Medicine®

OPEN

Polymorphisms and pharmacogenomics for the toxicity of methotrexate monotherapy in patients with rheumatoid arthritis

A systematic review and meta-analysis

Qi Qiu, PhD[a], Jing Huang, BS[b,c], Yang Lin, PhD[a], Xiaoming Shu, PhD[d], Huizheng Fan, MS[e], Zhihua Tu, BS[f], Youwen Zhou, PhD[g,h], Cheng Xiao, PhD[b,*]

Abstract

Background: Methotrexate (MTX) is widely used and considered a first-line disease modifying antirheumatic drug (DMARD) for the treatment of rheumatoid arthritis (RA). However, 10% to 30% of patients discontinue therapy within a year of starting the treatment, usually because of undesirable side effects. Many of the relevant genes have been investigated to estimate the association between gene polymorphisms and MTX toxicity in RA patients, although inconsistent results have been reported.

Methods: We searched EMBASE and PubMed in February 2016 for polymorphisms and pharmacogenomics study of the toxicity of MTX monotherapy in RA patients. The meta-analysis was stratified by whether genetic variants associated with MTX toxicity.

Results: A total of 42 publications that included 28 genes with 88 gene SNPs associated with the transporters, enzymes, and metabolites of MTX or the progression of RA were included in the SR, and 31 studies were included in 7 meta-analyses. The meta-analysis showed a significant association between the toxicity of MTX and the RFC-1 80G > A (rs1051266) polymorphism in the European RA patients.

Conclusion: RFC-1 80G > A (rs1051266) polymorphism was associated with MTX toxicity, and larger and more stringent study designs may provide more accurate results for the effect of these SNPs on the MTX toxicity.

Abbreviations: ABC = adenosine triphosphate-binding cassette, ADA = adenosine deaminase, ADP = adenosine diphosphate, AE = adverse event, AICAR = 5-aminoimidazole-4-carboxamide ribonucleotide, AMP = adenosine monophosphate, ATIC = aminoimidazole-4-carboxamide ribonucleotide transformylase, ATP = adenosine triphosphate, bDMARD = biologic disease-modifying antirheumatic drug, CBS = cystathionine-β-synthase, CCND1 = cyclin D1, CI = confidence interval, CL = cystathionine lyase, DHF = dihydrofolate, DHFR = dihydrofolate reductase, DMARDs = disease-modifying antirheumatic drugs, dTMP = deoxythymidine-5′-monophosphate, dUMP = deoxyuridine-5′-monophosphate, FAICAR = 10-formyl-AICAR, FPGS = folylpoly-glutamyl synthase, GGH = glutamyl hydrolase, IMP = inosine monophosphate, IMPDH2 = inosine 5′-monophosphate dehydrogenase, ITP = inosine triphosphate, ITPA = inosine triphosphate pyrophosphatase, MDR1 = multidrug resistance 1, MS = methionine synthase, MTHFD1 = methylenetetrahydrofolate dehydrogenase, MTHFR = methylenetetrahydrofolate reductase, MTR = methionine synthase, MTRR = methionine synthase reductase, MTX = methotrexate, MTX-PGs = methotrexate polyglutamates, OR = odds ratio, PRISMA = Preferred Reporting Items for SRs and Meta-Analyse, RA = rheumatoid arthritis, RFC-1 = reduced folate carrier 1, SHMT = serine hydroxymethyltransferase, SLC = solute carriers, SLCO = solute carrier organic anion transporter, SNPs = single nucleotide polymorphisms, SR = systematic review, THF = tetrahydrofolate, TSER = thymidylate synthase enhancer region, TYMS = thymidylate.

Keywords: meta-analysis, methotrexate, pharmacogenomics, polymorphisms, rheumatoid arthritis, systematic review, toxicity

[a] Institute of Clinical Pharmacology, Beijing Anzhen Hospital, Capital Medical University, [b] Institute of Clinical Medicine, China-Japan Friendship Hospital, [c] Beijing University of Chinese Medicine, [d] Department of Rheumatology, China-Japan Friendship Hospital, Beijing, [e] Department of Gastroenterology, People's Hospital of Yichun, Jiangxi Yichun, [f] Department of Rheumatology, Yili Kazak Autonomous Prefecture Hospital of Traditional Chinese Medicine, Xinjiang Yining, China, [g] Department of Dermatology and Skin Science, University of British Columbia, [h] Molecular Medicine Lab and Chieng Genomics Center, Vancouver Coastal Health Research Institute, Vancouver, BC, Canada.

* Correspondence: Cheng Xiao, Institute of Clinical Medicine, China-Japan Friendship Hospital, Beijing 100029, China (e-mail: xc2002812@126.com).

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic synovial joint inflammation, which leads to disability and diminished quality of life.[1,2] The main objectives for managing RA are to control pain, prevent or control joint damage, and avoid long-term loss of function. Disease-modifying antirheumatic drugs (DMARDs) are mainstay treatments for controlling the symptoms of RA and modifying its radiographic progression.[3] There are several DMARDs available; however, since the reintroduction of methotrexate (MTX) in the early 1980s, MTX has become the most highly effective, fast-acting, disease-modifying antirheumatic drug and is one of the most widely used and the first-line DMARD for the treatment of RA.[4,5] Accumulating evidence has indicated that earlier treatment with DMARD therapy improves long-term outcomes.[3,6,7]

Although the combined efficacy and continuation rates for MTX are superior to that of other DMARDs.[3] Responses to MTX in terms of both efficacy and toxicity vary considerably between patients implying the necessity to study factors that may contribute to such interindividual variability.[8] Estimates indicated that in 10% to 30% of the patients, MTX therapy is discontinued because of adverse effects.[9,10] Various factors, including individual patient factors, disease-specific factors, and genetic factors, have been shown to influence the toxicity of MTX. Therefore, consistently reliable clinical or molecular markers are not available to accurately predict the response to MTX therapy. Pharmacogenomics refers to the study of the entire genome (covering transcriptomic and proteomic fields) and the expression levels of individual genes (mRNA) to identify the genetic factors influencing adverse effects and toxicity to MTX treatment.[11] Researchers believe that pharmacogenetic markers may offer a strategy to help identify patients who are more likely to suffer the toxicity of MTX, although this hypothesis requires clinical evidence.

The reasons behind patient occurrence of adverse events remain unclear, but research into these issues has generated considerable interest. Many of the relevant genes involved in the metabolism of MTX have been investigated to estimate the association between gene polymorphisms and MTX toxicity in RA patients.[4] However, these studies have produced mixed results because of their small sample size and poor statistical power. A meta-analysis can provide a potential solution to this problem because these evaluations combine the results from several studies. Indeed, one of the major advantages of using meta-analyses is the ability to evaluate larger sample sizes, which reduces the likelihood of random errors producing false-positive or false-negative associations. Therefore, to overcome the limitations of individual studies, resolve inconsistencies, and increase precision, we performed a meta-analysis in our study to determine whether the gene polymorphisms in the evaluated studies can predict the adverse events or toxicity to MTX therapy in patients with RA.

Over the past 10 years, 7 meta-analyses[1,4,12–16] on the association between polymorphisms and the toxicity of MTX in RA patients were published in the PubMed and Embase databases. To the best of our knowledge, this is the first systematic review (SR) summarizing all of the available studies on the association between single nucleotide polymorphisms (SNPs) and responsiveness to MTX in RA patients. In the present study, we focused on studies that reported the toxicity of MTX monotherapy and utilized pharmacogenetics, or the analysis of

an individual's genetic variations to predict MTX toxicity in treatment. We also updated the meta-analysis of the MTHFR (677C > T (rs1801133) and 1298A > C (rs1801131)), ABCB1 3435C > T (rs1045642), RFC-1 80G > A (rs1051266), and ATIC 347C > G (rs2372536) polymorphisms and completed the first meta-analysis on the association between MTR A2756G (rs1805087) and MTRR 66A > G (rs1801394) SNPs and the toxicity of MTX in RA patients and the MTHFR (677C > T (rs1801133) and 1298A > C (rs1801131)) and RFC-1 80G > A (rs1051266) polymorphisms were included in the homology subgroup analysis.

2. Methods

The methodology for this study was based on the Preferred Reporting Items for SRs and Meta-Analyses (PRISMA) statement.[17] Ethical approval was not necessary for this meta-analysis because the results included pooled data from individual studies that received ethics approval.

2.1. Published study identification and selection for meta-analysis

All studies investigating the relationship between SNPs and MTX toxicity in RA published before February 2016 were identified using computer-based searches of the PubMed database and Embase database (OvidSP) using the following combination of keywords 'methotrexate[Title/Abstract] AND (polymorphism [Title/Abstract] OR polymorphisms[Title/Abstract] OR genetic [Title/Abstract]) AND ("arthritis, rheumatoid"[MeSH Terms] OR ("arthritis"[All Fields] AND "rheumatoid"[All Fields]) OR "rheumatoid arthritis"[All Fields] OR ("rheumatoid"[All Fields] AND "arthritis"[All Fields]))'. Details of the search flow are provided in Fig. 1. The titles alone were initially reviewed for suitability, and then the abstracts of these titles were obtained and reviewed to determine the full-text retrieval suitability. Data were then extracted as described in the following section from suitable full-text reports. Only studies of human subjects that used validated genotyping methods were included. Case reports, editorials, and review articles were excluded.

2.2. Data extraction

References were screened and data were extracted independently by 2 authors (QQ and JH) using a predetermined data collection template. To resolve discrepancies on the inclusion of studies and interpretation of data, a third investigator (YL) was consulted, and consensus was reached by discussion. The following data were recorded: first author's last name, year of publication, location of study, inclusion and exclusion criteria, sample size, MTX dose, SNP analysis results, treatment duration, and demographic details of patients, follow-up period, toxicity criteria and adverse events.

