Associations between the genetic polymorphisms of MTHFR and outcomes of methotrexate treatment in rheumatoid arthritis

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Abstract

Objectives

To determine whether 5, 10-methylenetetrahydrofolate reductase (MTHFR) rs1801133C/T, rs1801131A/C, rs2274976A/G, rs2066462C/T genetic polymorphisms are associated with clinical response and adverse effects (AEs) of methotrexate (MTX) treatment in Chinese Han patients with rheumatoid arthritis (RA).

Methods

One hundred and ten RA patients defined by the American College of Rheumatology (ACR) 1987 revised criteria were involved in this study. All patients were treated with low-dose MTX (10-15 mg/week) without concomitant uses of other DMARDs. Clinical response (using ACR20 criteria) and AEs were evaluated at 0, 4, 12, 16 and 24 weeks. The genotypes of MTHFR rs1801133C/T, rs1801131A/C, rs2274976A/G and rs2066462C/T were detected by real-time fluorescent quantitative PCR.

Results

The allele frequency of rs1801131C in the clinical response group was higher than in the non-response group (21.0% vs. 8.1%, $p<0.05$), and the patients with CC or AC genotype had greater clinical response than those with AA genotype. The allele frequencies of rs1801133T and rs2274976A were higher in the group with AEs than that without AEs (56.4% vs. 37.5% and 14.9% vs. 4.2%, respectively, both $p<0.05$). The patients with CT or TT genotype in rs1801133 had higher risks of AEs than those with CC genotype.

Conclusions

While rs1801131A/C genetic polymorphism is associated with the clinical response, rs1801133C/T and rs2274976A/G genetic polymorphisms are associated with MTX-related AEs in the treatment of RA. This suggests individualisation is necessary to achieve optimal outcomes in MTX therapy of RA.

Key words

5, 10-methylenetetrahydrofolate reductase, genetic polymorphisms, clinical response, adverse events, rheumatoid arthritis.

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Introduction

The anti-folate drug methotrexate (MTX) is the most commonly used disease-modifying anti-rheumatic drug (DMARD) for rheumatoid arthritis (RA) and other inflammatory autoimmune diseases. It is able to reduce disease activity and delay or stabilise the development of bone erosions (1). Most RA patients have improvement in disease activity with MTX treatment, but MTX-related adverse effects (AEs) and incomplete responses are quite common. While 45%~65% of the patients experience good clinical response, 10%~30% discontinue this therapy due to side effects (2, 3). Although the precise mechanism of MTX is unknown, it is believed to be related to the inhibition of folate pathway enzymes, such as dihydrofolate reductase (DHFR), methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthase (TS) (4-7).

Human MTHFR gene locates on 1p36.3 and the coding region is 1980bp, including 11 exons and 10 introns. To date, several single-nucleotide polymorphism (SNPs) of MTHFR genes such as rs1801133C/T (677), rs1801131A/C (1298), rs2274976A/G, and rs2066462C/T have been identified. The most studied two SNPs were rs1801133C/T (677) and rs1801131A/C (1298). Currently, the reports about the associations between these SNPs and MTX-related AEs and clinical responses are controversial (2, 3, 8, 9, 10). The aim of this study was, therefore, to determine whether MTHFR rs1801133C/T, rs1801131A/C, rs2274976A/G, rs2066462C/T genetic polymorphisms are associated with MTX-related clinical response and AEs in Chinese Han patients with RA. In Chinese Han people, the frequencies of the rs1801133T (677), rs1801131C (1298), rs2274976A and rs2066462T allele have been reported at 49%, 20%, 10% and 11%, respectively, in the current study.

Patients and methods

Patients

RA patients with active disease according to the American College of Rheumatology (ACR) revised criteria were recruited. Inclusion criteria were: >3 swollen joints (28-joint count), >5 tender joints (28-joint count), either an erythrocyte sedimentation rate (ESR) >28 mm/hour or a visual analogue scale for global health >20 mm (0 best, 100 worst), and joint function: II~III. We recruited 110 patients with 71% females, mean age 49.2±13.4 years old (range from 18 to 75) and average duration of disease 44±13.1 months (range from 3 to 96).

