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Research Article

Influence of Genetic Polymorphisms of *MDR1, RFC1, FPGS, GGH*, and *MTHFR* on Methotrexate Efficacy and Toxicity in Chinese Patients with Rheumatoid Arthritis

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Abstract

Aim: To investigate the influence of genetic polymorphisms of Multidrug Resistant Gene *(MDR1)*, Reduced Folate Carrier *(RFC1)*, Folylpolyglutamyl Synthase *(FPGS)*, Gamma Glutamyl Hydrolase *(GGH)*, and Methylenetetrahydrofolate Reductase *(MTHFR)* on Methotrexate (MTX) efficacy and toxicity in Chinese patients with Rheumatoid Arthritis *(RA)*.

Methods: One hundred and thirteen Rheumatoid Arthritis (RA) patients defined by the American College of Rheumatology (ACR) 1987 classification criteria were recruited in this study. All patients were treated with low-dose MTX (10-20 mg/week) without concomitant uses of other DMARDs for at least six months. *RFC1* 80G>A, *MDR1* 3435C>T, *FPGS* rs1544105 G>A, *GGH* - 401C>T, *MTHFR* 677C>T and *MTHFR* 1298A>C were genotyped by Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR–RFLP) methods. The MTX toxicity assessment was categorized according to the common toxicity criteria of the National Cancer Institute.

Result: The remission of RA symptoms was achieved in all the *MDR1* 3435TT genotype carriers (16/16), in 73.1% of subjects with 3435CT genotype (38/52), and in 75.6% of patients with 3435CC genotype (34/45) (P=0.046), respectively. Interaction between SNPs in *RFC1* 80 and *MTHFR* 1298 seemed to affect the efficacy of MTX with the best overall performances (accuracy of 0.67) and a CVC of 10/10 (P=0.002). *MTHFR* 677T allele carriers were more susceptible to MTX toxicity (P=0.007, OR:1.897, 95%CI: 1.725-2.087), compared to those with CC genotype. Of four diplotypes, patients with *MTHFR* CA-TA diplotype were more easy to experience toxicity (*P*=0.007, OR: 2.273, 95%CI: 1.303-3.964) when compared to patients without CA-TA diplotype.

Conclusion: *MDR1* 3435C>T might influence efficacy of RA therapy with MTX. Interaction between *RFC1* 80 and *MTHFR* 1298 seemed to impact MTX efficacy. Polymorphisms in the *MTHFR* gene were associated with toxicity of MTX. Further studies are warranted.

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease that leads to severe joint damage and affects around 1% of the population worldwide [1]. RA is associated with substantial disability and increased mortality. Of the several Disease-Modifying Antirheumatic Drugs (DMARDs) available, the folate antagonist methotrexate (MTX) have become one of the most commonly used drugs and the first choice to treat RA [2,3]. The dosage and administration of MTX for the treatment of RA are low dose of the range of 10-20 mg/week in China, which is different from the high-dose for the treatment of cancer, acute lymphoblastic leukemia. However, clinical response to MTX varies considerably among patients, ranging from 46 to 65% [4,5]. Toxicity also limits the use of MTX, and approximately 10-30% of patients with RA discontinued MTX because of toxicity [6]. There are no reliable molecular markers of response and toxicity of therapy, especially in Chinese RA patients. Therefore, identification of predictive markers is critical to the choice of appropriate treatment and avoiding unpredictable adverse reaction prior to initiation of MTX.