2.3. Statistical analyses

The gene SNPs detected in more than 2 studies were included in the meta-analysis. Genotype frequencies for the MTHFR (677C > T (rs1801133) and 1298A > C (rs1801131)), RFC-1 80G > A (rs1051266), ATIC 347C > G (rs2372536), MTR A2756G (rs1805087), MTRR 66A > G (rs1801394), and ABCB1 3435C > T (rs1045642) polymorphisms were determined. We examined the differences in CC versus (CT + TT) for the MTHFR 677C > T
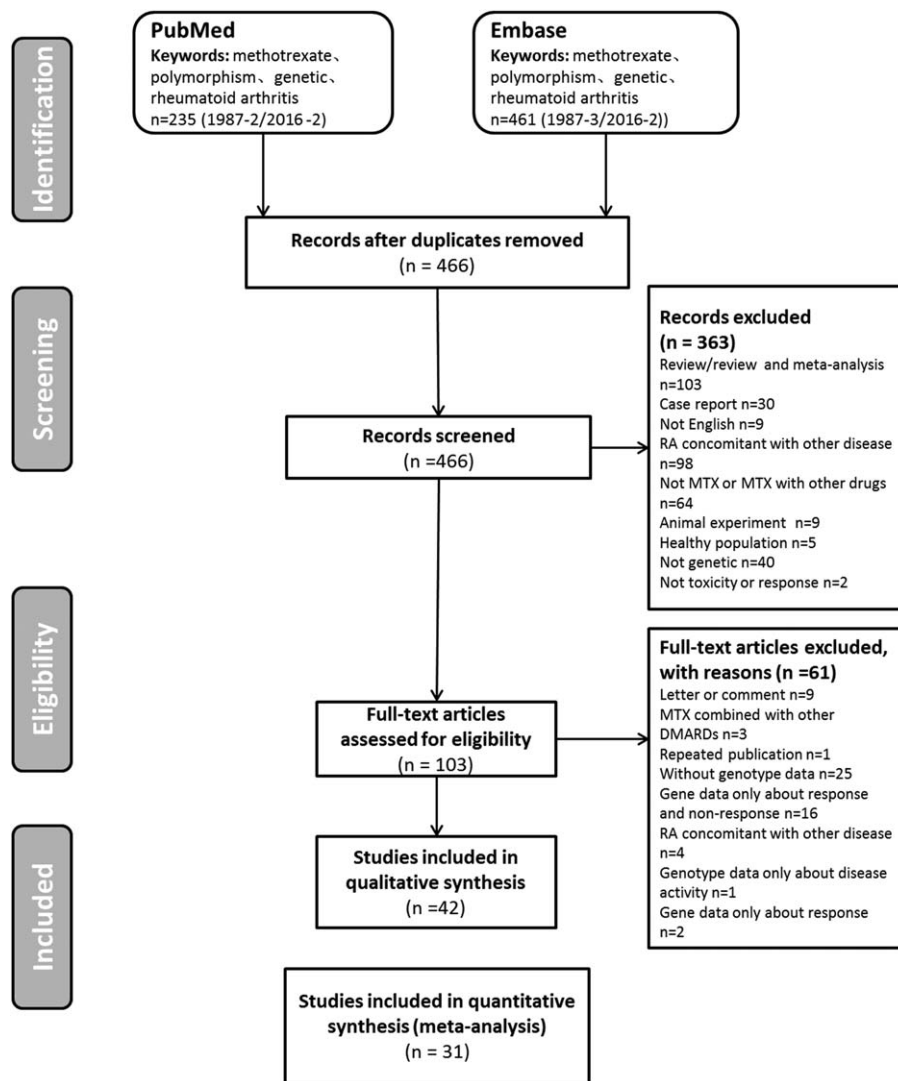
Figure 1. Study selection flow diagram adapted from the Preferred Reporting Items for SRs and Meta-Analyses (PRISMA) Statement.

(rs1801133) and ABCB1 3435C > T (rs1045642) polymorphisms; AA versus (AC + CC) for the MTHFR 1298A > C (rs1801131) polymorphism; GG versus (GA + AA) for the RFC-1 80G > A (rs1051266); CC versus (CG + GG) for the ATIC 347C > G (rs2372536) polymorphism; and AA versus (AG + GG) for the MTRR 66A > G (rs1801394) polymorphism. This process corresponded to a dominant model that assumes a dominant effect of the minor allele, which is consistent with a previous meta-analysis and allowed for the inclusion of a maximum number of studies.[9,14,15] For each study, the point estimate of risk, the OR, and the corresponding 95% CIs of MTX with AE versus without AE were calculated. Then, the overall pooled OR and corresponding 95% CIs were estimated using the Mantel–Haenszel method, and the fixed effect was the absence of moderate inconsistency (>50%) across studies.[3] A fixed effect framework assumes that the effect of allele frequency is constant across studies and between-study variations are caused by chance or random variation. The random effects model was used when heterogeneity > 50%, and it assumes different underlying effects, considers both within- and between-study variation, and is advantageous because it accommodates diversity between studies

and provides a more conservative estimate. The odds ratio (OR) was pooled using inverse variance methods to generate a summary OR and 95% confidence interval (CI). We assessed the heterogeneity between the included studies using the χ^2-based Cochran Q statistic. The percentage of across-study variability attributable to heterogeneity beyond chance was estimated using the I^2 statistic. Differences in the pooled ORs were compared using a Z test. A 2-sided P value of less than 0.05 was considered significant for all analyses. All statistical meta-analyses were completed with STATA (version 13.0; Stata Corp, College Station, TX).[18] The quantitative results are expressed as mean ± SD.[19,20]

3. Results

3.1. Study selection

Figure 1 shows the study selection process. The initial search identified 696 publications (PubMed: 235; and Embase: 461). The full text of 103 articles was reviewed in detail, and 61 articles were further excluded for the following reasons: letter or

Table 1

Studies reporting methods of associating polymorphisms with toxicity to MTX in RA.

Study, year	Number of patients	Patients countries (ancestry)	Toxicity criteria	Adverse events	Genotyping method	Genes	Conclusion for methotrexate-related toxicities
Chaabane S et al, 2015[30]	141	Tunisia (African)	Clinical findings or laboratory tests	Total (n = 81, 57.44%) gastric toxicity: 56%.	PCR-RFLP	MTHFR C677T (rs1801133), MTHFR A1298C (rs1801131), TYMS 2R/3R (rs1805087), MTR A2756G DHFR 19-base pair deletion allele (rs7091108), MTRR A66G (rs1801394)	The TYMS 2R/ 3R polymorphism is associated with a protective effect against overall MTX toxicity, DHFR 19-base pair deletion allele, MTR A2756G and MTRR A66G polymorphisms were not associated with increased MTX toxicity.
Muralidharan N et al, 2015[31]	336	India (South Asian)	Clinical findings or laboratory tests	Total (n = 67, 19.94%) Gastrointestinal: 37 (55.22%); Hematological manifestations1: 3 (19.40%); Patotoxicity: 5 (7.46%); Infections: 8 (11.94%); Pulmonary toxicity: 3 (4.47%); MTX-induced nodulosis: 1 (1.49%).	Real-time PCR	MDR1 3435C > T (rs1045642)	MDR1 3435C > T gene polymorphism influences the clinical phenotype and adverse events to MTX in the South Indian cohort of patients with RA.
Muralidharan N et al, 2015[28]	327	Portugal (European)	Clinical findings or laboratory tests	Total (n = 67, 20.48%) Gastrointestinal: 36 (53.73%); Hematological manifestations: 13 (19.40%); Hepatotoxicity: 5 (7.46%); Infections: 8 (11.94%); Pulmonary toxicity: 3 (4.47%); MTX-induced nodulosis: 1 (1.49%).	PCR-RFLP	RFC -1 80G > A (rs1051266)	RFC-1 80G > Agenepolymorphism is not associated with MTX treatment response and MTX-induced adverse effects in South Indian Tamil patients with RA.
Świerkot J et al, 2015[9]	240	Poland (European)	Medical history, physical examination, and selected laboratory tests	Total (n = 128, 53%) Gastrointestinal: 80 (33%); Hematological manifestations: 8 (3.3%); Hepatotoxicity: 18 (7.5%); Infections: 9 (3.8%); Pulmonary toxicity: 7 (2.9%); MTX-induced nodulosis: 2 (0.8%); Alopecia: 28 (12.0%); Fatigue, headache, myalgia: 34 (14.0%) Skin reactions: 2 (0.8%).	PCR-RFLP	MTHFRc.677C > T (rs1801133) and c.1298A > C (rs1801131), RFC-1 c.80A > G (rs1051266), TYMS 2R/3R (rs45455694) and 6bp ins/ del (rs16430), GGH c.-401C > T (rs3758149), TC c.593T > C	GGH 401TT and CT genotypes were associated with a reduction in the number of MTX-related adverse events
Saleh MM et al, 2015[11]	159	Jordan (East Asian)	Patients' medical files, laboratory results, and the questionnaire according to published data	Renal toxicity; liver cirrhosis; elevated liver enzymes; lung fibrosis; skin nodules; subcutaneous nodulosis; mouth ulcers; allergy; MTX gastrointestinal intolerance	PCR	MTHFR C677T (rs1801133),	There was no significant association between the C677T and A1298C polymorphisms and response to or specific toxicity of MTX. However, the C677T polymorphism was associated with "any MTX toxicity".
Lima A et al, 2014[32]	233	Portugal (European)	Clinical findings or laboratory tests	Total (n = 77, 33.0%) Gastrointestinal: 58 (75.3%); Skin andsubcutaneous tissue disorders: 9 (11.7%); Hepatobiliary disorders: 5 (6.5%); Respiratory, thoracic and mediastinal disorders : 5 (6.5%).	PCR-RFLP	TYMS 28 bp VNTR (rs34743033), TESR (rs2853542 and rs34743033), TYMS 1494del6 (rs34489327)	Regarding MTX-related toxicity, no statistically significant differences were observed in relation to TYMS genotypes and haplotypes
Lima A et al, 2014[29]	233	Portugal (European)	Clinical findings or laboratory tests	Total (n = 77, 33.0%) Gastrointestinal: 58 (75.3%); Skin andsubcutaneous tissue disorders: 9 (11.7%); Hepatobiliary disorders: 5 (6.5%) Respiratory, thoracic, and mediastinal disorders: 5 (6.5%)	PCR-RFLP	RFC -1 80G > A (rs1051266)	SLC19A1 G80A genotyping may be a useful tool for clinicians to identify patients at higher risk for developing gastrointestinal toxicity related to MTX treatment.
Jekic B et al, 2012[8]	184	Serbia (European)	Patient's reports, results of routine laboratory measurements and physical examinations	Total (n = 53, 28.8%); Hepatoxicity: 18 (9.8%); Nausea/vomitus: 21 (11.4%) Bone-marrow toxicity: 8 (4.3%) Stomatitis: 3 (1.6%) Hair loss: 7 (3.8%) Cough: 1 (0.5%)	PCR-RFLP	GGH 452 C >T, GGH -354 G > T, CCND1 870A > G, TYMS 2R/3R, TYMS 3RG/ 3RC	The 3 G/3 G genotype of the TYMS gene may indicate predisposition of poor response to MTX and GG genotype of GGH -354 T > G polymorphism may have high predictive value for myelosuppression in RA patients.