Exclusion criteria included treatment with MTX or other DMARDs in the previous three months, concomitant treatment with an experimental drug, a malignancy within the last 5 years, bone marrow hypoplasia, serum creatinine >1.3× upper limit of normal (ULN), serum aspartate transaminase/alanine transaminase (AST/ALT) >1.5×ULN, total white cell count $<3.9\times10^9$/L, Haemoglobin (Hb) <80g/L, platelet count $<75\times10^9$/L, severe cardiovascular diseases, immunodeficiency, diabetes mellitus, alcohol or drug abuse, pregnancy or the wish to become pregnant (11) during the study period.

All RA patients are Chinese Han people from Anhui Province. The study was approved by the Anhui Medical University Medical Human Research Ethics Committee, and written informed consent was obtained from all participants.

Treatment

All 110 RA patients were treated with the low-dose MTX (10-15mg/week) without concomitant other DMARDs for 24 weeks. During 0~12 weeks, NSAIDs and/or low-dose prednisone (≤10mg/d) were allowed to be used according to each patient's condition.

Clinical outcomes and laboratory assessments

Clinical outcomes (early morning stiffness, tender joints, swollen joints, physician's assessment, patient's assessment, Health Assessment Questionnaire (HAQ)) and laboratory markers (ESR, CRP, total white cell count, platelet count, routine urine examination, serum AST/ALT and serum creatinine) were evaluated at weeks 0, 4, 12, 16 and 24 to determine the clinical response of MTX and MTX-related AEs.

Clinical response evaluation

We used ACR20 criteria (12) to monitor clinical responses of treatment and utilised this for dosage adjustments. Clinical response was defined as 20% improvement in tender and swollen joint counts and 20% improvement in 3 of the 5 following measures: patient and physician global assessments, pain, disability, and an acute phase reactant (ESR or CRP). This was evaluated at week 24.

MTX-related AEs evaluation

Adverse events were categorised according to the common toxicity criteria of the National Cancer Institute. General: fatigue/malaise, fever (>37.7°C), headache, sweating, weight gain and weight loss. Gastrointestinal: anorexia, diarrhoea, dyspepsia, gastrointestinal bleed, hepatitis, nausea or vomiting, pancreatitis. Ear, nose, throat: stomatitis, tinnitus, xerostomia. Skin: alopecia, bullous eruption, petechiae, rash. Pulmonary: cough, dyspnoea, pleurisy, pneumonitis, decreased pulmonary function (<90% diffusing capacity of the lung for carbon monoxide or forced vital capacity of pretreatment value). Neuropsychiatric: anxiety or depression, somnolence, inability to concentrate, insomnia, decreased libido, peripheral neuropathy, vertigo. Blood chemistry: serum creatinine >1.3×upper limit of normal (ULN); serum AST >1.5×ULN; serum ALT >1.5×ULN; serum alkaline phosphatise >2.0×ULN. Haematological: anaemia (haemoglobin decrease >1.4g/dl from baseline); leucopenia (total white cell count <3900/mm^3); thrombocytopenia (platelet count <75,000/mm^3).

During follow-up, adverse events were recorded by the patients' self-reports, investigator's reports, physical examinations, or laboratory measurements. Each adverse event was described by its duration, frequency, severity, an assessment of its cause, its relationship to the study medication, whether it influenced the course of treatment, and whether it required specific therapy. AEs were determined if adverse events were judged to be related to MTX therapy.

Genotyping

DNA extraction was performed using Puregene DNA extraction kits (Qiagen, Valencia, CA) on an Autopure LS (Qiagen) using the whole-blood protocol (13). DNA was dissolved in Tris-EDTA buffer, aliquoted into a minimum of 2 vials, and stored at -80°C. The allelic discrimination of the *MTHFR* gene poly-morphisms rs1801133 C/T, rs1801131 A/C, rs2274976 G/A and rs2066462 C/T was assessed using TaqMan genotyping assays (assay ID: C_1202883_20 for rs1801133, C_850486_20 for rs1801131, C_16183363_10 for rs2274976 and C_11644767_10 for rs2066462) on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). The final volume for each reaction was 5 μL, consisting of 2.5 μL TaqMan Universal PCR Master Mix (Applied Biosystems), 0.25 μL of primers/TaqMan probe mix, and 5 ng DNA. The PCR profile consisted of an initial denaturation step at 95°C for 10 min and 50 cycles at 92°C for 10 sec and 60°C for 1 min. The fluorescence level was measured with the ABI PRISM 7900HT sequence detector (Applied Biosystems). Genotypes were determined by ABI SDS software. The laboratory staff was blinded to the identity of the subjects.

