Are gene polymorphisms related to adverse events of methotrexate in patients with rheumatoid arthritis? A retrospective cohort study based on an updated meta-analysis

Ther Adv Chronic Dis

2020, Vol. 11: 1–17

DOI: 10.1177/2040622320916026

Jing Huang*, Huizhen Fan*, Qi Qiu*, Kunpeng Liu*, Shuang Lv, Jiang Li, Hui Yang, Xiaoming Shu, Yuan Xu, Xiangchen Lu, Cheng Lu, Yunnan Zhang and Cheng Xiao

Correspondence to:
Cheng Xiao
Department of Emergency, China-Japan Friendship Hospital, Beijing, China

Institute of Clinical Medicine, China-Japan Friendship Hospital, Beijing, China

Beijing University of Chinese Medicine, Beijing, China
xc2002812@126.com

Jing Huang
Department of Emergency, China-Japan Friendship Hospital, Beijing, China

School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing, China

Huizhen Fan
Department of Gastroenterology, People's Hospital of Yichun, Jiangxi Yichun, China

Qi Qiu
Yunnan Zhang
Institute of Clinical Pharmacology, Beijing An Zhen Hospital, Capital Medical University, Beijing, China

Kunpeng Liu
Department of Anesthesiology, Peking University International Hospital, Beijing, China

Shuang Lv
Xiangchen Lu
Institute of Clinical Medicine, China-Japan Friendship Hospital, Beijing, China

Beijing University of Chinese Medicine, Beijing, China

Jiang Li
Hui Yang
Department of Laboratory Medicine, China-Japan Friendship Hospital, Beijing, China

Xiaoming Shu
Department of Rheumatology, China-Japan Friendship Hospital, Beijing, China

Abstract

Aims: We performed an updated meta-analysis to verify correlations between gene polymorphisms and adverse events in methotrexate (MTX)-treated rheumatoid arthritis (RA) patients. Then, we conducted a retrospective cohort study of Han Chinese in China.

Methods: Relevant studies were collected from the PubMed database and the EMBASE database until December 2017. Pre-allele, dominant, recessive, codominant, and homozygotic models were applied. In addition, a retrospective cohort study enrolling 162 RA patients treated with MTX was conducted. Single nucleotide polymorphism (SNP) genotyping was analyzed by PCR and product sequencing.

Results: A total of 39 studies were included in 20 meta-analyses; meta-analysis showed a significant association between MTX-related toxicity and 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C>T(rs1801133) polymorphism in East Asian RA patients, and significant associations were observed between MTX-related toxicity and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC) 347C>G (rs2372536), reduced folate carrier 1 (RFC-1) 80G>A (rs1051266), and adenosine triphosphate-binding cassette B1 (ABCB1) 3435C>T(rs1045642) polymorphisms in European RA patients but not in East Asian RA patients. Moreover, in our retrospective cohort study, ATIC 347C>G(rs2372536) and ABCB1 3435C>T(rs1045642) polymorphisms were not associated with MTX-related toxicity. However, a significant association was observed between MTX-related toxicity and RFC-1 80G>A (rs1051266) polymorphism in Chinese Han RA patients.

Conclusion: Evidence-based results suggest that the MTHFR 677C>T(rs1801133), ATIC 347C>G(rs2372536), RFC-1 80G>A (rs1051266), ABCB1 3435C>T(rs1045642) polymorphisms are associated with MTX-related toxicity. Larger and more stringent study designs may provide more accurate findings for the effects of these SNPs on MTX-related toxicity, and larger sample-size studies of the Chinese Han population should be conducted for further validation.

Keywords: adverse events, methotrexate, polymorphism, rheumatoid arthritis

Received: 31 August 2019; revised manuscript accepted: 27 February 2020.

Background

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that leads to progressive disability, systemic complications, early death, and socioeconomic cost.[1] RA affects approximately 1% of the global population,[2] and most of those affected are women. RA is a multifactorial autoimmune disease affected by environmental and genetic factors. Presenting at any age, RA often leads to irreversible deformity.

Yuan Xu
Department of TCM Rheumatology, China-Japan Friendship Hospital, Beijing, China

Cheng Lu
Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences, Beijing, China

*These authors contributed equally to this work and should be considered co-first authors.

Methotrexate (MTX) is a first-line disease-modifying antirheumatic drug (DMARD) for RA. Owing to its efficacy and low cost, both the American College of Rheumatology (ACR)[3] and the European League Against Rheumatism (EULAR)[4] currently recommend low-dose (5–25 mg/week) MTX monotherapy as the initial treatment for patients newly diagnosed with RA. Though MTX is indispensable in the treatment of RA, it is difficult for some patients to use due to adverse reactions or patient comorbidities.[5] About 30–45% of RA patients who receive MTX suffer from adverse events, and 16% of RA patients discontinue MTX due to adverse events.[6] MTX was reported to be associated with various adverse drug events such as hepatotoxicity, renal dysfunction, gastrointestinal dysfunction, pancytopenia, interstitial pneumonia, and increased susceptibility to infection.[7]

MTX is an antagonist of the essential nutrient folic acid and has anti-inflammatory and antiproliferative effects. MTX inhibits the progression of RA, alleviates joint pain and damage, and delays loss of mobility.[8] Interindividual differences in susceptibility to adverse events are suggested to be due to genetic polymorphisms related to the capacity of transporters and enzymes that mediate the biotransformation and accumulation/elimination of MTX in the body.[9] Therefore, identifying predictors of MTX toxicity is important for the management of RA to achieve individualized treatment. Accumulating evidence suggests associations between gene polymorphisms and MTX toxicity in RA patients. A number of meta-analyses,[10–21] focused mostly on 5,10-methylenetetrahydrofolate reductase (MTHFR) single nucle-otide polymorphisms (SNPs) have been conducted to determine whether gene polymorphisms can predict adverse events of MTX therapy in patients with RA. However, the results of these meta-analyses were inconsistent and inconclusive. In 2017, we published a systematic review and meta-analysis to evaluate the correlations between gene polymorphisms and MTX-related toxicity in RA patients under one genetic model. We found a significant association between the toxicity of MTX and the reduced folate carrier 1 (RFC-1) 80G>A (rs1051266) polymorphism in RA patients.[22] Here, we performed an updated meta-analysis to assess the relationships between gene polymorphisms and MTX-related toxicity under various genetic models. In addition, we conducted a retrospective cohort study to further validate these evidence-based findings.

Materials and methods

Updated meta-analysis

Search strategy and study selection. Relevant studies investigating the association between gene polymorphisms and MTX adverse events in RA were collected from the PubMed database and the EMBASE database (OvidSP) until December 2017. The search strategy has been detailed in our previous article.[22] Details of the search flow are summarized in Figure 1. Studies satisfying the following inclusion criteria were included in the meta-analysis: (1) articles investigating the associations between gene polymorphisms and toxicity in RA patients treated with MTX; (2) extractable genotype and allele frequencies to calculate odds ratios (ORs) and 95% confidence intervals (CIs); (3) full-text articles published in English. The major exclusion criteria were: (1) duplicate publications; (2) letter, comments, reviews, meta-analyses, editorials, or conference abstracts; (3) insufficient genotype or allele data for cases with and without adverse events; (4) non-English articles.

Data extraction. References were screened, and all available data were extracted carefully from each study by two of the authors independently using a predetermined data collection template. Potential conflicts were resolved by a third investigator. The following data were included: name of first author, year of publication, country, ethnicity of study population, sample size, numbers of cases with and without adverse events, genotype frequencies of adverse events, study conclusions, toxicity criteria, and adverse events such as overall adverse events and hepatic, central nervous system (CNS), hematological, gastrointestinal, and respiratory adverse events.

Statistical analysis. Gene SNPs detected in more than two studies were included in the meta-analysis. Genotype frequencies of the MTHFR 677C>T (rs1801133), 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC) 347C>G (rs2372536), RFC-1 80G>A (rs1051266), adenosine triphosphate-binding cassette B1 (ABCB1) 3435C>T (rs1045642), MTHFR 1298A>C (rs1801131), MTR2756 A>G (rs1805087), MTRR 66A>G

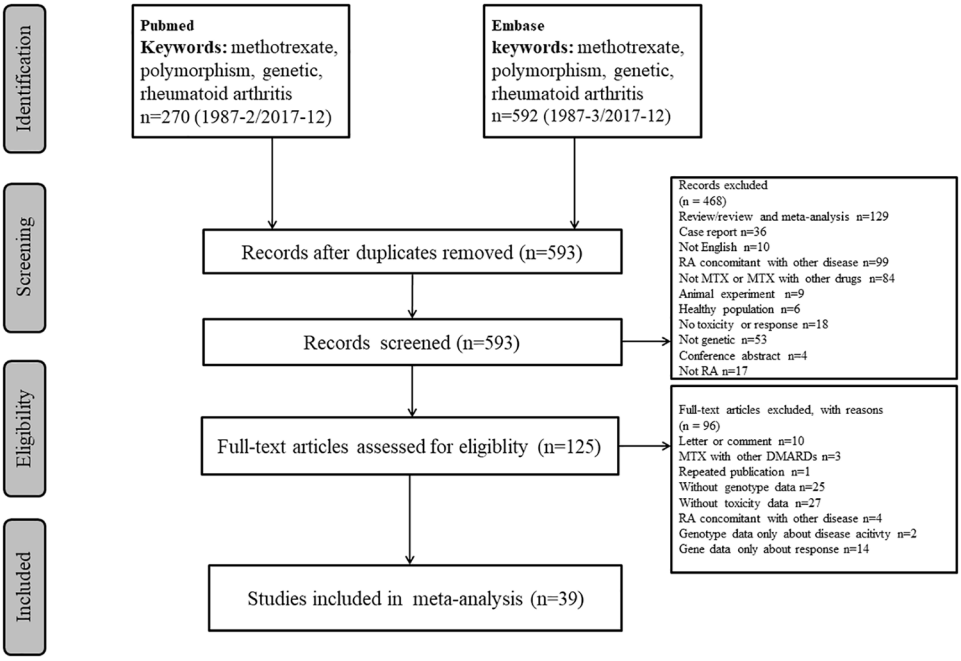

Figure 1. Flow chart summarizing study identification and selection.
DMARD, disease-modifying antirheumatic drug; MTX, methotrexate; RA, rheumatoid arthritis.

