The Association of MTHFR C677T and A1298C Polymorphisms with Methotrexate Response and Toxicity in Psoriasis

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ABSTRACT

Background: Methotrexate (MTX) is used to treat psoriasis with various side effects and responsiveness. No predictive indicator of responsiveness or toxicity is available.

Objective: To study the association of the C677T and A1298C polymorphisms in the methylenetetrahydrofolate reductase (*MTHFR*) gene with the responsiveness and side effects of MTX in psoriatic Thai patients.

Methods: The polymorphisms were identified from 86 MTX-treated patients in three hospitals located in southern Thailand using allele-specific PCR. The patients were monitored for the effectiveness and toxicity of the MTX.

Results: The C677T genotype frequencies for C/C, C/T, and T/T were 70.9, 26.8, and 2.3%, respectively, and those of the A1298C genotypes for A/A, A/C, and C/C were 57, 32.5, and 10.5%, respectively. Seventy-three patients (87.9%) responded to MTX. The non-responders had higher combined frequencies of C/T and T/T of the C677T (50%) than the responders (24.7%) with no statistical significance ($p = 0.131$), but had a lower frequency of CC genotype of the A1298C, although not statistically different ($p = 0.691$). Eleven patients (12.8%) suffered toxicity. No association of these polymorphisms and MTX toxicity was found.

Conclusion: The study did not show an association between these *MTHFR* polymorphisms with MTX response and toxicity in these patients.

Keywords: MTHFR; polymorphism; psoriasis; methotrexate

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INTRODUCTION

Psoriasis is a chronic inflammatory skin disease with a prevalence of approximately 1-5% in the general population.[1] It is characterized by an abnormally excessive and rapid growth of the epidermis. Methotrexate (MTX), a folic acid antagonist, is one of the principle treatments in moderate to severe psoriasis. Its action is via inhibiting dihydrofolate reductase (DHFR), an enzyme that converts dihydrofolate (DHF) into tetrahydrofolate (THF) which is a methyl group shuttle required for synthesis of purines, thymidylic acid, and certain amino acids.[2] As it affects DNA and protein synthesis, MTX interferes with the growth of normal cells along with the abnormal target cells and causes a number of side effects leading to discontinuing treatment in approximately 20-30% of the cases.[3,4] However, because of its high efficacy, low cost, and ease of administration, MTX is still used as the drug of

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choice in moderate to severe psoriasis. Therefore, searching for factors that may predict treatment responsiveness and risk of toxicity may improve efficacy and patient safety.

Methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme in folate metabolism. It catalyzes 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-methyl-THF) which is the methyl donor for synthesis of methionine from homocysteine. Reduced MTHFR activity results in an increased plasma homocysteine level which contributes to MTX toxicity.[3,5] *MTHFR* C677T and A1298C are the two most important polymorphisms of clinical significance. The enzymes coded by heterozygous C/T or homozygous T/T of the *MTHFR* C677T were associated with reduced enzyme activity.[5] Several published articles regarding the association of MTHFR C677T and A1298C polymorphisms with MTX response and adverse effects have been reported in rheumatoid arthritis (RA).[6,7]

Psoriasis is a chronic inflammatory disease treated with low dose MTX similar to RA. However, reports of low dose MTX treatment in psoriasis are scarce.[8,9] Currently, there is no predictive indicator before treatment of the risks and side effects or effectiveness of MTX. Therefore, we evaluated the association of *MTHFR* C677T and A1298C polymorphisms with the responsiveness and side effects of MTX in psoriatic Thai patients.

MATERIALS AND METHODS

Subjects

One-hundred and three psoriatic patients who were older than 18 years and had moderate to severe disease involving ≥20% of the body surface area were recruited from Songklanagarind Hospital, Hatyai Hospital, and Songkhla Hospital from September 2007 to March 2010. These three hospitals are located in Songkhla Province in southern Thailand. Exclusion criteria were pregnant women and lactating mothers, patients with a history of cirrhosis, kidney disease, blood disorders, diabetes, and tuberculosis. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University and all patients gave their written informed consent. All participants underwent a clinical interview and physical examination to determine the Psoriasis Area and Severity Index (PASI) score. Laboratory investigations included complete blood count, urinalysis, BUN, creatinine and liver function tests for the baseline. Blood samples (5 mL) were collected for genotypic study.

The protocol for the treatment was 7.5 to 15 mg of MTX per week (0.25 mg/kg of body weight per week) divided into 3 consecutive oral doses approximately every 12 hours until the lesion resolved and the dose was then tapered by 2.5-mg decrements. All patients received supplemental folic acid. The patients were scheduled for follow-up at weeks 4, 8, 16, and 24 to evaluate the treatment response and adverse effects. Laboratory tests, including complete blood count, BUN, creatinine, and liver function tests were done at weeks 4, 8, and 16 for toxicity monitoring. MTX-related adverse effects were defined as one or a combination of gastrointestinal symptoms (nausea, abdominal pain, and diarrhea) or increasing hepatic enzymes (aminotransferases, γ-glutamyltransferase) after taking MTX and resolved when the drug was discontinued. Drug discontinuance was indicated if the levels of transaminases exceeded three times the normal values. The treatment response was defined as a reduction of 50% or more of the PASI score at week 4 or 8.