Statistical analysis

Statistical analyses were performed using the statistical package SPSS10.0. $p<0.05$ was considered statistically significant. Hardy-Weinberg equilibrium was assessed using χ^2 analysis. Differences in allele frequencies between groups were evaluated using χ^2 test. Multiple logistic regression analysis was used to determine the associations between SNPs and clinical response or AEs. Haplotype analyses were performed using SHEsis (14) software (Http://analysis.bio-x.cn/myAnalysis.php).

Results

Eleven of the 110 RA patients were lost to follow-up. Among the remaining 99 RA patients, 95 had DNA samples, one failed to get the genotype of rs1801131A/C, and two patients discontinued after experiencing severe MTX-related AEs (one severe epispasis and one severe gastrointestinal symptom). Therefore, 93 patients were available for response evaluation at week 24, and 94-95 patients were available for AEs evaluation analysis. All SNPs were in Hardy-Weinberg equilibrium.

SNP allele frequencies and clinical response of MTX

The clinical response was 66.7% (62 out of 93 patients) at week 24, and the baseline variables were comparable between the response group and the non-response group (Table I). As shown in Table II, allele frequency of rs1801131C in response group was significantly higher than in non-response group (21.0% *vs*. 8.1%, $p<0.05$). The RA patients with CC or AC genotype had higher clinical response than those with AA genotype after the adjustment of sex and age (OR=3.53, 95%CI: 1.18–10.58). In contrast, there were no significant associations between the allele frequencies of another 3 SNPs and MTX-related to clinical response in both unadjusted and adjusted analyses.

Table I. The clinical baseline characteristics of the response group and the non-response group of RA patients.

	Responder (n= 62)	Non-responder (n=31)	*p*-value
Age (year)	49.6 ±14.1	48.6 ± 12.7	0.740
Female sex (%)	74.2 (46)	67.7 (21)	0.683
Disease duration (months)	44.7 ±13.9	43.1 ± 16.2	0.622
Swollen joint counts	7.6 ±3.6	7.1 ± 2.9	0.504
Tender joint counts	9.6 ±4.3	8.9 ± 2.4	0.402
Health assessment questionnaire (HAQ)	1.43 ±0.61	1.29 ± 0.58	0.292
Erythrocyte sedimentation rate (mm/h)	48.1 ±10.3	50.3 ± 8.3	0.305
C-reactive protein (mg/dl)	5.75 ±4.13	6.25 ± 3.95	0.578
MTX dose (mg/w)	10.70 ±1.38	10.92 ± 1.67	0.501

Table II. Genotype and allele frequencies of MTHFR genetic polymorphisms and clinical response of MTX.

		Responder (n=62)	Non-responder (n= 31)	χ^2 value	*p*-value
rs1801133C/T	*CC (%)*	13 (21.0)	9 (29.0)		
	CT (%)	38 (61.3)	17 (54.8)		
	TT (%)	11 (17.7)	5 (16.1)	0.74	0.689
	T (%)	60 (48.4)	27 (43.5)		
	C (%)	64 (51.6)	35 (56.5)	0.39	0.533
rs1801131A/C	*AA (%)*	37 (59.7)	26 (83.9)		
	AC (%)	24 (38.7)	5 (16.1)		
	CC (%)	1 (1.6)	0 (0)	5.54	0.018
	C (%)	26 (21.0)	5 (8.1)		
	A (%)	98 (79.0)	57 (91.9)	4.95	0.026
rs2274976A/G	*AA (%)*	0 (0)	0 (0)		
	AG (%)	11 (17.7)	7 (22.6)		
	GG (%)	51 (82.3)	24 (77.4)	0.31	0.578
	A (%)	11 (8.9)	7 (11.3)		
	G (%)	113 (91.1)	55 (88.7)	0.31	0.578
rs2066462C/T	*CC (%)*	53 (85.5)	27 (87.1)		
	CT (%)	9 (14.5)	4 (12.9)		
	TT (%)	0 (0)	0 (0)	0.04	0.833
	T (%)	9 (7.3)	4 (6. 5)		
	C (%)	115 (92.7)	58 (93.5)	0.01	0.919

Table III. Genotype and allele frequencies of MTHFR genetic polymorphisms and MTX-related adverse effects (AEs).