Study, year	Number of patients	Patients countries (ancestry)	Toxicity criteria	Adverse events	Genotyping method	Genes	Conclusion for methotrexate-related toxicities
Milic V et al, 2012[33]	125	Serbia (European)	Physical examinations and laboratory analysis	Hepatotoxicity: 13 (10.4%) Vomitus: 10 (8.0%) Stomatitis: 1 (0.8%) Hair loss: 7 (5.6%) Bone-marrow toxicity: 7 (5.6%) Cough: 1 (0.8%)	PCR-RFLP	DHFR 216T > C (rs6151599), DHFR 317A > G (rs408626), ATIC 129T > G (rs535042)	None of the analysed polymorphisms was associated with MTX toxicity.
Tasbas O et al, 2011[34]	64	Turkey (European)	Clinical interview and physical examination	Total: 36 (56.2%) Fatigue: 18 (28.1%) Malaise: 14 (21.9%) Nausea or vomiting: 20 (31.3%) Disturbed liver function tests: 6 (9.4%) Haematological: 2 (3.1%) Pulmonary: 5 (7.8%) Mucocutaneous: 15 (23.4%) Ear, nose, throat: 3 (4.7%) Neuropsychiatric: 2 (3.1%)	PCR	MTHFR C677T, MTHFR A1298C	A1298C and C677T polymorphisms in the MTHFR gene, were not related with MTX-related toxicity in RA patients receiving folate supplementation.
Cáliz R et al, 2012[35]	468	Spanish (European)	No information	Total: 84 (18%) Gastric toxicity: (21%) Hepatic toxicity: (15%) Neurological, dermatological (alopecia), oral ulceration, haematological, hepatic, pulmonary, gastrointestinal complications (nausea, vomiting, and dyspepsia).	PCR	MTHFR C677T (rs1801133), MTHFR A1298C (rs1801131)	These results demonstrate that the C677T polymorphism in the MTHFR gene is associated with MTX toxicity in a Spanish RA population.
Xiao H et al, 2010[36]	110	China (East Asian)	Patients' self-reports, investigator's reports, Physical examinations, or laboratory measurements.	No information	TaqMan	MTHFR 677C > T (rs1801133), MTHFR 1298A > C (rs1801131), MTHFR G > A (rs2274976) and MTHFR C > T (rs2066462)	SNP rs1801133C/T and rs2274976A/G genetic polymorphisms are associated with MTX-related AEs in the treatment of RA.
Mena JP et al, 2010[37]	70	Mexican (South American)	Laboratory measurements.	An increse of transaminasemia (n = 13, 19%)	PCR-RFLP	MTHFR C677T, MTHFR A1298C	The A1298C polymorphism was associated with elevation of transaminases.
Bohanec GP et al., 2008[38]	150	Slovenia (European)	Patients' file	Total: (n = 148, 69.5%) Gastrointestinal complaint: 68 (31.9%) Hepatotoxicity: 56 (26.3%) Bone-marrow toxicity: 24 (11.3%) Dermatological complaint: 22 (10.3%) Neurotoxicity: 15 (7.0%) Renal toxicity: 7 (3.3%) Infections: 9 (4.2%) Pulmonary toxicity: 5 (2.3%) Mucositis: 4 (1.9%) Other: 16 (7.5%)	PCR-RFL	RFC -1 80G > A (rs1051266), MDR1 3435C > T (rs1045642), MTHFR C677T, MTHFR A1298C, (MDR1 G2677T > A/C, TS 2R → 3R, MS A2756G, MTRR A66G,	Our results suggest that genetic polymorphisms in the folate metabolic pathway and MTX transporters modify the toxicity but not the efficacy of MTX treatment.
Berkun Y et al, 2007[39]	86	Israel (West Asian)	Clinical interview and physical examination	Total: (n = 30, 34.9%) MTX-induced nodulosis: 10 (11.6%) Ulcers: 8 (0.09%) Gastrointestinal: 11 (12.8%) Elevated liver function results: 3 (0.03%)	PCR	MTR A2756G	In our population of MTX-treated RA patients the 2756GG genotype of the MTR gene was more common than expected and was associated with MIARN.
Takatori R et al, 2006[24]	124	Japan (East Asian)	Physical findings and test findings	Total: (n = 48, 38.7%) Hepatic toxicity: 31 (64.6%) Gastrointestinal complaint: 8 (16.7%) Bone marrow toxicity: 2 (0.04%) Pulmonary toxicity: 2 (0.04%) Renal toxicity: 2 (0.04%) Rash: 2 (0.04%) Epilation: 2 (0.04%)	Real-time PCR	ABCB1 3435C > T (rs1045642), RFC-1 80G > A (rs1051266), ATIC 347C > G (rs2372536), TYMS 3UTR - > + 6 bp	There were no significant differences in MTX toxicity among the genotypes of all the genes.
Berkun Y et al, 2004[40]	93	Israel (West Asian)	Clinical interview and physical examination	No information	PCR	MTHFR C677T, MTHFR A1298C	1298CC polymorphism was associated with a reduction in methotrexate related adverse effects.

Study, year	Number of patients	Patients countries (ancestry)	Toxicity criteria	Adverse events	Genotyping method	Genes	Conclusion for methotrexate-related toxicities
Moya P et al, 2016[27]	194	Spanish (European)	Clinical interview and physical examination	Total: (n = 83, 42.8%) Gastrointestinal complaints: 30 (15.5%) Hepatotoxicity: 27 (13.9%) Alopecia: 10 (5.1%) Infections: 9 (4.6%) Lung disease: 8 (4.1%) Moderate-to-severe leukopenia: 6 (3.1%) Nodulosis: 3 (1.5%) Mucosal ulcerations: 2 (1.0%) Mild leukopenia: 2 (1.0%) Cancer of solid organ: 2 (1.0%)	Real-time PCR	SLC19A1 (rs1051266, rs4818789, rs2833957), ABCB1 (rs10267099, rs13233308, rs1858923, rs17327624, rs1202181, rs1202184, rs10280623, rs10264990, rs4148734, rs1202170, rs868755, rs6961419, rs11983225, rs4437575, rs1045642, rs3842), GGH (rs1800909, rs11545077, rs13248452, rs3780130, rs7010484, rs11995525), FPGS (rs10987742, rs10760502, rs10106)	The FPGS rs10106 variant was associated with MTX toxicity, 3 ABCB1 SNPs, rs868755, rs10280623 and rs1858923, were associated with toxicity.
Soukup T et al, 2015[41]	120	Czech epublic (European)	Patients' files	Total: (n = 16, 13.3%) Dyspepsia: 6 (37.5%); Hepatopathia:2 (12.5%); Diarrhea:2 (12.5%); Infection:4 (25%); Leukopenia:1 (6.25%); Alopecia: 1 (6.25%).	TaqMan	MTHRF 677C > T (rs1801133), MTHFR 1298A > C (rs1801131)	In this study, we did not find any association of C677T and A1298C variants on MTX treatment inefficacy
Plaza-Plaza JC et al, 2012[42]	53	Spanish (European)	Clinical findings or laboratory testsp	Total: (n = 24, 45.3%) Elevated liver enzyme levels: 21 (87.5%); Gastrointestinal symptoms: 2 (8.3%); Leukopenia: 1 (4.2%) Mucositis: (4.2%)	PCR-RFLP	MTHFR 677 (rs1801133), MTHFR 1298 (rs1801131), RFC1 80 (rs1051266), ABCB1 (rs1045642)	The presence of the MTHFR C677T and ABCB1 C3435T SNPs contribute to MTX toxicity in patients with RA.
Borman P et al, 2015[43]	64	Portugal (European)	Files and patient interviews	Total: (n = 36, 56.2%); Gastrointestinal toxicity was the most common.	PCR	TS 2R3R	2R and 3R polymorphisms in the TS gene were not related with MTX-related toxicity in RA patients receiving folate supplementation
Aggarwal P et al, 2006[44]	150	India (South Asian)	Routine clinical and laboratory assessments	Total: (n = 30, 20%) Hepatic toxicity: 10 (33%); Thrombocytopaenia: 3 (10%); Anaemia: 2 (6.6%); MTX induced pneumonitis: 3 (10%); Gastrointestinal toxicity: 2 (6.6%); MTX induced transaminitis: 1 (3.3%)	PCR-RFLP	MTHFR C677T	Our findings suggest that C677T polymorphism in the MTHFR gene is not predictive of toxicity or efficacy of MTX treatment in RA patients receiving folate supplementation.
Kumagai K et al, 2003[45]	115	Japan (East Asian)	Clinical and laboratory assessments	Total: (n = 52, 45%) Elevation of transaminase: 20 (17%) Hair loss: 11 (10%) Gastrointestinal intolerance: 8 (7%) Skin rashes, itching: 6 (5%) General fatigue: 4 (3%) Pulmonary toxicity: 5 (4%) Stomatitis: 3 (3%) Leukopenia: 2 (2%)	PCR-RFLP	TYMS 2R/3R, TYMS 3'UTR, MTHFR C677T, and A1298C	MTHFR C677T and A1298C polymorphisms showed no association with MTX-related toxicity or efficacy.
Ghodke Y et al, 2008[46]	34	India (South Asian)	No information	Total: (n = 13, 38.2%)	PCR-RFLP	MTHFR 677C > T (rs1801133),MTHFR 1298A > C (rs1801131), TS 5UTR 2R/3R, TS 3UTR -> + 6 bp	There was no statistically significant association observed for any of the allele/genotype and MTX-related efficacy and toxicity.
Droztzik M et al, 2007[28]	174	Poland (European)	No information	Total: (n = 10, 5.7%) An increase of aminotransferase activity: 10	PCR-RFLP	RFC-1 80G > A (rs1051266)	The increase of aminotransferase activity was noted more frequently in carriers of 80AA genotype.
van Ede AE et al, 2001[47]	236	Netherlands (European)	Routine laboratory measurement and standardtoxicityquestionnaire	Total: (n = 57, 24%) Elevated ALT values: 30 (52.6%) Gastrointestinal complaints and hair loss: 27 (47.4%)	PCR	MTHFR C677T	The presence of the C677CT or C677TT genotypes was associated with an increased risk of discontinuing MTX treatment because of adverse events.
Wessels JA et al, 2006[23]	205	Netherlands (European)	Nonspecific questioning; physical examination or determination of clinical laboratory parameters	Total: (n = 60, 29%) Skin and mucosa disorders: 17 (8%) Hepatic/elevated liver enzyme levels: 16 (8%) Gastrointestinal (general well-being, nausea, vomiting, diarrhea, constipation): 26 (13%)	Real-Time PCR	AMPD1 34C > T (rs17602729), ITPA 94C > A (rs1127354), and ATIC 347C > G (rs2372536)	ATIC G allele carriers experienced a greater frequency of adverse events.