(rs1801394), g-folylpolyglutamate synthetase (FPGS) 1994A>G (rs10106), AMPD1 34C>T (rs17602729), ITPA 94C>A (rs1127354), glutamyl hydrolase (GGH)-401C>T (rs3758149), GGH 16T>C (rs1800909), GGH 452C>T (rs11545078), ABCC2 C>T (rs4148396), ABCG2 914C>A (rs2231142), FPGS 2572C>T (rs1544105), thymidylate synthetase (TYMS) 28-bp tandem repeat (rs34743033), ADORA2A 4221164C>T (rs2267076), ADORA2A 24429 543C>T (rs2298383), and MTHFD1 1958G>A (rs2236225) polymorphisms were determined. Five comparison models were analyzed: the pre-allele model (A *versus* a), dominant model (AA *versus* Aa+aa), recessive model (aa *versus* AA+Aa), codominant model (Aa *versus* AA+aa), and homozygotic model (AA *versus* aa), where 'A' refers to the wild-type allele and 'a' refers to a mutated allele. Subgroup analysis according to ethnicity (European, African, and East Asian) was performed. The detailed method of statistical analysis has been described in our previous articles.[22,23]

Retrospective cohort study

Patient recruitment and assessment have been described in detail elsewhere.[24,25] Briefly, RA patients who fulfilled the 1987 RA criteria of the American College of Rheumatology (ACR) were recruited from April 2016 to April 2018 at China–Japan Friendship Hospital and the People's Hospital of Yichun, China. This study was registered on the Chinese Clinical Trial Registry (ChiCTR-RNC-14004887). Informed consent was obtained from all individual participants included in the study, which was approved by the Ethics Committee of the People's Hospital of Yichun (ethics ID: 2014-01).

All patients were treated with MTX orally for at least 3 months. Patients were monitored for MTX-related toxicity by clinical assessment and laboratory investigations. Genomic DNA was isolated from 2 ml ethylenediaminetetraacetic acid (EDTA)-anticoagulated whole blood samples using commercial DNA extraction kits (Qiagen, Hilden, Germany). For each DNA sample, MTHFR 677C>T (rs1801133), RFC-1 80G>A (rs1051266), ATIC 347C>G (rs2372536), and ABCB1 3435C>T (rs1045642) polymorphisms were detected by PCR and product sequencing.

The Hardy-Weinberg equilibrium (HWE) of the patients was calculated by chi-square statistics. All statistical data are described as numbers and frequencies. The mean and standard deviation (±SD) are used to describe sample tests, while the median

and interquartile range (± IQR) are used for non-Gaussian distributed variables. Allele and genotype association analyses with regard to MTX-related adverse events were performed using the χ^2 test. Correlation of the associated SNP and the adverse events was performed by logistic regression analysis. Logistic regression models were built depending on age and gender to estimate adjusted ORs. Allele and genotype risk were assessed using OR and 95% confidence interval (CI) values. $P<0.05$ was regarded as statistically significant. Statistical analysis was carried out using SPSS 20.0 Version (IBM Corporation, Armonk, NY, USA).

Results

Meta-analysis

We conducted an updated meta-analysis to evaluate the association between gene polymorphism and toxicity in MTX-treated RA patients. A total of 862 citations were collected from electronic databases, and, after excluding duplicates, screening titles and abstracts, and reading full articles, a total of 39 articles were included.[8,9,26-62] The study selection process is presented in Figure 1. The main characteristics of studies are summarized in Additional file 1: Table S1.

Overall, 23 studies (including 1433 cases presenting adverse events and 2384 cases without adverse events) were included in the meta-analysis of the MTHFR 677C>T (rs1801133) polymorphism. Regardless of ethnicity, the MTHFR 677C>T (rs1801133) polymorphism was related to presenting adverse events in the recessive model (OR = 1.438, 95% CI: 1.144–1.806, $p = 0.002$). No significant evidence of a relationship between the MTHFR 677C>T (rs1801133) polymorphism and MTX-related toxicity was observed in the pre-allele model, dominant model, codominant model, and homozygotic model (Additional file 2: Table S2). Moreover, significant between-study heterogeneity was observed in the pre-allele ($I^2 = 77.7$, $\chi^2 = 80.64$, $p<0.001$), dominant ($I^2 = 74.2$, $\chi^2 = 85.38$, $p<0.001$), codominant ($I^2 = 50.2$, $\chi^2 = 36.18$, $p = 0.007$), and homozygotic models ($I^2 = 59.7$, $\chi^2 = 42.21$, $p = 0.001$), but not in the recessive model (Additional file 2: Table S2).

In the East Asian population (549 presenting adverse events and 594 without adverse events), the MTHFR 677C>T (rs1801133) polymorphism was associated with the presence of adverse events in the recessive model (OR = 1.698, 95% CI: 1.176–2.451, $p = 0.005$) (Figure 2) but with the absence of adverse events in the dominant model (OR = 0.497, 95% CI: 0.248–0.998, $p = 0.049$) (Figure 3). No significant evidence of a relationship between the MTHFR 677C>T (rs1801133) polymorphism and MTX-related toxicity was observed in the pre-allele model, codominant model and homozygotic model (Additional file 2: Table S2). In addition, no significant association was observed in the European population in any of the five models. (Additional file 2: Table S2).

Seven studies (444 presenting adverse events and 920 without adverse events) were included in the meta-analysis of the ATIC 347C>G (rs2372536) polymorphism. Regardless of ethnicity, the ATIC 347C>G (rs2372536) polymorphism was not related to the presence and/or absence of adverse events in all five genetic models (Additional file 2: Table S2). Moreover, significant between-study heterogeneity was not observed in any of the five models (Additional file 2: Table S2).

By contrast, in the European population (161 presenting adverse events and 457 without adverse events), the ATIC 347C>G (rs2372536) polymorphism was correlated with the absence of adverse events in the pre-allele model (OR = 0.756, 95% CI: 0.575–0.995, $p = 0.046$) (Figure 4) and dominant model (OR = 0.679, 95% CI: 0.468–0.985, $p = 0.041$) (Figure 5) but not in the recessive model, codominant model, or homozygotic model (Additional file 2: Table S2). No significant association was observed in the East Asian population (Additional file 2: Table S2).

For the RFC-1 80G>A (rs1051266) polymorphism, 12 studies (906 presenting adverse events and 1278 without adverse events) were included in the meta-analysis. The RFC-1 80G>A (rs1051266) polymorphism was not related to the presence and/or absence of adverse events in the overall population (Additional file 2: Table S2). Moreover, significant between-study heterogeneity was not observed in all five models (Additional file 2: Table S2).

However, in the European population (503 presenting adverse events and 865 without adverse events), the RFC-1 80G>A (rs1051266) polymorphism was related to the presence of adverse events in the dominant model (OR = 1.352, 95%