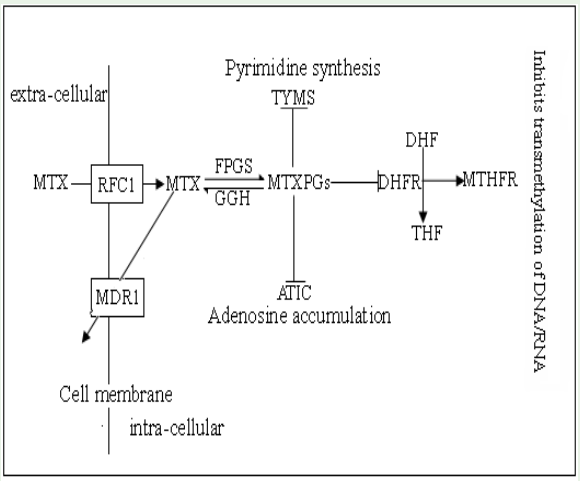

Figure 1: Illustrating some of the key enzymes involved in metabolism of MTX. RFC: Reduced Folate Carrier; MTXPGs: Polyglutamated MTX; FPGS: Folylpoly-Glutamate Synthase; GGH: Gamma-Glutamyl Hydrolase; DHF: Dihydrofolate; THF: Tetrahydrofolate; TYMS: Thymidylate Synthase; DHFR: Dihydrofolate Reductase; MTHFR: Methylenetetrahydrofolate Reductase; ATIC: 5-Aminoimidazole-4-Carboxamide Ribonucleotide Transformylase.

As shown in Figure 1, the folate pathway consists of MTX being transported into the cell by reduced folate carrier 1 (RFC1) [7] and pumped out of the cell by members of the Adenosine Triphosphate (ATP) - Adenosine Binding Cassette (ABC) family of transporters, such as P-glycoprotein (P-gp, encoded by *MDR1* gene) [8]. Intracellular MTX is polyglutamated by the enzyme Folylpolyglutamyl Synthase (FPGS). This process can be reversed by γ-glutamyl hydrolase (GGH) [9]. Polyglutamation of MTX (MTXPGs) helps to retain MTX within the cell, preventing drug efflux by the ABC transporters. MTXPGs inhibit Dihydrofolate Reductase (DHFR) which reduces dihydrofolate to tetrahydrofolate (THF) [10]. THF is converted to 5,10-methylene tetrahydrofolate (5-10-CH_2-THF) and subsequently to 5-methyl-THF (5-CH_3-THF) by Methylenetetrahydrofolate Reductase (MTHFR) which might affect anti rheumatic effect indirectly [11]. The above influx-metabolism-efflux pathway of MTX is well understood, polymorphisms of multiple factors involved in this pathway may contribute significantly to the variability of response and toxicity of MTX therapy.

RFC1 80 G>A, a nonsynonymous mutation in exon 3, has been usually reported to cause increased transcriptional activity [12]. *MDR1* 3435 C>T in exon 26 is found to influence the intestinal P-gp expression, and individuals homozygous for 3435T had decreased intestinal P-gp expression [13,14]. As for *FPGS*, a study showed an association between FPGS mRNA expression in peripheral blood mononuclear cells and poor response to MTX in RA patients [15], the functional roles of SNPs in this gene are yet to be determined. *GGH* -401 C>T in the promoter has been described to increase GGH expression [16,17]. *MTHFR* 677 C>T results in a thermolabile variant of *MTHFR* with decreased enzyme activity and subsequent increased plasma homocysteine levels [18]. Homozygotes (CC) and heterozygotes (AC) for 1298A>C have reduced activity of the MTHFR enzyme, although not a thermolabile variant [9].

All of these SNPs has been investigated in the association with response and toxicity to MTX, but with conflicting results that might be due to differences in sample size, ethnicities, therapy and co-administered drugs, or the confounding effects of other genetic polymorphisms. In Chinese, these SNPs was studied mainly in leukemia patients, whose therapy included high-dose MTX (up to 5 gram/m^2·course). There were few studies on these SNPs in Chinese RA patients who treated with low-dose MTX. Especially, *FPGS* and *GGH* genetic polymorphisms have not been investigated in Chinese RA patients. In sum, our aim was to investigate the influence of genetic polymorphisms of the factors involving in the folate pathway on MTX efficacy and toxicity in Chinese patients with Rheumatoid Arthritis (RA).