Study, year	Number of patients	Patients countries (ancestry)	Toxicity criteria	Adverse events	Genotyping method	Genes	Conclusion for methotrexate-related toxicities
Davis LA et al, 2014[48]	319	America (North American)	Participants' medical records	Dermatologic: 16 (2.49%); Gastrointestinal: 79 (12.31%); Haematologic: 195 (30.37%); Hepatic: 168 (26.17%); Infectious Disease: 81 (12.62%); Central Nervous System: 15 (2.34%); Respiratory: 45 (7.01%) Other: 46 (7.17%).	qPCR	MTHFR rs1801131, MTHFR rs1801133, FPGS, rs7033913, FPGS, rs10760503, FPGS, rs10106, GGH, rs12548933, GGH, rs7010484, GGH, rs4617146, GGH, rs719235, GGH, rs11988534	RA subjects taking MTX may have decreased time-to-SigAE with ≥ 1 copy of the minor allele in MTHFR rs1801131.
Muralidharan N et al, 2016[22]	319	India (South Asian)	Clinical history and laboratory investigations	Total: (n =65, 20.38%) Gastrointestinal adverse events: 36 (55.38%); Hematological manifestations in the form of anemia, leucopenia or Pancytopenia: 11 (16.92%); An increase in amino transferase levels more than 2-times the upper limit of normal range: 5 (7.69%); Infections like herpes zoster, oral candidiasis, cellulitis or urinary tract infection: 9 (13.84%); Pulmonary toxicity: 3 (4.61%); MTX-induced nodulosis: 1 (1.53%)	PCR-RFLP	ATIC 347C > G (rs2372536)	ATIC 347C > G gene polymorphism may be associated with the development of MTX induced gastrointestinal adverse events.
Stamp LK et al, 2010[25]	191	New Zealand (Oceanian)	No information	No information	TaqMan, PCR	ABCB13435C > T (rs1045642), AMPD134C > T,SLC19A1 80G > A (rs1051266), MTHFR1298A > C (rs1801131), MTHFR677C > T (rs1801133), MTRR66A > G (rs1801394), MTR2756A > G (rs1805087), MTHFD11958G > A, ABCC2 (rs2273697), ATIC347C > G (rs2372536), ITPA94C > A (rs41320251), SHMT1420C > T (rs1979277), TYMS allele 1 2R3R, TYMS allele 2 2R3R, TYMS allele 1 0/1, TYMS allele 2 0/1, ABCC1 (rs35592), ABCC1 (rs3784862), ABCC2 (rs4148396), GGH452C > T (rs11545078), ABCG2 (rs17731538), ABCG2 (rs2231142)	There were weak associations between central nervous system adverse effects and AMPD1 34C > T and between gastrointestinal adverse effects and MTHFD1 1958G > A and ABCC2 IVS23 + 56T > C. There was a stronger association between any adverse effect and ABCG2 914C > A
Wessels JA et al, 2006[49]	205	Netherlands (European)	ADEs were reported by the patients themselves, or were reported as a result of nonspecific questioning on patients' well-being by the investigator, physical examination, or Laboratory measurements	Total: (n =68, 34%) Skin and mucosal disorders: 17 (8.5%); Elevated liver enzyme levels: 18 (9%) Gastrointestinal: 26 (13.0%)	Sequenom	Data showed only MTHFR A1298C (rs1801131)[50]	Patients with MTHFR 1298AA and MTHFR 677CC showed greater clinical improvement with MTX, whereas only the MTHFR 1298C allele was associated with toxicity

7

Study, year	Number of patients	Patients countries (ancestry)	Toxicity criteria	Adverse events	Genotyping method	Genes	Conclusion for methotrexate-related toxicities
Taniguchi A et al, 2007[51]	156	Japan (East Asian)	Clinical records	Total: (n =43, 27.56%) ALT elevation: 37	TaqMan	MTHHR C677T and A1298C	Patients with the T allele at C677T in MTHFR were more susceptible to overall adverse events
Kim SK et al, 2006[52]	385	Korea (East Asian)	Medical records and patient interviews	Total: (n =154, 40%) Gastrointestinal dysfunction: 58 (15.1%); Abnormal transaminase: 48 (12.5%); Hair loss: 49 (12.7%); Nodulosis: 4 (1.0%); Oral ulcer: 3 (0.8%); Dizziness: 2 (0.5%); Leukopenia: 1 (0.3%); Megaloblastic anemia: 1 (0.3%); Iinterstitial pneumonitis: 1 (0.3%)	PCR-RFLP	MTHHR C677T	The MTHFR C677T polymorphism may be an important predictor of MTX-related toxicity in patients with RA.
Samara SA et al, 2014 [10]	120	Jordan (west Asian)	Patients' questionnaires and laboratory results	Total: (n =104, 86.6%); Gastrointestinal toxocity: 53 (51%); Fatigue: 17 (16.3%); Liver toxocity: 31 (29.8%);	PCR-RFLP	RFC1 80 (rs1051266), MDR1 C3435T (rs1045642),	Our results suggest that genetic polymorphisms in methotrexate transporters affect the toxicity but not the response of MTX treatment.
Choe JY et al, 2012[50]	167	Korea (East Asian)	No information	Abnormal liver function; Gastrointestinal disturbance; Oral ulceration; Leucopenia; Alopecia; Dizziness.	MTHFR genotyping kit	MTHFR C677T, MTHFR A1298C	MTHFR polymorphisms were associated with MTX toxicities in Korean patients with RA
Ghocke-Puranik Y et al, 2015[21]	322	India (South Asia)	Physical examination and laboratory parameters	Total: (n =170, 53%) Gastrointestinal tract: 101 (31%) Upper gastrointestinal tract: 96 (30%) Lower gastrointestinal tract: 6 (1.9%) Pain in abdomen: 19 (6%) Hepatic: 69 (21%) Mucositis: 43 (13.4%) Bone marrow: 24 (7.5%) Hair loss: 14 (4.3%) Central nervous system side-effects: 7 (2.2%)	PCR-RFLP	TS 5UTR, TS 3 UTR, GGH C401T, SHMT1 C1420T	Polymorphisms in GGH, SHMT1 and TS were associated with MTX-related adverse events while SNPs in MTHFR and RFC1/SLC19A1 were associated with MTX efficacy.
Urano W et al, 2002[53]	106	Japan (East Asia)	No information	Total: (n =21, 19.8%) Increase in transaminases: 12 (11.3%) Stomatitis: 3 (2.8%); Nausea/vomiting, anorexia: 2 (1.8); Fatigue: 2 (1.8%) Hair loss: 1 (0.9%) Rash: 1 (0.9)	PCR-RFLP	MTHFR C677T, MTHFR A1298C	Polymorphisms within the MTHFR gene are associated with both the efcacy and toxicity of MTX in rheumatoid arthritis patients.
Lima A et al, 2014[29]	233	Portugal (European)	Physician directly asked the patient	Total: (n =77, 33%)	Sequenom	SLC16A7 A > T (rs3763980), SLC16A7 T > G (rs10877333), SLC16A7 T > G (rs10877333), SLC19A1 G > A (rs1051266), SLC19A1 A > G (rs2838956), SLC19A1 G > A (rs3788200), SLC22A11 T > A (rs11231809), SLC46A1 G > A (rs2239907), SLC01B1 T > C (rs4149056), ABCB1 C > T (rs1045642), ABCB1 C > T (rs1128503), ABCB1 G > A/T (rs2032582), ABCC1 T > C (rs35592), ABCC1 A > G (rs246240), ABCC1 G > C (rs2074087), ABCC1 G > A (rs3784864), ABCC2 G > A (rs717620), ABCC2 C > T (rs4148396), ABCG2 T > C (rs13120400), ABCG2 G > A (rs17731538)	This study demonstrated that SLC19A1, SLC46A1and SLC01B1 genotypes may help to identify patients with increased risk of MTX-related overall toxicity and that SLC19A1 and SLC01B1 genotypes, and SLC19A1 haplotypes may help to identify patients with increased risk of MTX-related gastrointestinal toxicity.