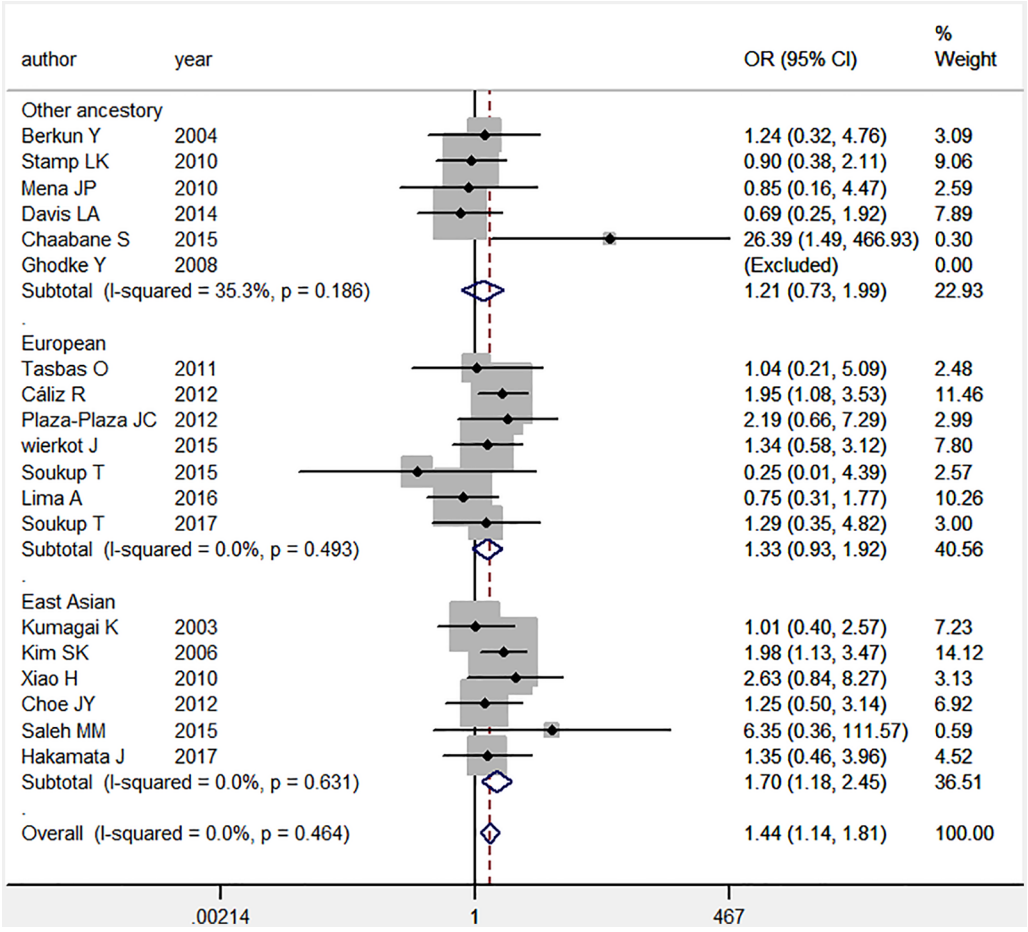

Figure 2. Forest plot showing the association between MTHFR 677C>T (rs1801133) polymorphism and MTX-related toxicity (recessive model).
CI, confidence interval; MTX, methotrexate; OR, odds ratio.

CI: 1.039–1.760, $p=0.025$) (Figure 6) but not the pre-allele model, recessive model, codominant model, or homozygotic model (Additional file 2: Table S2). No significant association was observed in the East Asian population (Additional file 2: Table S2).

For the ABCB1 3435C>T (rs1045642) polymorphism, seven studies (459 presenting adverse events and 586 without adverse events) were included in the meta-analysis. The ABCB1 3435C>T (rs1045642) polymorphism was not related to the presence and/or absence of adverse events in the overall population (Additional file 2: Table S2). Moreover, significant between-study heterogeneity was observed in the dominant ($I^2=66.5$, $\chi^2=17.91$, $p=0.006$), recessive ($I^2=67.8$, $\chi^2=18.62$, $p=0.005$), and codominant models ($I^2=81$, $\chi^2=26.35$, $p<0.001$) but not in other two models (Additional file 2: Table S2).

By contrast, in the European population (92 presenting adverse events and 296 without adverse events), the ABCB1 3435C>T (rs1045642) polymorphism was correlated with the absence of adverse events in the pre-allele model (OR = 0.622, 95% CI: 0.442–0.875, $p=0.006$) (Figure 7) and homozygotic model (OR = 0.389, 95% CI: 0.178–0.851, $p=0.018$) (Figure 8) but not in the dominant model, recessive model, or codominant model (Additional file 2: Table S2). No significant association was observed in the East Asian population (Additional file 2: Table S2).

The MTHFR 1298A>C (rs1801131), MTR2756 A>G (rs1805087), MTRR 66A>G (rs1801394),

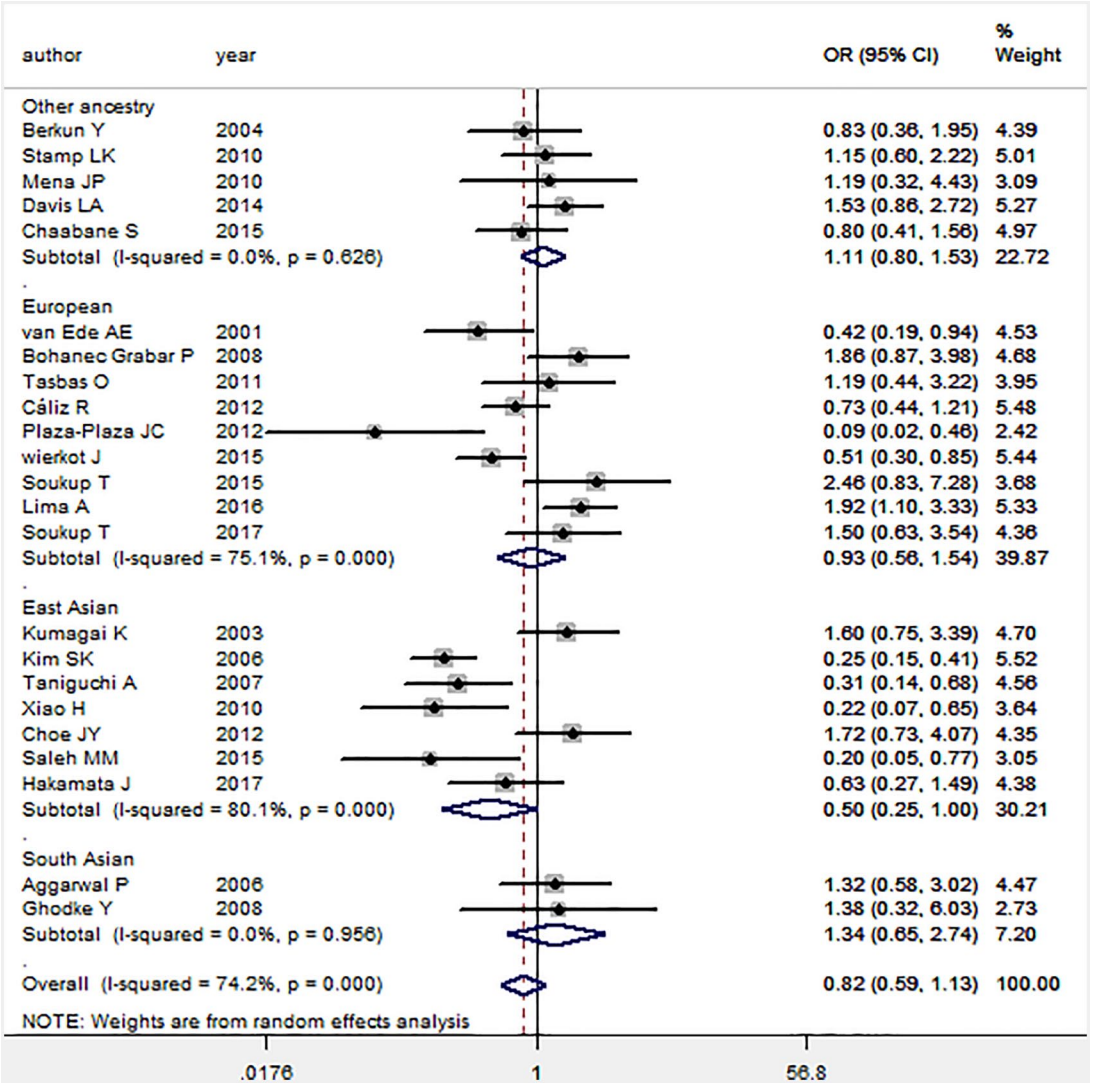

Figure 3. Forest plot showing the association between MTHFR 677C>T (rs1801133) polymorphism and MTX-related toxicity (dominant model).
CI, confidence interval; MTX, methotrexate; OR, odds ratio.

FPGS 1994A>G (rs10106), AMPD1 34C>T (rs17602729), ITPA 94C>A (rs1127354), GGH -401C>T (rs3758149), GGH 16T>C (rs1800 909), GGH 452C>T (rs11545078), ABCC2 C>T (rs4148396), ABCG2 914C>A (rs2231 142),FPGS 2572C>T(rs1544105), TYMS 28-bp tandem repeat (rs34743033), ADORA2A 4221 164C>T (rs2267076), ADORA2A 24429543C>T (rs2298383), and MTHFD1 1958G>A (rs223 6225) polymorphisms were not correlated with the presence and/or absence of adverse events in the overall population, the European population or the East Asian population under five genetic models. (Additional file 2: Table S2).

Characteristics of the study population in the retrospective cohort study

The present study included 162 patients with RA, of which 83.95% were females, treated with MTX. No heterogeneity was detected for any other SNPs in our study. Table 1 summarizes the detailed demographic characteristics of the RA patients.