Materials and Methods

Recruitment of subjects

A total of 113 patients with RA were recruited in our study from the Department of Rheumatology, the First Affiliated Hospital of Sun Yat-sen University from 2007 to 2009. All the patients conformed to the 1987 classification criteria of the American College of Rheumatology (ACR) [19] and underwent detailed clinical evaluation at the time of enrollment. All patients were treated with the low-dose MTX (10-20 mg/week) without concomitant other DMARDs for at least six months. Baseline data were obtained by chart review according to the ACR 20% response criteria. These included demographics, the number of swollen joints, the number of tender joints, physician's as well as patient's global assessment of disease activity on a scale of 0-10, patient's assessment of pain on a 100-mm Visual Analog Scale (VAS), functional status of the patient using the Health Assessment Questionnaire (HAQ) scored on a scale of 0-3, rheumatoid factor, C-reactive protein and details of treatment history. A 22-joint count (including the metacarpophalangeal joints, the proximal interphalangeal joints, wrists and elbows) was used [20,21]. A patient was classified as a good reactor when both the tender joint count and the swollen joint count were ≥20% improved from baseline after at least six months' therapy plus no less than three of the following criteria were met: VAS ≤20 mm, ≥20% improvement in Erythrocyte Sedimentation Rate (ESR), in both physician's and patient's global assessment of disease activity and in HAQ. Patients received additional DMARDs or biologic agents (e.g: infliximab, etanercept), together with those without weekly regular treating with MTX, were excluded from the study. All patients were prescribed with non-steroidal anti-inflammatory drugs and/or low-dose prednisone (≤10mg/d) at the beginning for providing symptomatic relief. MTX toxicities assessed in this study included gastrointestinal disturbances, liver function abnormalities, leucopenia, alopecia, oral ulceration, malaise and dizziness. These adverse effects were categorized according to the common toxicity criteria of the National Cancer Institute.

All the study participants were from South China. The study was approved by the local ethics committee and written informed consent was obtained from all subjects after a detailed briefing of the purpose and protocol of the study.

Genetic analysis

Venous blood of 2 ml was drawn from the patients and DNA was isolated according to the conventional phenol chloroform method and stored at −20°C for genetic analysis. *RFC1* 80G>A, *MDR1* 3435C>T, *FPGS* rs1544105 G>A, *GGH* - 401C>T, *MTHFR* 677C>T and *MTHFR* 1298A>C were genotyped using Polymerase Chain

Reaction–Restriction Fragment Length Polymorphism (PCR–RFLP) methods [22-24]. Samples confirmed by sequencing were included in each set of genotype assays as controls.

Statistical analysis

Variables were examined for normal distribution using Kolmogorov-Smirnov Test (KS Test). Normally distributed continuous variables were analyzed using Student's T-Test; otherwise a Mann-Whitney U Test was used for variables with skewed distribution. Categorical variables such as gender were compared using χ^2 Test. Haplotypes were reconstructed using PHASE version 2.0.1 (http://stephenslab.uchicago.edu/software.html) [25] and tested for association with the response status and toxicity using χ^2 Test. All the statistical analyses were performed using Statistical Product and Service Solutions (SPSS Inc., Chicago, Illinois, USA) version 21.0 software for windows. $P<0.05$ was considered statistically significant. The reported values of P were two-sided.

Multifactor dimensionality reduction [26], a non parametric and genetic model-free test, was used to test the interactions among genes. This is a powerful and flexible test, which enables detection and characterization of nonlinear interactions among discrete genetic and clinical/environmental attributes. In other words, this algorithm facilitates the simultaneous detection and characterization of multiple genetic loci which may contribute to a clinical trait by reducing the dimensionality of the multilocus data.

Results

Clinical analysis

Among the 113 patients (94 females and 19 males) analyzed in this study and categorized based on their evaluation, 88 were identified as good reactors and 25 as poor ones. 36 patients experienced toxicities of MTX. Demographic and clinical characteristics (sex, age, weight, MTX-duration and dose) of these four groups are presented in Table 1. No significant associations were observed between all the individual demographic factors and MTX response and toxicity using the univariate analysis.