		With AEs (n= 47)	Without AEs (n=48)	χ^2 value	*p*-value
rs1801133C/T	*CC (%)*	5 (10.6)	17 (35.4)		
	CT (%)	31 (66.0)	26 (54.2)		
	TT (%)	11 (23.4)	5 (10.4)	9.22	0.010
	T (%)	53 (56.4)	36 (37.5)		
	C (%)	41 (43.6)	60 (62.5)	6.80	0.009
rs1801131A/C	*AA (%)*	33 (71.7)	30 (62.5)		
	AC (%)	13 (28.3)	17 (35.4)		
	CC (%)	0 (0)	1 (2.1)	0.91	0.341
	C (%)	13 (14.1)	19 (19.8)		
	A (%)	79 (85.9)	77 (80.2)	1.07	0.302
rs2274976A/G	*AA (%)*	0 (0)	0 (0)		
	AG (%)	14 (29.8)	4 (8.3)		
	GG (%)	33 (70.2)	44 (91.7)	7.12	0.008
	A (%)	14 (14.9)	4 (4.2)		
	G (%)	80 (85.1)	92 (95.8)	6.37	0.012
rs2066462C/T	*CC (%)*	42 (89.4)	40 (83.3)		
	CT (%)	5 (10.6)	8 (16.7)		
	TT (%)	0 (0)	0 (0)	0.73	0.393
	T (%)	5 (5.3)	8 (8.3)		
	C (%)	89 (94.7)	88 (91.7)	0.68	0.411

SNP allele frequencies and MTX-related AEs

The incidence of AEs was 49.5% (47 out of 95 patients). The most commonly AEs were nausea with indigestion (70.2%), serum AST >1.5×ULN and/or serum ALT >1.5×ULN (10.7%), leucopenia (6.4%), rash (2.1%), alopecia (2.1%), headache (2.1%), others (6.4%). As shown in Table III, the allele frequencies of rs1801133T and rs2274976A were significantly higher in the group with AEs than that without AEs (56.4% *vs*. 37.5%, 14.9% *vs*. 4.2%, $p<0.05$). For rs1801133C/T, the RA patients with CT or TT genotype had higher MTX-related AEs risk than those with CC genotype after adjustment for sex and age (CT *vs*. CC: OR=3.55, 95% CI: 1.12–11.27; TT *vs*. CC: OR=5.33, 95% CI: 1.19–23.90). For rs2274976A/G, the RA patients with AG genotype had higher MTX-related AEs risk than those with GG genotype (OR=6.92, 95% CI: 1.83-26.14). There were no significant associations between the allele frequencies of rs1801131C and rs2066462T and MTX-related AEs.

Haplotype frequencies and MTX-related AEs

As shown in Table IV, the 2 SNPs (rs1801133C/T, rs2274976A/G) constructed 4 kinds of haplotype: C-A, C-G, T-A, T-G. In the group with AEs, the frequency of these haplotypes was 8.4%, 35.2%, 6.5% and 49.9% respectively. In that without AEs, it was 4.1%, 58.4%, 0 and 37.5%, respectively. The haplotype frequency of T-A in the group with AEs was significantly higher than in the group without AEs (OR=417.89, 95% CI: 24.24–7204.68, $p=0.02$), and the haplotype frequency of C-G in the group with AEs was significantly lower than in the group without AEs (OR=0.39, 95% CI: 0.22–0.70). There were no significant associations between the haplotype frequency of C-A or T-G and MTX-related AEs.

Discussion

To our knowledge, this study is the first to determine the associations between MTHFR genetic polymorphisms and MTX-related clinical response and AEs in Chinese Han patients with RA. We found that while allele frequency of rs1801131C was associated with increased clinical response, the allele frequencies of rs1801133T and rs2274976A were associated with increased AEs of MTX therapy.

Although the functionality of MTHFR genetic polymorphisms has not fully been explored, it has been shown that the effects of the mutant alleles of MTHFR rs1801133C/T (677) and rs1801131A/C (1298) lead to higher thermolability and a decrease in enzyme activity (15-17). The rs2274976A/G genetic polymorphism can result in Arg594Gln amino acid substitution (18) but its functional relevance is not clear, although higher homocysteine concentrations have been reported in association with the wild-type genotype among Swedish adolescents (19). In Chinese Han peo-

Table IV. The haplotype frequency and MTX-related AEs (rs1801133C/T, rs2274976A/G).