MTX-induced adverse events were observed in 39 patients (24.07%) during the course of treatment. Gastrointestinal side effects were the most frequent events (27/39 patients, 69.23%) including nausea, diarrhea, stomach pain, abdominal pain, indigestion, and anorexia, followed by

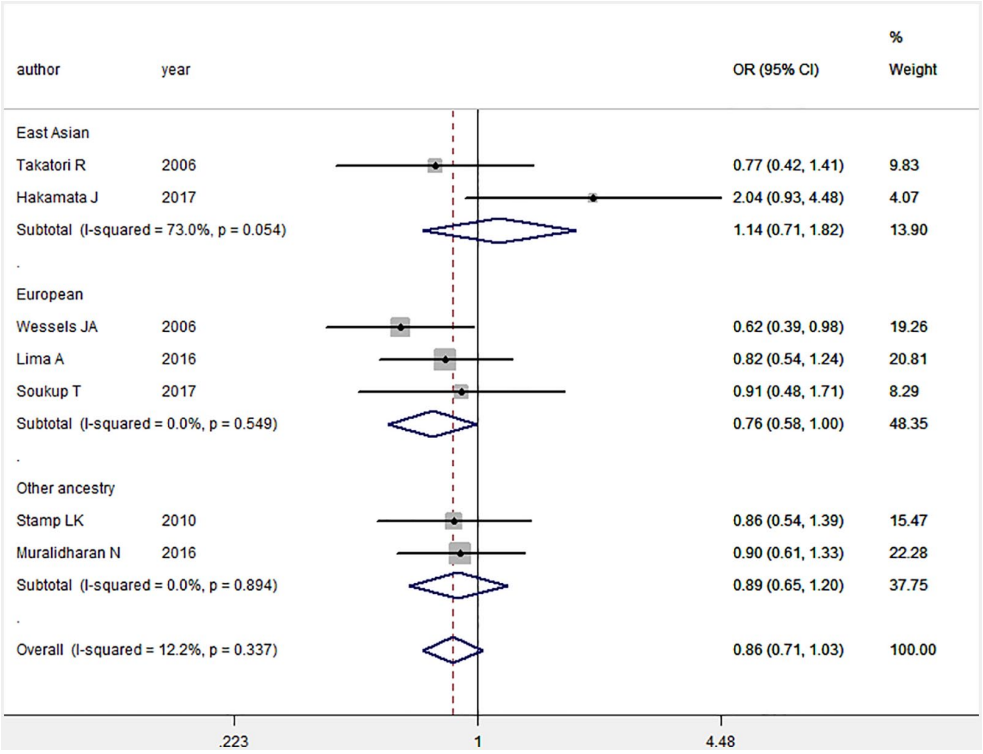

Figure 4. Forest plot showing the association between ATIC 347C>G (rs2372536) polymorphism and MTX-related toxicity (pre-allele model).
CI, confidence interval; MTX, methotrexate; OR, odds ratio.

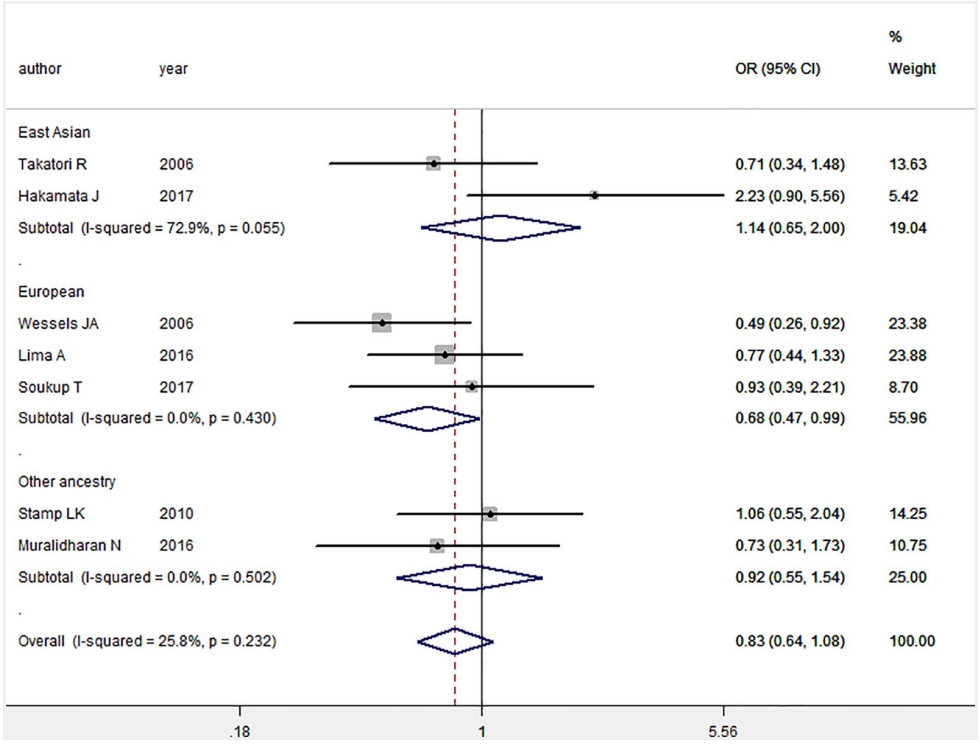

Figure 5. Forest plot showing the association between ATIC 347C>G (rs2372536) polymorphism and MTX-related toxicity (dominant model).
CI, confidence interval; MTX, methotrexate; OR, odds ratio.

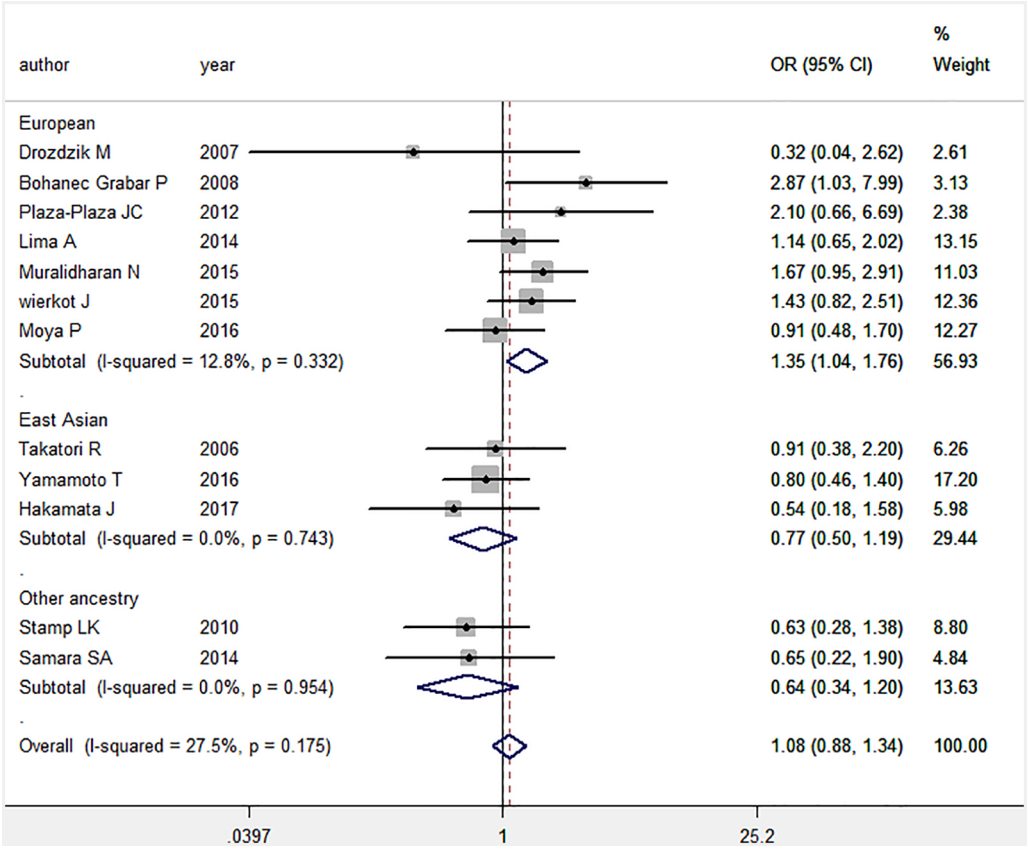

Figure 6. Forest plot showing the association between RFC-1 80G>A (rs1051266) polymorphism and MTX-related toxicity (dominant model).
CI, confidence interval; MTX, methotrexate; OR, odds ratio.

elevations in liver transaminases (12/39 patients, 30.78%). Tiredness (6/39 patients, 15.38%), pain (1/39 patients, 2.56%), alopecia (1/39 patients, 2.56%), erythema of the extremities (1/39 patients, 2.56%), and lung disease (1/39 patients, 2.56%) also occurred.

Associations between gene polymorphisms and MTX adverse events

Table 2 presents a comparison of the distributions of the MTHFR 677C>T (rs1801133), RFC-1 80G>A (rs1051266), ATIC 347C>G (rs2372536) and ABCB1 3435C>T (rs1045642) polymorphisms between the groups of RA patients with and without adverse events. The genotype frequencies of the RA patients conformed to HWE ($p > 0.05$). There was a significant difference in the RFC-1 80G>A (rs1051266) polymorphism distribution between patients with and without adverse events ($p = 0.02$); however, there were no significant differences in the frequency distribution of genotypes and alleles for the MTHFR 677C>T (rs1801133), ATIC 347C>G (rs2372536), and ABCB1 3435C>T (rs1045642) polymorphisms. We were unable to obtain results for MTHFR 677C>T (rs1801133) polymorphism genotyping in one case and ABCB1 3435C>T (rs1045642) polymorphism genotyping in five cases because the 260/280 ratios of these samples were not between 1.8 and 1.9. Finally, the total number of patients analyzed was 161 for the MTHFR 677C>T (rs1801133) polymorphism and 157 for the ABCB1 3435C>T (rs1045642) polymorphism.[24,25]

Discussion

MTX is a structural analog of folic acid that has anti-inflammatory and antiproliferative effects. However, its exact mode of action, predictors of treatment response, and adverse events in RA are not fully understood. Many studies have attempted to identify associations between MTX-related

