that determination of the MTHFR genotype would be clinically useful when JIA patients are treated with MTX to estimate the risk of developing adverse events and the risk of treatment failure. However, these results are preliminary and will need confirmation by further observation, since the number of patients with the 677T allele in our study population was low.

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