Study, year	Number of patients	Patients countries (ancestry)	Toxicity criteria	Adverse events	Genotyping method	Genes	Conclusion for methotrexate-related toxicities
Dervieux T et al, 2006[26]	48	American (North America)	Questionnaire	Total: 45% Gastrointestinal tract: 32%; Nausea: 17%; Diarrhea: 11% Dyspepsia: 14%; Stomatitis: 4% AST > 40 units/liter: 2% Central nervous system: 28%; Headache: 13%; Lethargy: 20%; Alopecia: 3% Cough: 9% Dyspnea: 0.3%	real-time TaqMan	GGH -401C > T, MTHFR 1298A > C, ATIC 347C > G, MS 2756A > G, MTRR 66A > G.	Risk genotypes associated with toxicity were in GGH -401CC, ATIC 347CC, MTHFR 1298AC/CC, MS 2756AA and MTRR 66GG.
Owen SA et al, 2013[54]	309	UK (European)	Medical record review	Total: n =61 Gastrointestinal: n =24; Abnormal liver function: n =20; Haematological: n =7 Skin rashes:n =6; Renal n =1; Headaches and pneumonitis: n =3	Sequenom	ATIC (rs7563206, rs3821353, rs12995526, rs16853834), GGHrs12681874, SLC19A1 (rs11702425, rs2838956, rs7499, rs2274808, rs9977268, rs7279445)	Five SNPs were significantly associated with adverse events; 3 in the DHFR gene (rs12517451, rs10072026, and rs1643657) and 2 of borderline significance in the FPGS gene.
Kooloos WM et al, 2010[55]	205	Netherlands (European)	The patients themselves, or were reported as a result of nonspecific questioning on patients' well-being by the investigator, physical examination, or laboratory measurements	Total: n =60 (30%)	Real-time PCR	ABCB1 3435C > T, TLR4 + 896A > G	Our data indicate that MTX toxicity was potentially associated with ABCB1 3435C > T and TLR4 + 896A > G. However, after correction, none of these associations remained significant.
Salazar J et al, 2014[56]	124	Spain (European)	Evaluated by the same rheumatologist	Total: (n =59, 47.6%); Hepatotoxicity: 18 (14.3%); Liver disease: 3 (2.4%); Gastrointestinal complaints: 24 (19%); Mucosal ulcerations: 1 (0.8%); Nodulosis: 2 (1.6%); Infections: 5 (4.0%); Alopecia: 10 (7.9%); Lung disease: 4 (3.2%); Mild leukopenia: 1 (0.8%); Leukopenia (moderate–serious): 4 (3.2%); Cancer of solid organ: 2 (1.6%)	Real-time PCR	ATIC rs16853826, ATIC rs10197559	The ATIC rs16853826 variant was associated with toxicity.

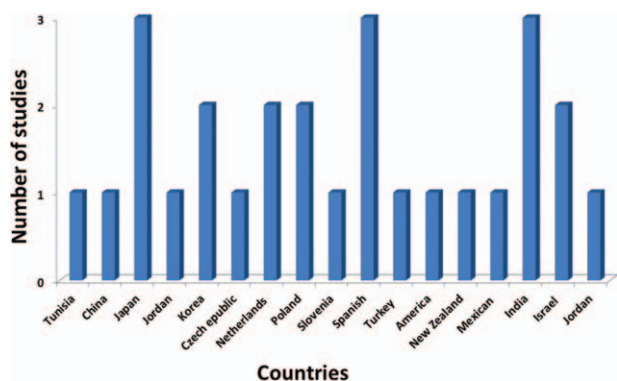MTX = methotrexate, RA = rheumatoid arthritis.

9

Figure 2. Distribution of countries in 31 studies that measured the association between polymorphisms and the toxicity to MTX in RA. MTX = methotrexate, RA = rheumatoid arthritis.

comment (n=9), MTX combined with other DMARDs (n=3), repeated publication (n=1), without genotype data (n=25), gene data only about response and nonresponse (n=16); RA concomitant with other disease (n=4); genotype data only about disease acitivity (n=1); and gene data only about response (n=2). Ultimately, 42 publications were included in the SR and 31 studies were included in 7 meta-analyses.

3.2. Study characteristics

For the analyzed studies, the characteristics and detected genes are shown in Table 1. The number of papers from Europe accounts for a large proportion of the total number of papers (Figs. 2 and 3).

3.3. Pharmacogenetic markers of RA response to MTX treatment

A total of 28 genes with 88 gene SNPs associated with the transporters, enzymes, and metabolites of MTX or the progression of RA were evaluated to explore the association between the gene polymorphisms and the MTX toxicity in previous studies (Table 1).
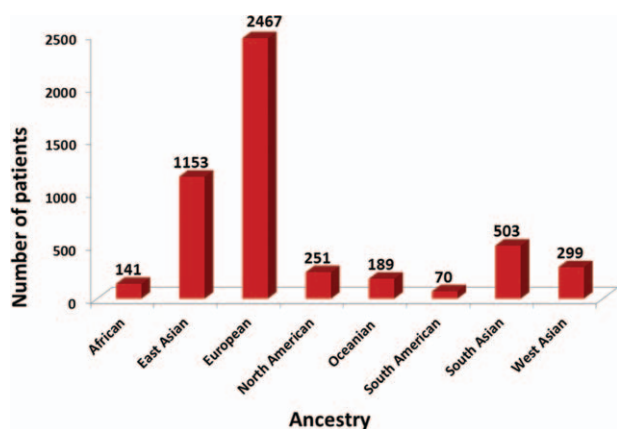

Figure 3. Distribution of ancestry in 31 studies that measured the association between polymorphisms and the toxicity to MTX in RA. MTX = methotrexate, RA = rheumatoid arthritis.

The main action of MTX is to inhibit the folate pathway and exert anti-inflammatory and anti-proliferative effects in RA. The present researches of the MTX metabolic pathway showed that MTX enters target cells through reduced folate carriers (SLC19A1 (RFC-1)) and effluxes from target cells through ATP-binding cassettes (ABCs), predominantly ABCC1–2, ABCB1, and ABCG2.[57] After polyglutamated by the enzyme FPGS, the polyglutamated MTX (MTX-PG) can be reversed by the enzyme GGH, and retained within the cells. The MTX-PG can inhibit the activity of DHFR competitively and reduce the dihydrofolation of tetrahydrofolate (THF), which is the precursor of the biologically active folate cofactor 5-methyl-THF, and this conversion is catalyzed by MTHFR. MTHFR, SHMT, and other enzymes in 1 carbon pool (MS, MTR, and MTRR) are not directly inhibited by MTX, although their expression level may contribute to the antifolate effects of MTX through subtle alterations in the folate pools.[21,58] MTX-PG can inhibit the TYMS (TSER)-mediated conversion of deoxyuridylate to deoxythymidylate in the de novo pyrimidine biosynthetic pathway and can also inhibit the activity of the enzyme ATIC and promote the intracellular accumulation of adenosine (AICAR), which, through a series of enzymatic reactions, leads to the generation of adenosine and increased extracellular concentrations of adenosine, an anti-inflammatory agent. This pathway includes the intermediates inosine monophosphate and inosine triphosphate and the key enzymes ITPA, IMP (IMPDH), and AMP (AMPD1 and ADA). CCND1 controls cell progression through the G1/S phase and is also involved in the regulation of TYMS (TSER) and DHFR.

The aforementioned genes are commonly used as important candidate genes in studies of RA response to MTX treatment. All of the genes and pathways included in the present SR are summarized in Fig. 4, where they are highlighted in green.

3.4. MTHFR 677C > T (rs1801133)

Twenty studies were included in the meta-analysis of MTHFR 677C > T (rs1801133), which contained data from a combined total of 1330 patients with adverse event (AE) and 1941 patients without AE and included 7 European studies (433 patients with AE and 897 patients without AE), 6 East Asian studies (577 patients with AE and 593 patients without AE), and 2 South Asian studies (43 patients with AE and 141 patients without AE). The characteristics of these studies are described in Table 2.

When all of the samples were included, the association between the frequency of 3 MTHFR 677C > T (rs1801133) alleles and MTX toxicity was not significant (OR=0.75, 95% CI: 0.53–1.06, Z=1.61, P=0.107). Moreover, significant between-study heterogeneity was observed (I^2=73.6%, χ^2= 71.86, P=0.000) (Fig. 5).

Stratification by ethnicity did not identify a significant association between the MTHFR 677C > T (rs1801133) 3 allele frequency and MTX toxicity in the European (OR=0.76, 95% CI: 0.43–1.34, Z=0.94, P=0.348), East Asian populations (OR=0.48, 95% CI: 0.21–1.07, Z=1.79, P=0.074) or South Asian (OR=1.34, 95% CI: 0.65–2.74, Z=1.02, P= 0.309) (Fig. 5).