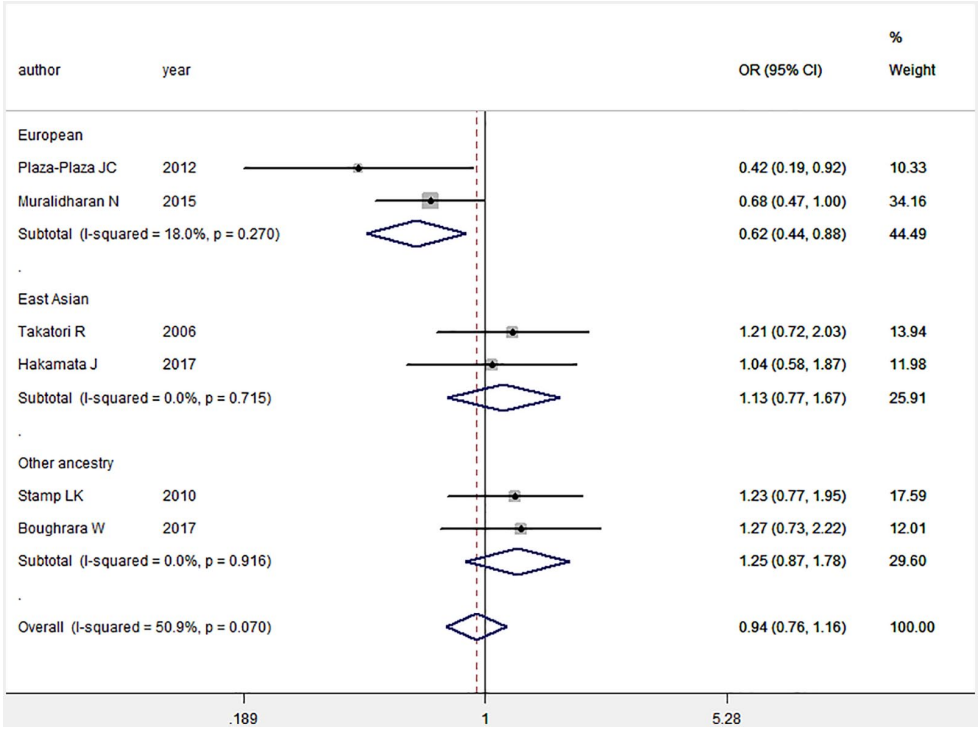

Figure 7. Forest plot showing the association between ABCB1 3435C>T (rs1045642) polymorphism and MTX-related toxicity (pre-allele model).
CI, confidence interval; MTX, methotrexate; OR, odds ratio.

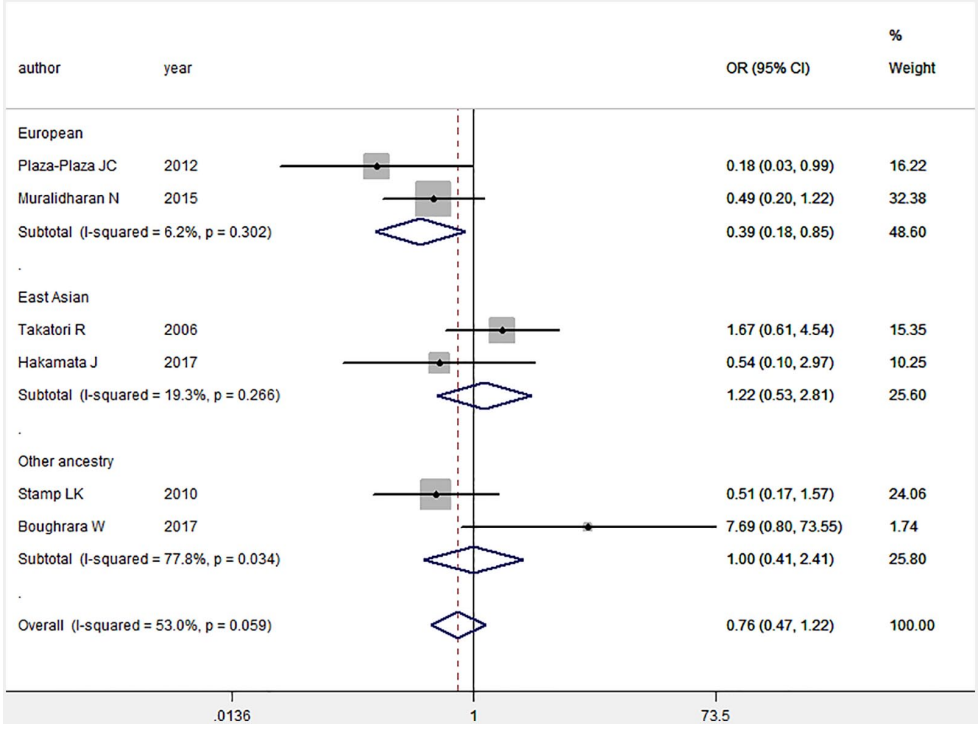

Figure 8. Forest plot showing the association between ABCB1 3435C>T (rs1045642) polymorphism and MTX-related toxicity (homozygotic model).
CI, confidence interval; MTX, methotrexate; OR, odds ratio.

Table 1. Characteristics of the patients in the retrospective cohort study.

	RA patients, *N* = 162	Patients with AEs, *N* = 39
Age (years)	52.99 ± 13.81	55 (48–64)
Female (%)	136 (83.95)	36/3
BMI (kg/m^2)	23.09 (20.70–25.53)	22.86 (21.05–22.45)
Disease duration (years)	4 (2–10)	7 (2.00–13.00)
Dose of MTX (mg/week)	10 (7.5–10)	10.00 (7.50–10.00)
CRP (mg/dl)	1.49 (0.64–3.99)	1.28 (0.80–2.36)
ESR (mm/hour)	30 (16.75–59.50)	25 (18.00–66.00)
RF positive (%)	142 (95.95%)	100%
CCP positive (%)	111 (82.84%)	87.88%
AKA positive (%)	62 (54.87%)	58.62%
ANA positive (%)	73 (58.87%)	66.67%
DAS28-ESR	5.17 ± 1.50	4.97 (4.01–6.25)

AE, adverse event; AKA, antikeratin antibody; ANA, antinuclear antibody; BMI, body mass index; CRP, C-reactive protein; CCP, cyclic peptide containing citrulline; DAS, disease activity score; ESR, erythrocyte sedimentation rate; MTX, methotrexate; RA, rheumatoid arthritis; RF, rheumatoid factor.

adverse events in RA and modifications of genes involved in the MTX mechanism of action, such as RFC-1, also known as the solute carrier family 19 folate transporter member 1 (SLC19A1); ABC, also called multidrug resistance protein 1 (MDR1), which is involved in the MTX membrane transport pathway; MTHFR, which is the most-studied genetic variant; dihydrofolate reductase (DHFR), FPGS, GGH, TYMS, ATIC, and ADORA2A, which are involved in MTX intracellular pathways.

Two SNPs in the MTHFR gene, rs1801133 and rs1801131, are the best known polymorphisms and are associated with a decline in enzyme activity that might be the potential cause of MTX-related toxicity in RA patients.[63] Previous meta-analyses conducted to verify the associations between MTHFR gene polymorphisms and MTX-related adverse events in RA patients have yielded inconsistent results. Fisher *et al.* performed a meta-analysis that showed that the 677C>T polymorphism was associated with increased toxicity (OR 1.71, 95% CI 1.32–2.21, $p < 0.001$), whereas the 1298A>C polymorphism was not (OR 1.12, 95% CI 0.79–1.6, $p = 0.626$).[10] However, a meta-analysis performed by Lee *et al.* showed that neither the 677C>T nor the 1298A>C polymorphism of MTHFR was associated with MTX toxicity in RA, both in all patients and in Asian patients.[11] A meta-analysis performed by Song and colleagues showed that the MTHFR 677C>T (rs1801133) polymorphism was associated with MTX-induced toxicity in RA patients in the East Asian population,[15] which is consistent with our findings.

ATIC is involved in the *de novo* synthesis of purine and converts aminoimidazole carboxamide adenosine ribonucleotide (AICAR) to formyl-AICAR,[64] which inhibits adenosine and AMP deaminase, resulting in increased adenosine and AMP concentrations.[65] MTX is transformed to MTX polyglutamates (MTX-PGs) after entering cells and directly inhibits ATIC,[66] leading to intracellular accumulation of AICAR and extracellular release of adenosine, which produces anti-inflammatory effects.[67] A meta-analysis by Lee *et al.*[19] indicated a significant association between the ATIC 347 GG + GC

Table 2. Frequencies of genotypes in RA patients with and without MTX-induced adverse events.