Table 1: Clinical data grouped by treatment response and toxicity.

	Response (n)		Toxicity (n)	
	Good (88)	Poor (25)	With (36)	Without (77)
Sex as a female	73	21	30	64
Age (y) (mean±SD)	43.4±14.5	48.3±15.1	44.7±15.0	45.2±14.5
Weight (kg) (mean±SD)	56.4±12.2	52.9±7.4	54.8±10.5	55.5±11.0
MTX-Duration (m) (mean±SD)	10.6±11.2	6.6±6.7	9.6±10.4	9.4±10.2
Dose (mg) (mean±SD)	14.4±2.3	14.2±2.3	14.3±2.4	14.4±2.3

Genetic analysis

The genotype frequency and allelic frequency distribution of *MDR1*, *RFC1*, *GGH*, *FPGS* and *MTHFR* genes are shown in Table 2. The frequencies of the six SNPs were in Hardy-Weinberg equilibrium. There were no statistic difference of allele frequencies between our study population and Han Chinese in Beijing (data obtained from the Hapmap database, www.hapmap.org). The frequencies of four haplotypes of *MTHFR* gene were 677C-1298A 57.5%, 677C-1298C 19.0%, 677T-1298A 23.0%, 677T-1298C 0.5% respectively. These four haplotypes combined into seven diplotypes, whose frequencies were CC-TA 14.2%, CA-CC 20.3%, CA-TA 29.2%, CA-CA 32.7%, TA-TA 0.9%, CC-CC 1.8%, TA-TC 0.9%, respectively. The last three diplotypes were not investigated in this study because the frequencies were too low.

Table 2: Allelic frequency and genotype frequency distribution of MDR1, RFC1, GGH, FPGS and MTHFR in Chinese Han RA patients.

Gene	SNP	Genotype	Frequency% (n)	Allele	Frequency% (n)
MDR1	3435C>T	CC	40(45)	C	63(142)
		CT	46(52)	T	37(84)
		TT	14(16)		
RFC1	80G>A	GG	24(27)	G	51(115)
		GA	54(61)	A	49(111)
		AA	22(25)		
GGH	-401 C>T	CC	73(82)	C	84(190)
		CT	23(26)	T	16(36)
		TT	4(5)		
FPGS	rs1544105G>A	GG	5(6)	G	25(56)
		GA	39(44)	A	75(170)
		AA	56(63)		
MTHFR	677 C>T	CC	55(62)	C	77(173)
		CT	43(49)	T	23(53)
		TT	2(2)		
MTHFR	1298A>C	AA	63(71)	A	81(182)
		AC	35(40)	C	19(44)
		CC	2(2)		

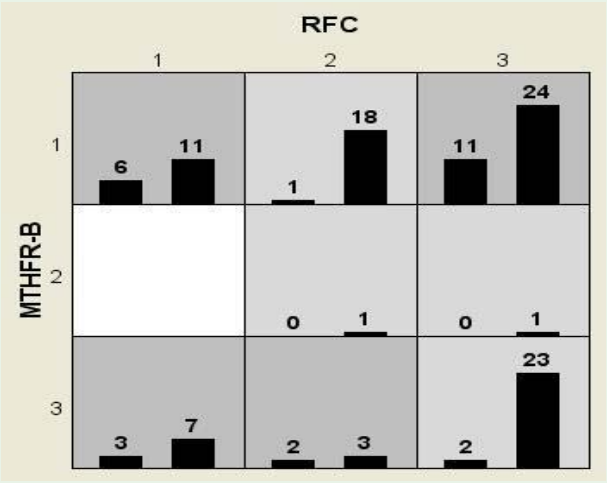

Figure 2: Multifactor dimensionality reduction analysis for MTX efficacy. Summary of two-locus genotype combinations associated with high versus low likelihood of efficacy to MTX. Number of alleles (RFC1 80G>A and MTHFR 1298A>C) was given. Combinations of higher likelihood of efficacy genotypes were depicted as darkly shaded cells, combinations of lower likelihood of efficacy genotype were depicted as lightly shaded cells; empty cells were left blank. The corresponding number of poor responders (left bars) and good responders (right bars) was indicated for each cell.
Note: RFC, RFC1 80G>A; MTHFR-B, MTHFR 1298A>C; number (1, 2, 3) on the abscissa axis and ordinate axis means wild type, homozygote and heterozygote, respectively.