3.5. MTHFR 1298A > C (rs1801131)

Sixteen studies were included in the meta-analysis of MTHFR 1298A > C (rs1801131), which contained data from a combined
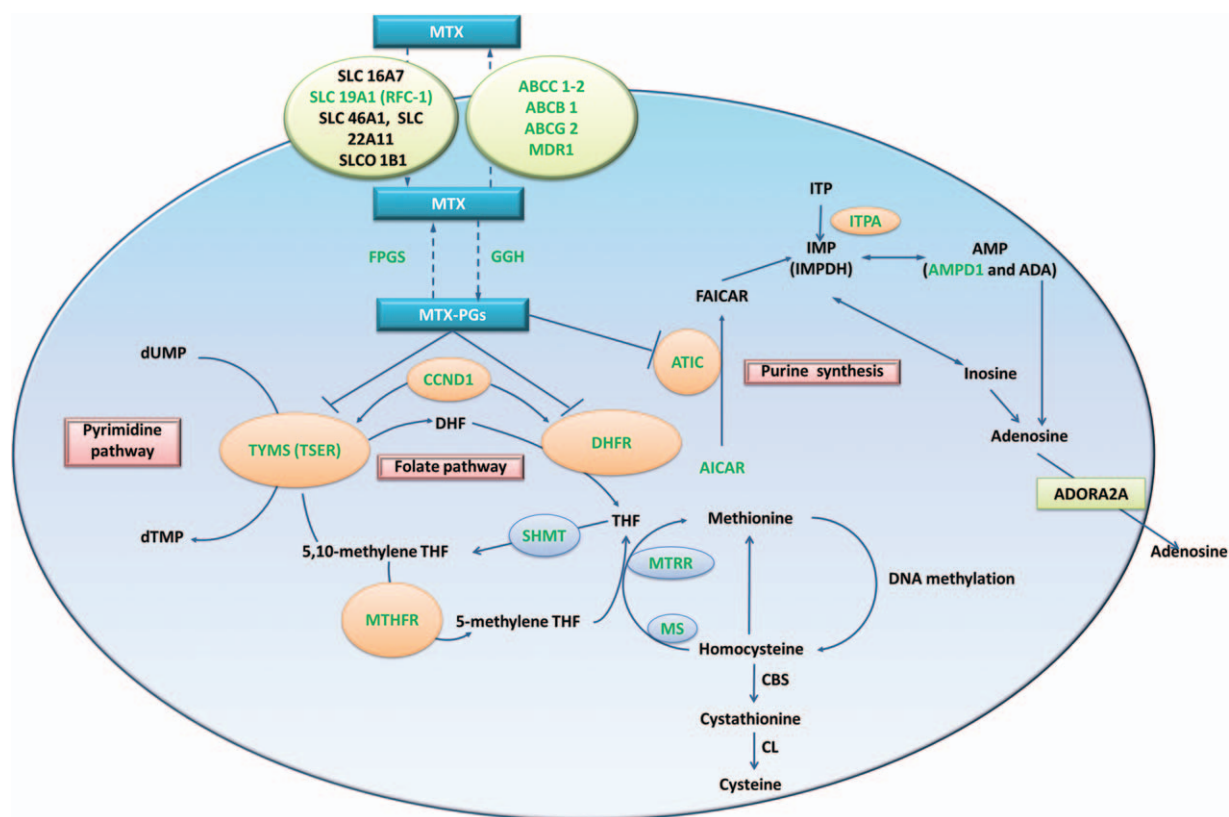
Figure 4. Summary of detected genes associated with the MTX toxicity in RA patients in previous studies. Schematic representation of the intracellular folate biosynthetic pathway and the genes detected in previous studies (in green). ABCC1–4, ABCB1, and ABCG2 = adenosine triphosphate-binding cassette (ABC) transporters, ADA = adenosine deaminase, ADP = adenosine diphosphate, AICAR = 5-aminoimidazole-4-carboxamide ribonucleotide, AMP = adenosine monophosphate, ATIC = -aminoimidazole-4-carboxamide ribonucleotide transformylase/IMP cyclohydrolase, ATP = adenosine triphosphate, CBS = cystathionine-β-synthase, CCND1 = cyclin D1, CH3 = methyl group, CL = cystathionine lyase, DHF = dihydrofolate, DHFR = dihydrofolate reductase, dTMP = deoxythymidine-5′-monophosphate, dUMP = deoxyuridine-5′-monophosphate, FAICAR = 10-formyl-AICAR, FPGS = folylpolyglutamyl synthase, GGH = glutamyl hydrolase, IMP = inosine monophosphate, IMPDH2 = inosine 5′-monophosphate dehydrogenase, ITP = inosine triphosphate, ITPA = inosine triphosphate pyrophosphatase, MDR1 = multidrug resistance 1, MS = methionine synthase, MTHFD1 = methylenetetrahydrofolate dehydrogenase, MTHFR = methylenetetrahydrofolate reductase, MTR = methionine synthase, MTRR = methionine synthase reductase, MTX = methotrexate, MTX-PGs = methotrexate polyglutamates, RFC-1 = reduced folate carrier 1, SHMT = serine hydroxymethyltransferase, SLC16A7, SLC19A1, SLC46A1, and SLC22A11 = solute carriers, SLCO 1B1 = solute carrier organic anion transporter, THF = tetrahydrofolate, TSER = thymidylate synthase enhancer region, TYMS = thymidylate.

total of 987 patients with AE and 1460 patients without AE and included 6 European studies (357 patients with AE and 783 patients without AE), 4 East Asian studies (266 patients with AE and 266 patients without AE). The characteristics of these studies are described in Table 3.

When all of the samples were included, the association between the MTHFR 1298A > C (rs1801131) 3 allele frequency and MTX toxicity was not significant (OR = 1.02, 95% CI: 0.74–1.39, $Z = 0.10$, $P = 0.923$). Moreover, significant between-study heterogeneity was observed ($I^2 = 61.8\%$, $\chi^2 = 39.30$, $P = 0.001$) (Fig. 6).

Stratification by ethnicity did not identify a significant association between the MTHFR A1298C (rs1801131) 3 allele frequency and MTX toxicity in the European (OR = 0.86, 95% CI: 0.52–1.42, $Z = 0.59$, $P = 0.558$), or the East Asian (OR = 1.22, 95% CI: 0.83–1.81, $Z = 1.02$, $P = 0.309$) (Fig. 6).

3.6. ATIC 347C > G (rs2372536)

Four studies were included in the meta-analysis of ATIC 347C > G (rs2372536), which contained data from a combined total of 311 patients with AE and 521 patients without AE. The characteristics of these studies are described in Table 4.

When all of the samples were included, the association between the ATIC 347C > G (rs2372536) and MTX toxicity was not significant (OR = 0.71, 95% CI: 0.50–1.01, $Z = 1.88$, $P = 0.060$). Moreover, significant between-study heterogeneity was not observed ($I^2 = 0.0\%$, $\chi^2 = 2.71$, $P = 0.438$) (Fig. 7).

3.7. MTR 2756A > G (rs1805087)

Three studies were included in the meta-analysis of MTR 2756A > G (rs1805087), which contained data from a combined total of 228 patients with AE and 188 patients without AE. The characteristics of these studies are described in Table 5.

When all of the samples were included, the association between the MTR 2756A > G (rs1805087) allele frequency and MTX toxicity was significant (OR = 0.99, 95% CI: 0.62–1.60, $Z = 0.03$, $P = 0.977$). Moreover, significant between-study heterogeneity was not observed ($I^2 = 14.1\%$, $\chi^2 = 2.33$, $P = 0.312$) (Fig. 8).

3.8. MTRR 66A > G (rs1801394)

Two studies were included in the meta-analysis of MTRR 66A > G (rs1801394), which contained data from a combined total of

Table 2

Summary of the analyzed studies and the distribution of methylenetetrahydrofolate reductase MTHFR 677C > T (rs1801133) genotypes.

Study	Study design	With AE (Case) CC	With AE (Case) CT+TT	Without AE (Control) CC	Without AE (Control) CT+TT	Mean age, y	Mean disease duration, y	MTX dose (mg per week) (range or mean±SD)	Date of end point, wk
Chaabane S et al, 2015[30]	Prospective cohort	27	33	41	40	52.08±12.48	12.20±9.03	11±2.46	No information
Świerkot J et al, 2015[9]	Prospective cohort	50	78	62	49	52±11.9	No information	15 (12.5–25)	24
Saleh MM et al, 2015[11]	Prospective cohort	43	53	12	3	49.2±13.4	11.16	15.92±5.35 (5–25)	16
Cáliz R et al, 2012[35]	Retrospective cohort	27	57	151	233	49±13.4	10.9±4.9	10–25	No information
Xiao H et al, 2010[36]	Prospective cohort	5	42	17	31	49.2	3.7	17.0±1.38	24
Soukup T et al, 2015[41]	Prospective cohort and retrospective cohort	10	6	42	62	58.5	No information	11.7	24
Plaza-Plaza JC et al, 2012[42]	Prospective cohort	2	24	13	14	54.7±14.37	6.9±3.92 (2–22)	10±2.10 (7.5–15)	52
Ghodke Y et al, 2008[46]	Retrospective cohort	9	4	13	8	No information	No information	7.5–17.5	No information
Bohanec GP et al, 2008[38]	Retrospective cohort	60	53	14	23	61 (51–69)	69.0 (40.5–137.5) mo	10.0 (10.0–12.5)	104
Davis LA et al, 2014[48]	Retrospective cohort	39	26	92	94	68.75±10.89	No information	No information	16
Stamp LK et al, 2010[25]	Retrospective cohort	58	78	20	31	60.5	10.4	15.0 (median) range 2.5–25.0	52
Tasbas O et al, 2011[34]	Prospective cohort	17	19	12	16	48.7±12.5	6.5 (0.50–34)	15 (10–20)	No information
Mena JP et al, 2010[37]	Prospective cohort	16	43	4	9	No information	No information	7.5±2.5	No information
Berkun Y et al, 2004[40]	Cross-sectional study	15	18	30	30	58.74	No information	12.03±3.86	No information
Aggarwal P et al, 2006[44]	Retrospective cohort	19	11	68	52	42.9±11.1	7.65±5.2	11.1±3.1	26.1±20.6 (month)
Kumagai K et al, 2003[45]	Prospective cohort	24	28	22	41	59.7±9.8	10.8±7.3	5.7±2.3	No information
Kim SK et al, 2006[52]	Retrospective study	27	127	106	125	50.4±11.0	13.9±7.5	5.0–20.0	52
Taniguchi A et al, 2007[51]	Retrospective study	10	33	56	57	55.79±12.2	9.4±11.1	>6 in 64 patients	>1 y
Choe JY et al, 2012[59]	Prospective cohort	36	89	8	34	53.9±10.4	13.7±7.8	10±1.9	No information
van Ede AE et al, 2001[47]	Multicenter, doubleblind, placebo-controlled tria	10	20	112	94	CC:56±12.5 CT and TT:55±13.1	CC:83±95.1 (mo) CT and TT:75±81.0 (mo)	7.5–25	No information

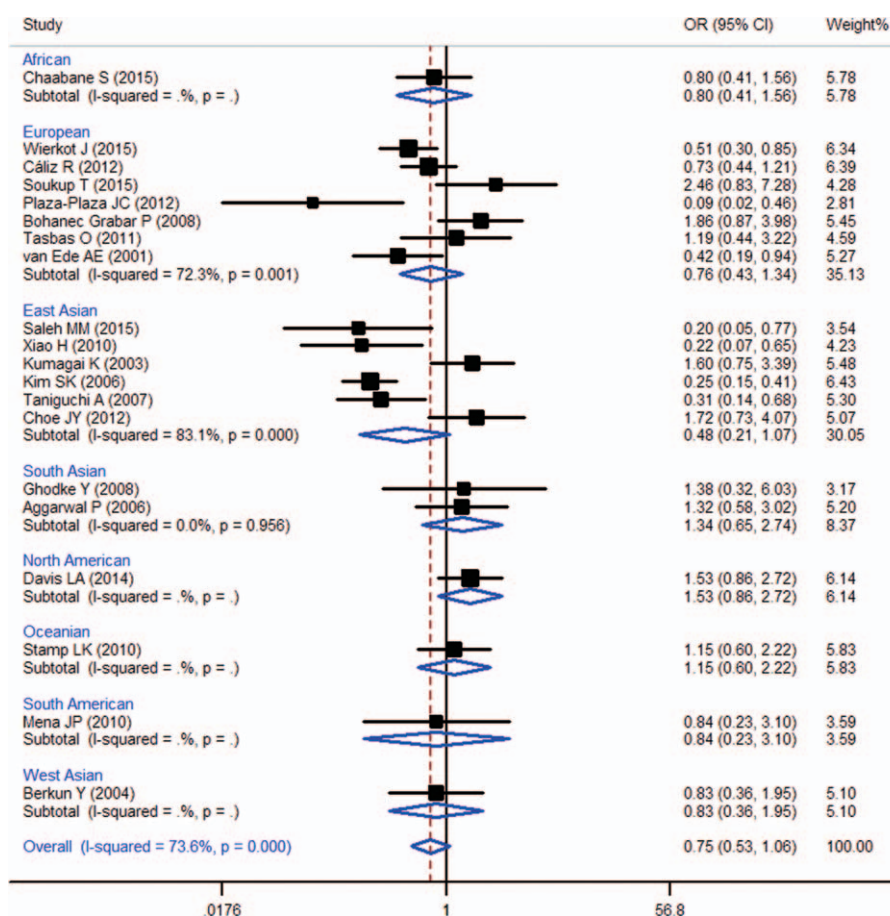MTHFR = methylenetetrahydrofolate reductase, MTX = methotrexate.