Genotypes or alleles	With AE (*n* = 39)	Without AE (*n* = 123)	*p* value	OR	95%CI
MTHFR 677C>T rs1801133 genotype					
CC	7 (20.0)	22 (18.03)			
CT	17 (42.5)	73 (59.84)	0.566	0.741	(0.266,2.064)
TT	15 (37.5)	27 (22.13)	0.284	1.814	(0.610,5.393)
Allele					
C	31 (41.25)	117 (47.95)			
T	47 (58.75)	127 (52.05)	0.205		
RFC-1 80G>A rs1051266 genotype					
GG	16 (42.5)	29 (23.58)			
GA	20 (50.0)	69 (56.09)	0.144	2.923	(0.774,11.041)
AA	3 (7.5)	25 (20.33)	0.009	6.523	(1.596,26.655)
Allele					
G	52 (67.5)	127 (51.63)			
A	26 (32.5)	119 (48.37)	0.020		
ATIC 347C>G rs2372536 genotype					
CC	26 (67.5)	71 (62.83)			
CG	12 (30.0)	45 (30.97)	0.423	0.725	(0.330,1.594)
GG	1 (2.5)	7 (6.2)	0.338	0.342	(0.038,3.077)
Allele					
C	64 (82.5)	187 (78.32)			
G	14 (17.5)	59 (21.68)	0.476		
ABCB1 3435C>T rs1045642 genotype					
CC	16 (43.24)	38 (31.67)			
CT	14 (37.84)	54 (45.0)	0.273	0.625	(0.270,1.448)
TT	7 (18.92)	28 (23.33)	0.270	0.561	(0.201,1.568)
Allele					
C	46 (62.16)	130 (54.17)			
T	28 (37.84)	110 (45.83)	0.226		

ABC, adenosine triphosphate-binding cassette; AE, adverse event; ATIC, aminoimidazole-4-carboxamide ribonucleotide transformylase; CI, confidence interval; MTHFR, methylenetetrahydrofolate reductase; MTX, methotrexate; OR, odds ratio; RA, rheumatoid arthritis; RFC-1, reduced folate carrier 1.

genotype and MTX toxicity in Caucasian (OR = 1.741, 95% CI 1.080–2.806, $p = 0.023$) but not in Asian patients, which is consistent with our findings. However, Chen *et al.*[68] found that ATIC 347 C>G was not associated with MTX toxicity in Caucasians.

After entering the body, MTX enters cells *via* the action of RFC, a member of the solute carrier (SLC) family of uptake-type transporters. MTX is transported outside cells by the actions of ABCC1, ABCC2, ABCC3, ABCC4, and ABCB1, which are members of the ABC family of discharge-type transporters.[54] The SLC19A1/RFC-1 80G>A (rs1051266) polymorphism is one of the most well-studied polymorphisms of the SLC/RFC gene. A total of 12 studies included in our meta-analysis investigated the association between MTX treatment toxicity and the SLC19A1/RFC-1 80G>A (rs1051266) polymorphism. The results showed that the SLC19A1/RFC-1 80G>A (rs1051266) polymorphism was associated with MTX treatment toxicity in the European population under the dominant model (OR = 1.352, 95% CI: 1.039–1.760, $p = 0.025$) but not in the East Asian population. However, this finding is not consistent with two previous meta-analyses that found that the SLC19A1/RFC-1 80G>A (rs1051266) polymorphism was not related to MTX treatment toxicity.[16,18] Differences in the inclusion and exclusion criteria are the main reasons for these inconsistent findings. The ABCB1/MDR1 3435C>T (rs1045642) polymorphism is one of the most-studied polymorphisms of the ABCB1 gene, and seven studies included in our meta-analysis investigated the association between MTX-related toxicity and the ABCB1/MDR1 3435C>T (rs1045642) polymorphism. The results showed that ABCB1 3435C>T (rs1045642) was associated with MTX treatment toxicity in the European population under the pre-allele (OR = 0.622, 95% CI: 0.442–0.875, $p = 0.006$) and homozygotic (OR = 0.389, 95% CI: 0.178–0.851, $p = 0.018$) model but not in the East Asian population. Moreover, in 2016, a meta-analysis conducted by Lee *et al.* indicated that MTX toxicity was associated with the ABCB1 3435C>T (rs1045642) polymorphism in RA (TC *versus* TT + CC; OR 0.483, 95% CI 0.259–0.900, $p = 0.022$).[17] Taken together, these findings suggested that the ABCB1 3435C>T (rs1045642) polymorphism might be associated with MTX toxicity in RA.

In this updated meta-analysis, five genetic models were performed to test the association of the polymorphisms and adverse events of MTX, which showed superiority compared with our previous meta-analysis,[22] which using a specific dominant model. Various genetic models ensured some interesting findings were not missed and the results more rigorously demonstrated absence and presence of association. Moreover, different from our previous meta-analysis, in which 31 studies were included in seven meta-analyses, there were 39 articles included in 20 meta-analyses in this updated-analysis. As for the results, different from our previous study, significant associations were observed between the MTHFR (677C > T (rs1801133) and MTX toxicity in East Asian RA patients, and ATIC 347C > G (rs2372536) and ABCB1 3435C > T (rs1045642) in European RA patients in this updated meta-analysis, and FPGS 1994A>G (rs10106), AMPD1 34C>T (rs17602729), ITPA 94C>A (rs1127354), GGH -401C>T (rs3758149), GGH 16T>C (rs1800909), GGH 452C>T (rs11545078), ABCC2 C>T (rs4148396), ABCG2 914C>A (rs2231142),FPGS 2572C>T(rs1544105), TYMS 28-bp tandem repeat (rs34743033), ADORA2A 4221164C>T (rs2267076), ADORA2A 24429543C>T (rs2298383) and MTHFD1 1958G>A (rs2236225) polymorphisms were not associated with the presence and/or absence of adverse events in the overall population, the European population, or the East Asian population.

As mentioned above, when stratification analysis was conducted by ethnicity, we observed that the ABCB1 3435C>T (rs1045642)polymorphism and ATIC 347C>G (rs2372536) polymorphism were not associated with MTX-related toxicity in the East Asian population of RA patients, and the results of our retrospective cohort study were consistent with these findings. However, the results of our retrospective cohort study showed that the MTHFR 677C>T (rs1801133) polymorphism was not associated with MTX-induced toxicity, but RFC-1 80G>A (rs1051266) polymorphism was associated with MTX-induced toxicity, which was not consistent with the evidence-based findings derived from our meta-analysis.

Although we conducted a meta-analysis and obtained convincing evidence-based results that were also validated by a retrospective study, certain limitations of our study should be noted.

First, only studies published in English were included, which may result in potential study selection bias. Second, publication bias could have distorted our meta-analysis because of the small number of included studies. We included 23, 20, 7, 5, 4, 12, 7, 4, 4, and 3 studies in the meta-analyses of the MTHFR 677C>T (rs1801133), MTHFR 1298A>C (rs1801131), ATIC 347C>G (rs2372536), MTR 2756A>G (rs1805087), MTRR 66A>G (rs1801394), RFC-1 80G>A (rs1051266), ABCB1 3435C>T (rs1045642), FPGS 1994A>G (rs10106), AMPD1 34C>T (rs17602729), and ITPA 94C>A (rs1127354) polymorphisms, respectively, and 2 studies in the meta-analyses of the GGH -401C>T (rs3758149), GGH 16T>C (rs1800909), GGH 452C>T (rs11545078), ABCC2 C>T (rs4148396), ABCG2 914C>A (rs2231142), FPGS 2572C>T (rs1544105), TYMS 28-bp tandem repeat (rs34743033), ADORA2A 4221164C>T (rs2267076), ADORA2A 24429543C>T (rs2298383), and MTHFD1 1958G>A (rs2236225) polymorphisms. Third, heterogeneity and confounding factors may have affected the meta-analysis. Variables such as study design, sex, rheumatoid factor status, disease duration, dose of MTX, folic acid supplementation, limited number of tested SNPs, and even patient's medical files all have the potential influence to this analysis. Fourth, for the retrospective cohort study, we performed a genetic analysis only of the MTHFR 677C>T (rs1801133), ATIC 347C>G (rs2372536), RFC-1 80G>A (rs1051266), and ABCB1 3435C>T (rs1045642) polymorphisms in a Chinese Han population, and the sample size was also limited.

The major issue surrounding low-dose MTX pharmacogenetics is the failure of reported associations to survive replication in one or more independent cohorts. Nonreplication across studies is attributed largely to insufficient statistical power and an array of pharmacological and clinical confounders.[69] In this up-dated meta-analysis, a total of 39 pharmacogenetic studies on low-dose MTX were included, and most of them (23/39) had less than 200 RA patients (Additional file 1: Table S1), and therefore have limited power to detect the effect sizes routinely reported. Statistical power is then further reduced by the requirement to adjust for multiple testing,[26] but, besides that, variability in the outcome definitions and the heterogeneity of the cohorts, limited number of tested SNPs, small effect for the selected variant, and a lack of consideration of epigenetic factors, may contribute to the inconsistency observed. The use of more powerful technologies, including proteomics and transcriptomic studies, genome-wide association studies (GWAS), together with epigenetic factors, large samples and high-quality associated clinical data, will be essential to detect more genetic factors responsible for variability in MTX responses.[70]

Conclusion

In summary, our updated meta-analysis showed that one SNP in the MTHFR gene, rs1801133, was significantly associated with MTX-related adverse events in the East Asian population, while one SNP in the ATIC gene, rs2372536; one SNP in the RFC-1 gene, rs1051266; and one SNP in the ABCB1 gene, rs1045642; were significantly associated with MTX-related adverse events in the European population. However, the retrospective cohort study indicated that MTHFR 677C>T (rs1801133) polymorphism was not associated with MTX-induced toxicity, but RFC-1 80G>A (rs1051266) polymorphism was associated with MTX-induced toxicity in the Chinese Han population, which is inconsistent with the results of our meta-analysis. Although meta-analysis is a powerful tool for evaluating and summarizing knowledge in a research field through statistical instruments, it can also be limited by confounding factors. All of these findings may require well-designed studies with larger sample sizes for further validation.