Table 3: Association between single nucleotide polymorphisms and efficacy and toxicity.

Gene	Genotype	RA		Toxicity	
	(n)	Good response (%)	Poor Response (%)	With (%)	Without (%)
MDR1 3435 C>T	C/C(45)	38.6	44.0	36.1	41.6
	C/T(52)	43.2	56.0	55.6	41.6
	T/T(16)	18.2	0	8.3	16.8
		P=0.046			
RFC1 80 G>A	G/G(27)	20.5	36.0	25.0	23.4
	G/A(61)	54.5	52.0	50.0	55.8
	A/A(25)	25.0	12.0	25.0	18.2
GGH -401 C>T	C/C(82)	72.7	72.0	75.0	71.4
	C/T(26)	22.7	24.0	22.2	23.4
	T/T(5)	4.6	4.0	2.8	5.2
FPGS rs1544105 G>A	G/G(6)	5.7	4.0	11.1	2.6
	G/A(44)	37.5	44.0	36.1	40.3
	A/A(63)	56.8	52.0	52.8	57.1
MTHFR 677 C>T	C/C(62)	55.7	52.0	33.4	64.9
	C/T(49)	42.0	48.0	63.9	33.8
	T/T(2)	2.3	0.0	2.7	1.3
				P=0.007	
MTHFR 1298 A>C	A/A(71)	60.2	72.0	72.2	58.4
	A/C(40)	37.5	28.0	27.8	39.0
	C/C(2)	2.3	0.0	0	2.6

Effect of genetic polymorphisms of *MDR1*, *RFC1*, *GGH*, *FPGS* and *MTHFR* on MTX efficacy

Of the six SNPs, statistic association was only found between *MDR1* 3435 C>T and MTX response by the univariate analysis (*P*=0.046) as shown in Table 3. The remission of RA symptoms was achieved in all the *MDR1* 3435TT genotype carriers (16/16), in 73.1% of subjects with 3435CT genotype (38/52), and in 75.6% of patients with 3435CC genotype (34/45). The SNPs in *RFC1*, *GGH*, *FPGS* and *MTHFR* genes did not show any correlations with RA response. None of *MTHFR* diplotypes was found to be associated with MTX response (Data not show).

Effect of genetic polymorphisms of *MDR1*, *RFC1*, *GGH*, *FPGS* and *MTHFR* on MTX toxicity

Significant association was observed between *MTHFR* C677T and MTX toxicity (*P*=0.007). *MTHFR* 677T allele carriers were more susceptible to MTX toxicity (OR:1.897, 95%CI:1.725-2.087), compared to those with CC genotype (see table 3). There were no significant correlation between other SNPs and toxicity. Four diplotypes of *MTHFR* gene (CC-TA, CA-CC, CA-TA, CA-CA) were investigated in our study. Of these diplotypes, patients with CA-TA diplotype were more easy to experience toxicity (*P*=0.007, OR: 2.273, 95%CI: 1.303-3.964) when compared to patients without CA-TA diplotype. No correlation was found between other diplotypes and MTX toxicity (see table 5).

Effect of gene-gene interaction on MTX efficacy and toxicity

Multifactor dimensionality reduction is a non parametric and a model free test which was used to test all patients in our study. We conducted the analysis of the entire data set using imputation software (MDRDT) (http://sourceforge.net/projects/mdr). All 6 variants from the *RFC*, *MDR1*, *FPGS*, *GGH* and *MTHFR* genes were analyzed. Interactions were assessed by Cross-Validation Consistency (CVC)

Table 4: Results from multifactor dimensionality reduction test.