Figure 5. Meta-analysis of MTHFR 677C > T (rs1801133) single-nucleotide polymorphism and associated risk of toxicity of MTX (CC vs CT + TT genotypes). % weight = the percentage weight attributed to each study in the meta-analysis, CI = confidence interval, OR = odds ratio. Squares represent point estimates for effect size expressed as an OR with the size proportional to the inverse variance of the estimate. Lines represent 95% CIs. The diamonds represent the overall pooled estimate.

194 patients with AE and 132 patients without AE. The characteristics of these studies are described in Table 6.

When all of the samples were included, the association between the MTRR 66A > G (rs1801394) allele frequency and MTX toxicity was not significant (OR = 1.41, 95% CI: 0.83–2.38, $Z = 1.27$, $P = 0.203$). Moreover, significant between-study heterogeneity was not observed ($I^2 = 0.0\%$, $\chi^2 = 0.35$, $P = 0.551$) (Fig. 9).

3.9. RFC −1 80G > A (rs1051266)

Ten studies were included in the meta-analysis of RFC -1 80G > A (rs1051266), which contained data from a combined total of 791 patients with AE and 1008 patients without AE and included 7 European studies (503 patients with AE and 865 patients without AE). The characteristics of these studies are described in Table 7.

When all of the samples were included, no significant association was found between the RFC −1 80G > A (rs1051266) 3 allele frequency and MTX toxicity was identified (OR = 1.18, 95% CI: 0.90–1.54, $Z = 1.21$, $P = 0.225$). Moreover, significant between-study heterogeneity was not observed ($I^2 = 18.4\%$, $\chi^2 = 11.04$, $P = 0.273$) (Fig. 10).

Stratification by ethnicity identified a significant association between the RFC −1 80G > A (rs1051266) 3 allele frequency and MTX toxicity in Europeans (OR = 1.36, 95% CI 1.01–1.83, $Z = 2.05$, $P = 0.041$) (Fig. 10).

3.10. ABCB1 3435C > T (rs1045642)

Five studies were included in the meta-analysis of ABCB1 3435C > T (rs1045642), which contained data from a combined total of 391 patients with AE and 460 patients without AE. The characteristics of these studies are described in Table 8.

When all of the samples were included, no significant association between the ABCB1 3435C > T (rs1045642) 3 allele frequency and MTX toxicity was found (OR = 1.36, 95% CI: 0.54–3.44, $Z = 0.65$, $P = 0.518$). Moreover, significant between-study heterogeneity was not observed ($I^2 = 79.3\%$, $\chi^2 = 19.32$, $P = 0.001$) (Fig. 11).

4. Discussion

The pathogenesis of RA is not well understood, and there are considerable challenges in the design of effective medicines to cure RA. MTX is still the gold standard drug for RA and plays antiproliferative and anti-inflammatory roles in RA therapy.[57,60] The toxicity of MTX comes to the most important factor in the failure of RA treatment. Although the factors influencing the toxicity in MTX remain unclear, genetic factors related to drug metabolism may play an important role in this variability. A single nucleotide polymorphism (SNP) is a common genetic variant that consists of a single DNA base pair change.

Table 3

Summary of the analyzed studies and the distribution of methylenetetrahydrofolate reductase MTHFR 1298A > C (rs1801131) genotypes.

Study	Study design	Genotype counts With AE (Case) AA	AC+CC	Without AE (Control) AA	AC+CC	Mean age, y	Mean disease duration, y	MTX dose (mg per week) (range or mean ± SD)	Date of end point, wk
Chaabane S et al, 2015 [30]	Prospective cohort	33	27	39	42	52.08±12.48	12.20±9.03	11±2.46	No information
Świerkot J et al, 2015 [9]	Prospective cohort	74	53	63	48	52±11.9	No information	15 (12.5–25)	24
Cáliz R et al., 2012 [35]	Retrospective cohort	49	35	199	185	49±13.4	10.9±4.9	10–25	No information
Xiao H et al, 2010 [36]	Prospective cohort	33	13	30	18	49.2	3.7	17.0±1.38	24
Soukup T et al, 2015 [41]	Prospective cohort and retrospective cohort	5	11	52	52	58.5	No information	11.7	24
Plaza-Plaza JC et al, 2012 [42]	Prospective cohort	18	8	13	14	54.7±14.37	6.9±3.92 (2–22)	10±2.10 (7.5–15)	52
Ghodke Y et al, 2008 [46]	Retrospective cohort	5	8	5	16	No information	No information	7.5–17.5	No information
Davis LA et al, 2014 [48]	Retrospective cohort	16	49	93	92	68.75±10.89	No information	No information	16
Wessels JA et al, 2006 [49]	"Prospective, subgroup of a RCT"	19	49	64	65	54.6	No information	7.5 (initialy)/15 (wk 4)/25 (wk 12)	24
Stamp LK et al, 2010 [25]	Retrospective cohort	57	79	27	24	60.5	10.4	15.0 (median) range 2.5–25.0	52
Tasbas O et al, 2011 [34]	Prospective cohort	12	24	12	16	48.7±12.5	6.5 (0.50–34)	15 (10–20)	No information
Mena JP et al, 2010 [37]	Prospective cohort	35	22	4	9	No information	No information	7.5±2.5	No information
Berkun Y et al, 2004 [40]	Cross-sectional study	22	11	28	32	58.74	No information	12.03±3.86	No information
Kumagai K et al, 2003 [45]	Prospective cohort	36	16	44	19	59.7±9.8	10.8±7.3	5.7±2.3	No information
Taniguchi A et al, 2007 [51]	Retrospective study	28	15	74	39	55.79±12.2	9.4±11.1	>6 in 64 patients	>1 y
Choe JY et al, 2012 [59]	Prospective cohort	90	35	26	16	53.9±10.4	13.7±7.8	10±1.9	No information

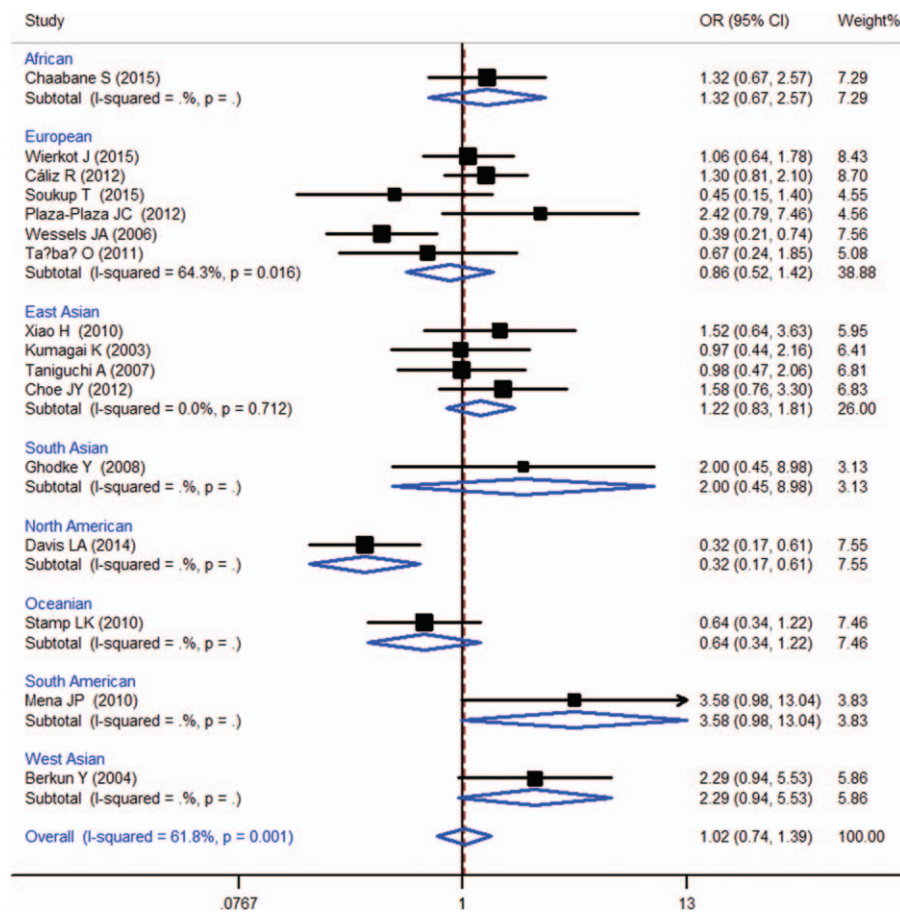MTHFR = methylenetetrahydrofolate reductase, MTX = methotrexate.

Figure 6. Meta-analysis of MTHFR 1298A > C (rs1801131) single-nucleotide polymorphism and the associated risk of of MTX (AA vs AC + CC genotypes). % weight = the percentage weight attributed to each study in the meta-analysis, CI = confidence interval, OR = odds ratio. Squares represent point estimates for effect size expressed as a OR with the size proportional to the inverse variance of the estimate. Lines represent 95% CIs. The diamonds represent the overall pooled estimate.