Acknowledgements

We thank our colleagues at China-Japan Friendship Hospital, Beijing University of Chinese Medicine and the People's Hospital of Yichun. We thank the staff who helped us during the study.

Author contributions

Design: CX; Searches: JH, QQ, HZF, and QPL; Study appraisal: JH, QQ, and CX; Data extraction: JH, QQ, and XCL; Clinical research: JH, HF, SL, XS, and YX; Experimental research: JH, SL, JL, CL, and HY; Data analysis: SL, JH, XCL and CX; Writing: JH and CX. Revised manuscript: JH, CX, and YNZ. All authors read and approved the final manuscript.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was funded by the International Cooperation Project of the Ministry of Science and Technology (Project ID: 2014DFA31490).

Conflict of interest statement

The authors declare that there is no conflict of interest.

ORCID iD

Cheng Xiao https://orcid.org/0000-0002-5601-9670

Supplemental material

Supplemental material for this article is available online.

References

1. McInnes IB and Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med* 2011; 365: 2205–2219.
2. McInnes IB and Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. *Lancet* 2017; 389: 2328–2337.
3. Singh JA, Saag KG, Bridges SL Jr, *et al*. 2015 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis. *Arthritis Care Res (Hoboken)* 2016; 68: 1–25.
4. Smolen JS, Landewe R, Bijlsma J, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann Rheum Dis* 2017; 76: 960–977.
5. Kaneko A. Tocilizumab in rheumatoid arthritis: efficacy, safety and its place in therapy. *Ther Adv Chronic Dis* 2013; 4: 15–21.
6. Lopez-Olivo MA, Siddhanamatha HR, Shea B, *et al*. Methotrexate for treating rheumatoid arthritis. *Cochrane Database Syst Rev* 2014; (6): Cd000957.
7. Shaikh N, Sardar M and Raj R. A rapidly fatal case of low-dose methotrexate toxicity. *Case Rep Med* 2018; 2018: 9056086.
8. Boughrara W, Benzaoui A, Aberkane M, *et al*. No correlation between MTHFR c.677 C > T, MTHFR c.1298 A > C, and ABCB1 c.3435 C > T polymorphisms and methotrexate therapeutic outcome of rheumatoid arthritis in West Algerian population. *Inflamm Res* 2017; 66: 505–513.
9. Hakamata J, Hashiguchi M, Kaneko Y, *et al*. Risk factors for abnormal hepatic enzyme elevation by methotrexate treatment in patients with rheumatoid arthritis: a hospital based-cohort study. *Mod Rheumatol* 2018; 28: 611–620.
10. Fisher MC and Cronstein BN. Metaanalysis of methylenetetrahydrofolate reductase (MTHFR) polymorphisms affecting methotrexate toxicity. *J Rheumatol* 2009; 36: 539–545.
11. Lee YH and Song GG. Associations between the C677T and A1298C polymorphisms of MTHFR and the efficacy and toxicity of methotrexate in rheumatoid arthritis: a meta-analysis. *Clin Drug Investig* 2010; 30: 101–108.
12. Spyridopoulou KP, Dimou NL, Hamodrakas SJ, *et al*. Methylene tetrahydrofolate reductase gene polymorphisms and their association with methotrexate toxicity: a meta-analysis. *Pharmacogenet Genomics* 2012; 22: 117–133.
13. Owen SA, Lunt M, Bowes J, *et al*. MTHFR gene polymorphisms and outcome of methotrexate treatment in patients with rheumatoid arthritis: analysis of key polymorphisms and meta-analysis of C677T and A1298C polymorphisms. *Pharmacogenomics J* 2013; 13: 137–147.
14. Morgan MD, Al-Shaarawy N, Martin S, *et al*. MTHFR functional genetic variation and methotrexate treatment response in rheumatoid arthritis: a meta-analysis. *Pharmacogenomics* 2014; 15: 467–475.
15. Song GG, Bae SC and Lee YH. Association of the MTHFR C677T and A1298C polymorphisms with methotrexate toxicity in rheumatoid arthritis: a meta-analysis. *Clin Rheumatol* 2014; 33: 1715–1724.
16. Kung TN, Dennis J, Ma Y, *et al*. RFC1 80G>A is a genetic determinant of methotrexate efficacy in rheumatoid arthritis: a human genome epidemiologic review and meta-analysis of observational studies. *Arthritis Rheumatol* 2014; 66: 1111–1120.
17. Lee YH, Bae SC and Song GG. Association of the ABCB1 C3435T polymorphism with responsiveness to and toxicity of DMARDs in rheumatoid arthritis: a meta-analysis. *Z Rheumatol* 2016; 75: 707–715.
18. Li X, Hu M, Li W, *et al*. The association between reduced folate carrier-1 gene 80G/A polymorphism and methotrexate efficacy or methotrexate related-toxicity in rheumatoid

arthritis: a meta-analysis. *Int Immunopharmacol* 2016; 38: 8–15.

19. Lee YH and Bae SC. Association of the ATIC 347 C/G polymorphism with responsiveness to and toxicity of methotrexate in rheumatoid arthritis: a meta-analysis. *Rheumatol Int* 2016; 36: 1591–1599.
20. Shao W, Yuan Y and Li Y. Association between MTHFR C677T polymorphism and methotrexate treatment outcome in rheumatoid arthritis patients: a systematic review and meta-analysis. *Genet Test Mol Biomarkers* 2017; 21: 275–285.
21. Fan H, Li Y, Zhang L, *et al*. Lack of association between MTHFR A1298C polymorphism and outcome of methotrexate treatment in rheumatoid arthritis patients: evidence from a systematic review and meta-analysis. *Int J Rheum Dis* 2017; 20: 526–540.
22. Qiu Q, Huang J, Lin Y, *et al*. Polymorphisms and pharmacogenomics for the toxicity of methotrexate monotherapy in patients with rheumatoid arthritis: a systematic review and meta-analysis. *Medicine (Baltimore)* 2017; 96: e6337.
23. Qiu Q, Huang J, Shu X, *et al*. Polymorphisms and pharmacogenomics for the clinical efficacy of methotrexate in patients with rheumatoid arthritis: a systematic review and meta-analysis. *Sci Rep* 2017; 7: 44015.
24. Lv S, Fan H, Li J, *et al*. Genetic Polymorphisms of TYMS, MTHFR, ATIC, MTR, and MTRR are related to the outcome of methotrexate therapy for rheumatoid arthritis in a Chinese population. *Front Pharmacol* 2018; 9: 1390.
25. Lv S, Fan H, Yang H, *et al*. Membrane-spanning protein genetic polymorphisms related to methotrexate therapeutic outcomes in a chinese rheumatoid arthritis population. *J Clin Pharmacol* 2019; 59: 1471–1476.
26. Muralidharan N, Gulati R, Misra DP, *et al*. Nonassociation of homocysteine gene polymorphisms with treatment outcome in South Indian Tamil Rheumatoid Arthritis patients. *Clin Exp Med* 2018; 18: 101–107.
27. Muralidharan N, Misra DP, Jain VK, *et al*. Effect of thymidylate synthase (TYMS) gene polymorphisms with methotrexate treatment outcome in south Indian Tamil patients with rheumatoid arthritis. *Clin Rheumatol* 2017; 36: 1253–1259.
28. Soukup T, Dosedel M, Nekvindova J, *et al*. The plausible association of MTHFR and ADORA2A polymorphisms with nodules in rheumatoid arthritis patients treated with methotrexate. *Pharmacogenet Genomics* 2017; 27: 43–50.
29. Yamamoto T, Shikano K, Nanki T, *et al*. Folylpolyglutamate synthase is a major determinant of intracellular methotrexate polyglutamates in patients with rheumatoid arthritis. *Sci Rep* 2016; 6: 35615.
30. Lima A, Bernardes M, Azevedo R, *et al*. Moving toward personalized medicine in rheumatoid arthritis: SNPs in methotrexate intracellular pathways are associated with methotrexate therapeutic outcome. *Biomed Res Int* 2016; 17: 1649–1674.
31. Moya P, Salazar J, Arranz MJ, *et al*. Methotrexate pharmacokinetic genetic variants are associated with outcome in rheumatoid arthritis patients. *Pharmacogenomics* 2016; 17: 25–29.
32. Muralidharan N, Mariaselvam CM, Jain VK, *et al*. ATIC 347C>G gene polymorphism may be associated with methotrexate-induced adverse events in south Indian Tamil rheumatoid arthritis. *Pharmacogenomics* 2016; 17: 241–248.
33. Chaabane S, Marzouk S, Akrout R, *et al*. Genetic determinants of methotrexate toxicity in tunisian patients with rheumatoid arthritis: a study of polymorphisms involved in the MTX metabolic pathway. *Eur J Drug Metab Pharmacokinet* 2016; 41: 385–393.
34. Muralidharan N, Antony PT, Jain VK, *et al*. Multidrug resistance 1 (MDR1) 3435C>T gene polymorphism influences the clinical phenotype and methotrexate-induced adverse events in South Indian Tamil rheumatoid arthritis. *Eur J Clin Pharmacol* 2015; 71: 959–965.
35. Muralidharan N, Mariaselvam CM, Mithun CB, *et al*. Reduced folate carrier-1 80G > A gene polymorphism is not associated with methotrexate treatment response in South Indian Tamils with rheumatoid arthritis. *Clin Rheumatol* 2016; 35: 879–885.
36. Swierkot J, Slezak R, Karpinski P, *et al*. Associations between single-nucleotide polymorphisms of RFC-1, GGH, MTHFR, TYMS, and TCII genes and the efficacy and toxicity of methotrexate treatment in patients with rheumatoid arthritis. *Pol Arch Med Wewn* 2015; 125: 152–161.
37. Saleh MM, Irshaid YM and Mustafa KN. Methylene tetrahydrofolate reductase genotypes frequencies: association with toxicity and response to methotrexate in rheumatoid arthritis

patients. *Int J Clin Pharmacol Ther* 2015; 53: 154–162.