Analysis[a]	Model	Traning	Testing	Sign test	CVC
		accuracy	accuracy	*P*[b]	
1	RFC1 G80A- MTHFR A1298C	0.68	0.67	0.002	10/10

Note: CVC, cross-validation consistency.
a Unpaired analysis.
b1000-fold permutation testing.

Table 5: Association between diplotypes of MTHFR and toxicity.

n=113	diplotypes							
	CC-TA		CA-CC		CA-TA*		CA-CA	
Toxicity	Carriers	Noncarriers	Carriers	Noncarriers	Carriers	Noncarriers	Carriers	Noncarriers
With%	16.7	83.3	11.1	88.9	47.2	52.8	22.2	77.8
without%	13.0	87.0	24.7	75.3	20.8	79.2	37.7	62.3

**P*=0.007

frequency. CVC is the number of times that a particular combination of loci/variables (model) is identified in each possible testing set, with the corresponding *P* value. The model with maximum CVC value is the best one.

The best model was generated by a moderately significant gene-gene interaction between *RFC1* 80G>A and *MTHFR* 1298A>C to the efficacy of MTX with the best overall performances (accuracy of 0.67) and a CVC of 10/10 (*P*=0.002). The graphical model and table showing results of the best model, along with its classification accuracy CVC evaluated by sign test, are given in Figure 2 and Table 4, respectively. As shown in figure 2, genotype combination is labeled as high versus low likelihood of efficacy to MTX. In each box, the left and the right bars represent the number of poor responders and good responders for each combination of genotypes, respectively. If the ratio of poor responders to good responders exceeds a threshold (the ratio of total number of poor responders to total number of good responders in the present analysis), the combination is labeled as high likelihood of response to MTX (dark boxes). For example, interaction of *RFC1* 80GA with *MTHFR* 1298AA fell in the high likelihood category because their ratio (11/24=0.46) was greater than the ratio of the number of poor responders to the number of good responders for the response dataset (25/88=0.28). No correlation was found between gene-gene interaction and MTX toxicity (data not show).

Discussion

Methotrexate, one of the most widely used anti-inflammatory agents, is usually administered orally or parenterally at a weekly low pulse dose in RA therapy. It has been reported that certain SNPs in the genes of the folate pathway affect the efficacy and toxicity of MTX [9]. However, there are few researches on the relationship between these SNPs and MTX efficacy and toxicity in Chinese patients with RA. Thus, in the present study, we have evaluated the effects of various factors, including the SNPs of genes involving in the folate metabolic pathway such as *RFC1*, *MDR1*, *GGH*, *FPGS* and *MTHFR*, on the anti-inflammatory efficacy and toxicity of MTX. This is the first study of the effect of *FPGS*, *GGH* genetic polymorphisms on MTX efficacy, and the interaction of genes effect on MTX efficacy and toxicity is also investigated firstly in Chinese patients with RA.

Among the 6 SNPs analyzed in this study, only *MDR1* rs1045642 (C3435T) (*P*=0.046) was observed to have a marginal association with efficacy. The homozygote (TT) genotype of this marker seemed to confer more possibility of good response in our study patients. This result was consistent with the P-gp function altered by this SNP.

Homozygous for *MDR1* 3435T decreased intestinal P-gp expression [13, 14], which resulted in decreasing of the efflux of MTX. This might lead to more remission of RA made by the higher concentration of MTX and MTXPGs. Our results were consistent with most of previous studies in different ethnic groups. In which, individuals with 3435TT genotype were more likely to have remission of RA compared to patients with the 3435CT and CC genotypes (P<0.05) [13,27].

RFC1 80AA, which causes increased transcriptional activity [12], was reported to be associated with better response [28]. A similar trend was observed in our study (see table 3) by univariate analysis, but the association was not significant, possibly due to inadequate sample size. *FPGS* rs1544105 G>A and *GGH* -401C>T were reported to be with limited helpfulness to MTX therapy [9,22], which was confirmed by our study.