DNA extraction and genotyping methods

DNA was extracted using the standard phenol-chloroform method and precipitated by conventional ethanol precipitation. Both C677T and A1298C were genotyped using allele-specific PCR. The forward primer (5'- CATATCAGT-CATGAGCCCAGCCACTCACTG -3') and the reverse primer (5'- GAGGACGGTGCGGT-GAGAGTGGG -3') for C677T were designed to expand the C677T polymorphic site. The primer specific for the C allele (5'- TTGAAGGAGA-AGGTGTCTGCGGGCGC -3') was concurrently designed to produce a PCR product of 200 bp with the previous reverse primer whereas that for the T allele (5'- GCCTCAAAGAAAAGCT-GCGTGATGATGAAATGGA -3') was made to produce a 292-bp product with the previous

forward primer. Therefore, in a single PCR reaction, the possible PCR products included the 433-bp of the first primer pair which served as a control and either the 200-bp of the C allele or the 292-bp of the T allele. The same principle was applied to the A1298C polymorphism. The forward primer (5'-GGAGCGGGAGGGCAGAAGAAGTTTGC-3') and the reverse primer (5'- GGGAAGTCA-CAGCCCCGCAGCC -3') produced a control product of 416 bp. The PCR product of 174-bp for the A allele was produced with the primer (5'-GGGGGAGGAGCTGACCAGTGACGA -3') and the reverse primer of the control whereas the 300-bp for the C allele was made with the primer (5'- CGAGAGGTAAAGAACGAAGACTT-CAAAGACACGTG -3') and the forward primer of the control. All PCR reactions were performed in a final volume of 10 µL containing 1X PCR buffer (20 mM Tris pH 8.4, 50 mM KCl), 200 µM dNTPs; 0.5 mM $MgCl_2$, 50 ng DNA; 0.25 µM of each of the 4 primers; 1x Q-solution (Qiagen), and 0.05 U Taq polymerase. PCR reactions were contained in 0.2 mL thin walled tubes. PCR was conducted using a PTC-100 HB Programmable Thermal Controller (MJ Research, Watertown, MA, USA) with the following parameters: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 63°C for 2 min, extension at 72°C for 1 min, and final extension at 72°C for 5 min. PCR products were run by electrophoresis on 6% polyacrylamide gel in 1X TBE buffer (0.04 M Tris-Boric acid, 0.001 M EDTA) and visualized with ethidium bromide under a UV transilluminator.

Statistical analysis

The associations between the polymorphisms and the response and side effects to MTX treatment were assessed by Fisher's exact test. Statistical significance was considered when the *p* value was ≤0.05.

RESULTS

Of the 103 cases of psoriatic patients treated with MTX, 17 cases were lost to follow-up and were excluded from the analysis. The patient characteristics are presented in Table 1. The mean PASI score at the beginning of the study was 29.1±13.8 and after treatment was 7.1±9.1. The genotype frequencies for *MTHFR* C677T in the remaining 86 cases were 70.9% (C/C), 26.8% (C/T), and 2.3% (T/T). Those for A1298C were 57.0% (A/A), 32.5% (A/C), and 10.5% (C/C). Three patients suffered from side effects before week 4 and treatment responsiveness could not be evaluated, so the remaining 83 patients were analyzed for MTX response. The characteristics of lesions of both response and non- response groups before treatment were not significantly different as shown in Table 2. Some patients had more than one type of lesions. Seventy-three patients (87.9%) achieved response to treatment at weeks 4 or 8. The responders were found to be older than the non-responders (45.4±14.1 vs. 37.1±8.9, $p = 0.025$).

Table 3 shows the frequencies of genotypes in the C677T and A1298C. The frequency of TT genotype of the C677T was higher in the non-responders group compared to the responders group (10% vs. 1.4%) with marginal statistical significance ($p = 0.074$). When we combined C/T and T/T genotypes of the C677T, we found that the non-responders still had a higher frequency of the combined genotypes (50%) compared to the responders (24.7%), but no statistical significance ($p = 0.131$). By contrast, the non-responders had a lower frequency of C/C genotype of the A1298C, but it was not a statistically significant difference ($p = 0.691$).

Eleven patients (12.8%) suffered from side effects. These include the three patients who developed side effects before week 4 and withdrew from the study of responsiveness. The remaining eight patients developed side effects after week 8. The side effects found included nausea, vomiting, rash, elevated liver enzymes, decreased neutrophils, and red blood cells. Clinical characteristics of patients with and without side effects were not significantly different (data not shown). Patients who had side effects had a higher proportion of the T/T of *MTHFR* C677T and the C/C of A1298C polymorphism (9.1, 18.2%, respectively) compared with patients without side effects (1.4, 9.3%) (Table 4). However, this was not statistically significant. This may be due to the small number of subjects in this group.