38. Soukup T, Dosedel M, Pavek P, *et al*. The impact of C677T and A1298C MTHFR polymorphisms on methotrexate therapeutic response in East Bohemian region rheumatoid arthritis patients. *Rheumatol Int* 2015; 35: 1149–1161.

39. Lima A, Seabra V, Bernardes M, *et al*. Role of key TYMS polymorphisms on methotrexate therapeutic outcome in portuguese rheumatoid arthritis patients. *PLoS One* 2014; 9: e108165.

40. Lima A, Bernardes M, Sousa H, *et al*. SLC19A1 80G allele as a biomarker of methotrexate-related gastrointestinal toxicity in Portuguese rheumatoid arthritis patients. *Immunol Cell Biol* 2014; 15: 807–820.

41. Davis LA, Polk B, Mann A, *et al*. Folic acid pathway single nucleotide polymorphisms associated with methotrexate significant adverse events in United States veterans with rheumatoid arthritis. *Clin Exp Rheumatol* 2014; 32: 324–332.

42. Samara SA, Irshaid YM and Mustafa KN. Association of MDR1 C3435T and RFC1 G80A polymorphisms with methotrexate toxicity and response in Jordanian rheumatoid arthritis patients. *Int J Clin Pharmacol Ther* 2014; 52: 746–755.

43. Caliz R, del Amo J, Balsa A, *et al*. The C677T polymorphism in the MTHFR gene is associated with the toxicity of methotrexate in a Spanish rheumatoid arthritis population. *Scand J Rheumatol* 2012; 41: 10–14.

44. Plaza-Plaza JC, Aguilera M, Canadas-Garre M, *et al*. Pharmacogenetic polymorphisms contributing to toxicity induced by methotrexate in the southern Spanish population with rheumatoid arthritis. *OMICS* 2012; 16: 589–595.

45. Choe JY, Lee H, Jung HY, *et al*. Methylenetetrahydrofolate reductase polymorphisms, C677T and A1298C, are associated with methotrexate-related toxicities in Korean patients with rheumatoid arthritis. *Rheumatol Int* 2012; 32: 1837–1842.

46. Tasbas O, Borman P, Gurhan Karabulut H, *et al*. The frequency of A1298C and C677T polymorphisms of the methylentetrahydrofolate gene in Turkish Patients with rheumatoid arthritis: relationship with methotrexate toxicity. *Open Rheumatol J* 2011; 5: 30–35.

47. Xiao H, Xu J, Zhou X, *et al*. Associations between the genetic polymorphisms of MTHFR and outcomes of methotrexate treatment in rheumatoid arthritis. *Clin Exp Rheumatol* 2010; 28: 728–733.

48. Stamp LK, Chapman PT, O'Donnell JL, *et al*. Polymorphisms within the folate pathway predict folate concentrations but are not associated with disease activity in rheumatoid arthritis patients on methotrexate. *Pharmacogenet Genomics* 2010; 20: 367–376.

49. Bohanec Grabar P, Logar D, Lestan B, *et al*. Genetic determinants of methotrexate toxicity in rheumatoid arthritis patients: a study of polymorphisms affecting methotrexate transport and folate metabolism. *Eur J Clin Pharmacol* 2008; 64: 1057–1068.

50. Ghodke Y, Chopra A, Joshi K, *et al*. Are Thymidylate synthase and Methylene tetrahydrofolate reductase genes linked with methotrexate response (efficacy, toxicity) in Indian (Asian) rheumatoid arthritis patients? *Clin Rheumatol* 2008; 27: 787–789.

51. Berkun Y, Abou Atta I, Rubinow A, *et al*. 2756GG genotype of methionine synthase reductase gene is more prevalent in rheumatoid arthritis patients treated with methotrexate and is associated with methotrexate-induced nodulosis. *J Rheumatol* 2007; 34: 1664–1669.

52. Drozdzik M, Rudas T, Pawlik A, *et al*. Reduced folate carrier-1 80G>A polymorphism affects methotrexate treatment outcome in rheumatoid arthritis. *Pharmacogenomics J* 2007; 7: 404–407.

53. Taniguchi A, Urano W, Tanaka E, *et al*. Validation of the associations between single nucleotide polymorphisms or haplotypes and responses to disease-modifying antirheumatic drugs in patients with rheumatoid arthritis: a proposal for prospective pharmacogenomic study in clinical practice. *Pharmacogenet Genomics* 2007; 17: 383–390.

54. Takatori R, Takahashi KA, Tokunaga D, *et al*. ABCB1 C3435T polymorphism influences methotrexate sensitivity in rheumatoid arthritis patients. *Clin Exp Rheumatol* 2006; 24: 546–554.

55. Aggarwal P, Naik S, Mishra KP, *et al*. Correlation between methotrexate efficacy & toxicity with C677T polymorphism of the methylenetetrahydrofolate gene in rheumatoid arthritis patients on folate supplementation. *Indian J Med Res* 2006; 124: 521–526.

56. Wessels JA, Kooloos WM, De Jonge R, *et al*. Relationship between genetic variants in the adenosine pathway and outcome of methotrexate treatment in patients with recent-onset rheumatoid arthritis. *Arthritis Rheum* 2006; 54: 2830–2839.

57. Wessels JA, de Vries-Bouwstra JK, Heijmans BT, *et al*. Efficacy and toxicity of methotrexate

in early rheumatoid arthritis are associated with single-nucleotide polymorphisms in genes coding for folate pathway enzymes. *Arthritis Rheum* 2006; 54: 1087–1095.

58. Kim SK, Jun JB, El-Sohemy A, *et al.* Cost-effectiveness analysis of MTHFR polymorphism screening by polymerase chain reaction in Korean patients with rheumatoid arthritis receiving methotrexate. *J Rheumatol* 2006; 33: 1266–1274.
59. Berkun Y, Levartovsky D, Rubinow A, *et al.* Methotrexate related adverse effects in patients with rheumatoid arthritis are associated with the A1298C polymorphism of the MTHFR gene. *Ann Rheum Dis* 2004; 63: 1227–1231.
60. Kumagai K, Hiyama K, Oyama T, *et al.* Polymorphisms in the thymidylate synthase and methylenetetrahydrofolate reductase genes and sensitivity to the low-dose methotrexate therapy in patients with rheumatoid arthritis. *Int J Mol Med* 2003; 11: 593–600.
61. van Ede AE, Laan RF, Blom HJ, *et al.* The C677T mutation in the methylenetetrahydrofolate reductase gene: a genetic risk factor for methotrexate-related elevation of liver enzymes in rheumatoid arthritis patients. *Arthritis Rheum* 2001; 44: 2525–2530.
62. Mena JP, Salazar-Paramo M, Gonzalez-Lopez L, *et al.* Polymorphisms C677T and A1298C in the MTHFR gene in Mexican patients with rheumatoid arthritis treated with methotrexate: implication with elevation of transaminases. *Pharmacogenomics J* 2011; 11: 287–291.
63. Te Loo DM, Hagleitner MM and Coenen MJ. Is there a role for the MTHFR 677C>T and 1298A>C polymorphisms in methotrexate-induced liver toxicity? *Pharmacogenomics* 2014; 15: 1401–1403.
64. Klein TE, Chang JT, Cho MK, *et al.* Integrating genotype and phenotype information: an overview of the PharmGKB project. Pharmacogenetics Research Network and Knowledge Base. *Pharmacogenomics J* 2001; 1: 167–170.
65. Chan ES and Cronstein BN. Methotrexate–how does it really work? *Nat Rev Rheumatol* 2010; 6: 175–178.
66. Dervieux T, Furst D, Lein DO, *et al.* Polyglutamation of methotrexate with common polymorphisms in reduced folate carrier, aminoimidazole carboxamide ribonucleotide transformylase, and thymidylate synthase are associated with methotrexate effects in rheumatoid arthritis. *Arthritis Rheum* 2004; 50: 2766–2774.
67. Riksen NP, Barrera P, van den Broek PH, *et al.* Methotrexate modulates the kinetics of adenosine in humans in vivo. *Ann Rheum Dis* 2006; 65: 465–470.
68. Chen Y, Zou K, Sun J, *et al.* Are gene polymorphisms related to treatment outcomes of methotrexate in patients with rheumatoid arthritis? A systematic review and meta-analysis. *Pharmacogenomics* 2017; 18: 175–195.
69. Stamp LK and Roberts RL. Effect of genetic polymorphisms in the folate pathway on methotrexate therapy in rheumatic diseases. *Pharmacogenomics* 2011; 12: 1449–1463.
70. Tarnowski M, Paradowska-Gorycka A, Dabrowska-Zamojcin E, *et al.* The effect of gene polymorphisms on patient responses to rheumatoid arthritis therapy. *Expert Opin Drug Metab Toxicol* 2016; 12: 41–55.