Although not all the independent contribution evaluated in our study was significant to RA response, the results of gene-gene interaction analysis using model-free statistical methods seemed promising. Using the multifactor dimensionality reduction method, a suggestive interaction between polymorphisms of *RFC1* 80G>A and *MTHFR* 1298A>C genes were identified with a CVC of 10/10 (P=0.002). In our study, *RFC1* 80G>A was found to associate with the efficacy of MTX by univariate analysis, but without statistic significance. Some studies had also reported *MTHFR* 1298A>C was associated with MTX efficacy [29,30]. Our results of gene-gene interaction displayed the hidden effect of genes in the folate pathway on MTX efficacy. In essence, the multifactor dimensionality reduction method can capture hidden, discrete, and nonlinear interactions in tightly regulated and intricate networks of genes [31,32]. The analysis revealed that interactions among various SNPs were associated with efficacy of low-dose MTX therapy. This is the first study about gene-gene interaction from the folate pathway of MTX treated on Chinese population with RA. The similar conclusion that gene-gene interactions impact MTX efficacy in RA was also reported by Dervieux [31] and Sharma [22] with the same multifactor dimensionality reduction analyzer, though the results were not totally the same. The discrepancy may be explained by the differences in study population, sample size, therapy plan, study design, etc. A study [33] about breast cancer also reported a significant gene-gene interaction between *RFC1* 80 G>A, *MTHFR* 677 C>T and TYMS (Thymidylate Synthetase) loci showing inflated risk for breast cancer. The interaction result which being similar to our result validated that gene-gene interaction among *RFC1* and *MTHFR* might generate and affect clinical outcome. However, replicate studies and inter-population comparisons are still warranted.

MTHFR 677C>T, a thermolabile variant, was reported to result in decreased enzyme activity and subsequent increased plasma homocysteine levels [18], then leading to toxicity, such as arteriosclerosis, liver function abnormalities, etc. Consistently, a significant association was observed between *MTHFR* 677C>T and MTX toxicity (P=0.007) in our study. Compared to those with CC genotype, patients with *MTHFR* 677T allele were more susceptible to MTX toxicity, which was in line with other studies [34, 35]. About *MTHFR* 1298 A>C, it was found to be associated with reduced activity of the MTHFR enzyme [36]. Some studies reported that *MTHFR* 1298 AA might be a risk factor of MTX toxicity [37-39]. It was possible that lower the MTHFR function produced by *MTHFR* 1298A>C resulted in retention of 5,10-methylenetetrahydrofolate (5-10-CH_2-THF). 5-10-CH_2-THF was beneficial for the synthesis of thymine nucleotides, which might avoid from the occurrence of MTX toxicity in certain extent. Moreover, 677 C>T was reported to be in linkage disequilibrium with 1298 A>C [39]. Some studies demonstrated that subjects with *MTHFR* the 677T-1298A haplotype had a higher frequency of MTX toxicity [39,40]. Similarly, in our study, carriers of CA-TA diplotype which also contains the 677T-1298A haplotype were more easy to experience toxicity than noncarriers. However, since only one patient with TA-TA diplotype was found in our study population, further investigation with a larger sample size that sufficient TA-TA carriers could be enrolled is required to confirm whether 677T-1298A haplotype is a real risk factor for MTX toxicity.

There are some limitations in our study. First, not all genes of the folate pathway are investigated in our study, but only the main important SNPs was explored. The most comprehensive study is warranted. Second, the toxicities discussed in our study were not divided to sub classifications for further study because of the limited sample size.

In summary, this study demonstrated that the *MDR1* 3435C>T might influence the efficacy of RA therapy with MTX and the interaction between *RFC1* 80G>A and *MTHFR* 1298A>C seemed to affect MTX efficacy. The polymorphisms in the *MTHFR* gene were shown to be associated with toxicity of MTX. These results may be useful for individualized therapy of MTX in Chinese RA patients.

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