TABLE 1. Characteristics of patients.

Variables	Category (n = 86)	Values (%)
Age (years)	Mean (range)	43.1 (21- 72)
Age of onset (years)	Mean	31.7
Gender	Male	59 (68.6)
	Female	27 (31.4)
Family history	Yes	16 (18.6)
	No	65 (75.6)
	Unknown	5 (5.8)
PASI score at baseline	Mean±SD	29.1±13.8
PASI score after treatment	Mean±SD	7.1±9.1

DISCUSSION

A number of studies have been conducted to elucidate whether polymorphisms of MTHFR or other enzymes in the folate pathway affect MTX efficacy. However, many studies in rheumatoid arthritis and two studies in psoriasis revealed no significant associations[6-11] while only a few noted significant associations.[12] In the present study, we found that C/T and T/T genotypes of the C677T are likely to be associated with non-response to MTX. The TT and CT genotypes of the C677T have been reported to be associated with reduced enzyme activity.[5] The effect of MTHFR activity

TABLE 2. The characteristic of lesions in the response and non- response group before treatment.

Variables	Responder (n=73)	Non responder (n=10)	Not known (n=3)	*P* value
PASI score	28.73±13.6	29.22±14.3		0.92
Plaque	40	6	1	
Guttate	4	0	0	
Erythroderma	3	0	0	
Plaque and Guttate	22	2	1	0.67
Plaque and Erythroderma	2	1	1	
Plaque and Guttate and Erythroderma	3	0	0	

TABLE 3. Association between *MTHFR* C677T and A1298C polymorphisms and response to MTX treatment (n = 83).

Polymorphism	Genotype	Response Number (%)	Non-response Number (%)	*p* value
C677T	C/C	55 (75.3)	5 (50)	0.074
	C/T	17 (23.3)	4 (40)	
	T/T	1 (1.4)	1 (10)	
A1298C	A/A	40 (54.8)	7 (70)	0.691
	A/C	25 (34.2)	3 (30)	
	C/C	8 (11.0)	0	

TABLE 4. Association between *MTHFR* C677T and A1298C polymorphisms and side effects to MTX treatment (n = 86).

Polymorphism	Genotype	Side effects Number (%)	No side effects Number (%)	*p* value
C677T	C/C	6 (54.5)	55 (73.3)	0.164
	C/T	4 (36.4)	19 (25.3)	
	T/T	1 (9.1)	1 (1.4)	
A1298C	A/A	7 (63.6)	42 (56.0)	0.346
	A/C	2 (18.2)	26 (34.7)	
	C/C	2 (18.2)	7 (9.3)	

on MTX response may be explained by the interaction of various enzymes in the folate pathway.[13] In the folate pathway, DHF is converted to THF by DHFR which is inhibited by MTX. The conversion of THF to 5, 10-methylene-THF is a crucial step as it provides major carbon sources for de novo purine synthesis. MTHFR irreversibly reduces the portion of 5, 10-methylene-THF to the 5-methyl-THF. Therefore, the reduced MTHFR activity may affect the balance of THF and the 5, 10-methylene-THF which affects the purine metabolism and subsequent DNA and RNA synthesis.

In our study, only 11 (12.8%) patients suffered from side effects. This incidence of adverse events is quite low compared to 20-30% reported in RA.[3] It has been reported that concomitant administration of folic acid, which reduceshomocysteine level, can lower the side effects of the drug.[14] Supplemental folic acid in all patients in our study may account for the low incidence of side effects.

Regarding the association of polymorphism with the risk of side effects, we did not find a statistically significant association between genotypes of either polymorphisms and MTX toxicity in psoriatic Thai patients, probably due to the small number of subjects in each group. The T/T and C/T genotypes of the C677T have been reported to be associated with diminished enzyme activity and increased plasma homocysteine level.[5] In addition, an increased homocysteine level from the homocysteine/methionine pathway is usually reported to be related to the toxicity in low-dose MTX therapy. The AC and CC genotypes of A1298C were also associated with lower enzyme activity, but their relation to plasma folate and homocysteine were not convincing.[15-17] From meta-analysis evidence in RA, it was indicated that C677T polymorphism was likely to be associated with an elevated risk of MTX toxicities.[18] Up to this date, there are very few studies regarding these two polymorphisms and the risks of adverse effects of MTX treatment in psoriatic patients. Our study provided similar findings to those of Campalani et al., and Warren et al., which showed no association between these two polymorphisms and clinical outcomes of MTX treatment.[8,9]

In conclusion, our study did not show an association between genotypes of the C677T and A1298C in the *MTHFR* gene or MTX response and toxicity in psoriatic Thai patients. This finding may be due to the small number of studied subjects. Further studies in a larger cohort are warranted.

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Declaration of Interest statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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