	With AEs (%)	Without AEs (%)	OR (95%CI)	*p*-value
C-A	7.87 (8.4)	3.98 (4.1)	2.11 (0.61-7.29)	0.23
C-G	33.13 (35.2)	56.02 (58.4)	0.39 (0.22-0.70)	0.00
T-A	6.13 (6.5)	0.02 (0)	417.89 (24.24-7204.68)*	0.02
T-G	46.87 (49.9)	35.98 (37.5)	1.66 (0.93-2.96)	0.09

The data presented in the first two columns are the number of individual haplotype and its frequency. The high OR was a consequence of the small number of individuals carrying this haplotype.

ple, we reported that the frequencies of the rs1801133T (677), rs1801131C (1298), rs2274976A and rs2066462T allele were 49%, 20%, 10% and 11%, respectively, in the current study. So far, studies about the relationship between MTHFR genetic polymorphisms and outcomes of MTX treatment in RA patients have produced controversial results. Urano *et al*. (8) reported that 1298A/C gene polymorphism was associated with the efficacy of MTX treatment. Weisman *et al*. (2) reported that there was significant correlation between MTX-related AEs and 677C/T gene polymorphisms, and the mutation of this SNP increased the MTX toxicity. Wessels *et al*. (3) found that RA patients with genotype of 1298AA or 677CC obtained better efficacy from MTX treatment. From a study of 167 Japanese RA patients, Kumagaik *et al*. (9) reported there was no correlation between the genetic polymorphism of 677C/T or 1298A/C and the efficacy of MTX treatment. Taraborelli M *et al*. did not find any association between MTHFR genotype/allele and MTX response or toxicity either (20). Hughes *et al*. (10) reported that allele 1298A was associated with the AEs of MTX in Caucasians. These discrepancies may be related to differences in factors such as race, region, polygenic inheritance, sample size and different criteria of the disease outcome evaluation and methods, educational status of patients (21). A recent meta-analysis suggested that the 677C/T polymorphism was associated with increased toxicity while 1298A/C polymorphism was not associated with increased toxicity (22).

In the current study, we found that in Chinese Han RA patients, the rs1801131A/C (1298) genetic polymorphisms were associated with the clinical response of MTX treatment, and rs1801133C/T (677)/rs2274976A/G genetic polymorphisms were associated with MTX-related AEs. These are consistent with the results reported by the Urano (8) and Weisman (2) groups. Both rs1801133C/T and rs1801131A/C variants are associated with decreased MTHFR activity, yet the effects on efficacy and toxicity are different. The 677C/T polymorphism results in an alanine to valine substitution, leading to the thermolabile variant of MTHFR with decreased enzyme activity, and subsequent increased plasma homocysteine levels; in contrast, the 1298A/C polymorphism results in glutamine to alanine substitution and does not result in increased plasma homocysteine levels (23). These may partly explain their difference in efficacy and toxicity.

In addition, this inconsistency may also reflect gene-gene interactions underlying the effects and/or toxicity of MTX treatment. For example, Dervieux *et al*. (24) measured fourteen SNPs in folate and adenosine biosynthesis pathways in 255 RA patients treated with MTX for at least 3 months, and reported that some gene-gene interactions impacted MTX efficacy and tolerability in RA patients. It is plausible that there exist gene-gene interactions between MTHFR genetic polymorphisms and other polymorphisms on MTX-related response and AEs, and this is required to be determined in further studies.

The haplotype frequencies of MTHFR genes may also be associated with the efficacy and toxicity of MTX treatment. Urano *et al*. (8) reported that patients with the rs1801133C- rs1801131C haplotype were receiving lower doses of MTX than those without this haplotype, while subjects with rs1801133T-rs1801131A had a higher frequency of AEs from MTX treatment. Using the haplotype analysis for rs1801133C/T and rs2274976A/G, we found that the haplotype T-A increased the risk of MTX-related AEs, while the haplotype C-G decreased the risk. These results suggest that careful patient selection is necessary to achieve optimal outcomes in MTX therapy. Clearly, further studies with large sample size are needed to determine the associations of all these MTHFR gene polymorphisms and outcomes of MTX therapy.

In conclusion, while the rs1801131A/C genetic polymorphism is associated with the clinical response, rs1801133C/T and rs2274976A/G genetic polymorphisms are associated with MTX-related AEs in the treatment of RA, suggesting individualisation is necessary to achieve optimal outcomes in MTX therapy of RA.

Key messages

5, 10-methylenetetrahydrofolate reductase (MTHFR) rs1801131A/C genetic polymorphism is associated with the clinical response in the treatment of rheumatoid arthritis. MTHFR rs1801133C/T and rs2274976A/G genetic polymorphisms are associated with MTX-related adverse events in the treatment of rheumatoid arthritis.

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