The SNP association study has become a very popular method for identification of genetic factors for complex disease traits.[61,62] Several studies have shown that SNPs could explain differences in genetic susceptibility to different diseases.[63,64] In recent years, extensive pharmacogenomics investigations have been performed to optimize MTX therapy for RA patients through genotyping and/or gene-expression-based tests. These tests were primarily based on mRNA and included transporters, enzymes, and metabolites genes[57]; however, the majority of the findings were inconclusive and inconsistent, even for classical candidate gene polymorphisms. Thus, developing effective and practical biomarkers to aid in the prediction of MTX toxicity in routine clinical practice remains a challenge. The present study performed an SR on the association between polymorphisms and the toxicity of MTX in RA patients using papers published in the PubMed and Embase databases. Furthermore, this review focused on studies that reported the toxicity of MTX mono-therapyand utilized pharmacogenetics, or the analysis of an

Table 4

Summary of the analyzed studies and the distribution of methylenetetrahydrofolate reductase ATIC 347C > G (rs2372536) genotypes.

Study	Study design	With AE (Case) CC	With AE (Case) CG + GG	Without AE (Control) CC	Without AE (Control) CG + GG	Mean age, y	Mean disease duration, y	MTX dose (mg per wk) (range or mean ± SD)	Date of end point, wk
Takatori R et al, 2006 [24]	Retrospective cohort	27	21	49	27	No information	No information	6.0 (median)	12
Wessels JA et al, 2006 [23]	Retrospective cohort	21	39	73	67	54.6	No information	7.5 (initialy)/15 (week 4)/25 (week 12)	24
Muralidharan N et al, 2016 [22]	Prospective cohort	7	58	36	218	42.59	3.54	16.88 ± 0.22	24
Stamp LK et al, 2010 [25]	Retrospective cohort	56	82	20	31	60.5	10.4	15.0 (median) range 2.5–25.0	52

ATIC = aminoimidazole-4-carboxamide ribonucleotide transformylase, MTX = methotrexate.
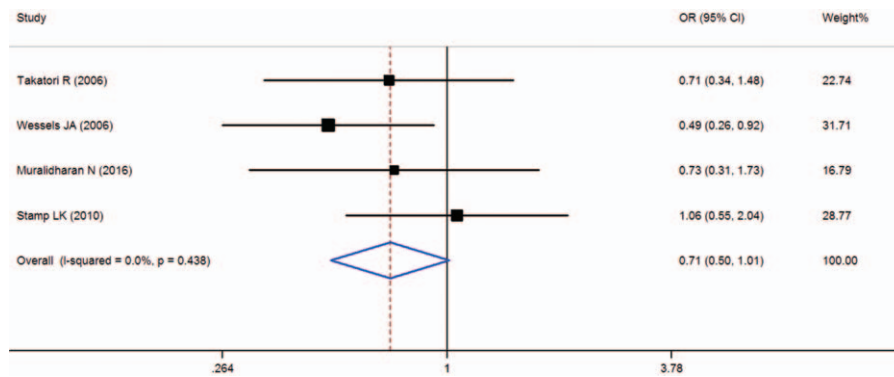
Figure 7. Meta-analysis of ATIC 347C > G (rs2372536) polymorphism and associated risk of toxicity of MTX (CC vs CG + GG genotypes). % weight = the percentage weight attributed to each study in the meta-analysis, CI = confidence interval, OR = odds ratio. Squares represent point estimates for effect size expressed as an OR with the size proportional to the inverse variance of the estimate. Lines represent 95% CIs. Diamond represents the overall pooled estimate.

Table 5

Summary of the analyzed studies and the distribution of methylenetetrahydrofolate reductase MTR 2756A > G (rs1805087) genotypes.

| Study | Study design | Genotype counts | | | | Mean age, y | Mean disease duration, y | MTX dose (mg per week) (range or mean ± SD) | Date of end point, wk |
		With AE (Case)		Without AE (Control)					
		A (AA)	G (AG + GG)	A (AA)	G (AG + GG)				
Chaabane S et al, 2015 [30]	Prospective cohort	45	15	54	27	52.08 ± 12.48	12.20 ± 9.03	11 ± 2.46	No information
Berkun Y et al, 2007 [39]	cross-sectional study	9	21	23	32	58.4 ± 13.8	No information	12.03 ± 3.86	No information
Stamp LK et al, 2010 [25]	Retrospective cohort	87	51	33	18	60.5	10.4	15.0 (median) range 2.5–25.0	52

MTR = methionine synthase, MTX = methotrexate.
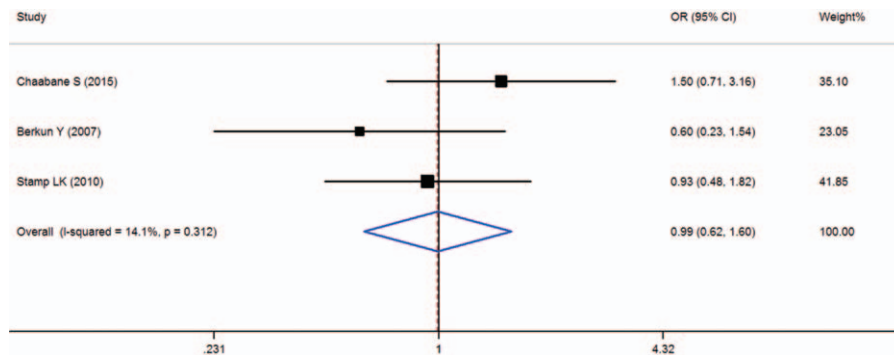
Figure 8. Meta-analysis of MTR 2756A > G (rs1805087) single-nucleotide polymorphism and associated risk of toxicity of MTX (AA vs AG + GG genotypes). % weight = the percentage weight attributed to each study in the meta-analysis, CI = confidence interval, OR = odds ratio. Squares represent point estimates for effect size expressed as a OR with the size proportional to the inverse variance of the estimate. Lines represent 95% CIs. Diamond represents the overall pooled estimate.

individual's genetic variation, to predict the toxicity in MTX treated RA patients.

MTHFR is the most extensively studied MTX-related gene because it plays an important role in both responses and toxicity to MTX treatment in RA.[14] MTHFR 677C > T (rs1801133) and 1298A > C (rs1801131) are 2 important polymorphisms that affect enzyme activity and MTX metabolism. MTHFR 677C > T is a nonsynonymous polymorphism that results in the substitution of alanine with valine at codon 222 of the MTHFR enzyme; MTHFR 1298A > C is another nonsynonymous polymorphism that leads to the substitution of glutamine with alanine in the C-terminal regulatory domain of the MTHFR enzyme, which results in decreased enzyme activity.[58] In recent years, extensive investigations have been performed to identify the association

Table 6

Summary of the analyzed studies and the distribution of methylenetetrahydrofolate reductase MTRR 66A>G (rs1801394) genotypes.

		Genotype counts							
		Responders (Case)		Nonresponders (Control)			Mean disease	MTX dose (mg per week)	Date of end
Study	Study design	AA	AG+GG	AA	AG+GG	Mean age, y	duration, y	(range or mean±SD)	point, wk
Chaabane S et al, 2015 [30]	Prospective cohort	21	39	20	61	52.08±12.48	12.20±9.03	11±2.46	No information
Stamp LK et al, 2010 [25]	Retrospective cohort	36	98	12	39	60.5	10.4	15.0 (median) range 2.5–25.0	52

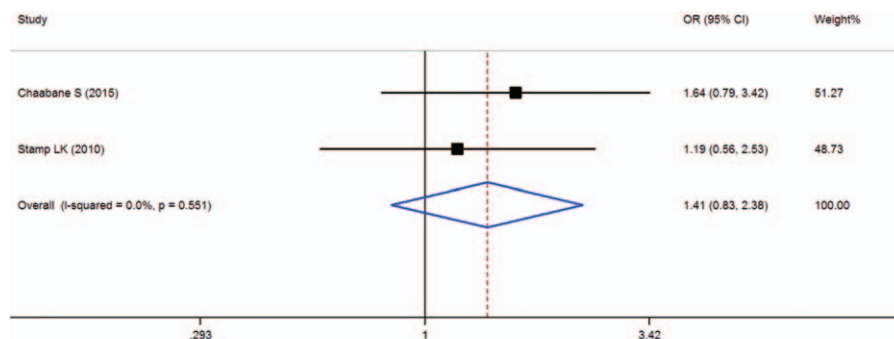MTRR = methionine synthase reductase, MTX = methotrexate.

Figure 9. Meta-analysis of MTRR 66A>G(rs1801394) single-nucleotide polymorphism and associated risk of toxicity of MTX (AA vs AG+GG genotypes). % weight = the percentage weight attributed to each study in the meta-analysis, CI = confidence interval, OR = odds ratio. Squares represent point estimates for effect size expressed as a OR with the size proportional to the inverse variance of the estimate. Lines represent 95% CIs. Diamond represents the overall pooled estimate.

Table 7

Summary of the analyzed studies and the distribution of methylenetetrahydrofolate reductase RFC −1 80G>A (rs1051266) genotypes.

		Genotype counts							
		With AE (Case)		Without AE (Control)			Mean disease	MTX dose (mg per week)	Date of end
Study	Study design	GG	GA+AA	GG	GA+AA	Mean age, y	duration, y	(range or mean±SD)	point, wk
Muralidharan N et al, 2015[28]	Prospective cohort	27	40	75	185	42.73±0.56	3.76±0.23	16.75±4	16
Świerkot J et al, 2015[9]	Prospective cohort	43	84	29	81	No information	No information	15 (12.5–25)	24
Lima A et al, 2014[29]	Retrospective cohort	28	49	52	104	52.0 (26.0–87.0)	10 (0.3–51.0)	15 (2.5–25)	No information
Bohanec GP et al, 2008[38]	Retrospective cohort	35	78	5	32	61 (51–69)	69.0 (40.5–137.5)mo	10.0 (10.0–12.5)	104
Takatori R et al, 2006[24]	Retrospective cohort	10	38	17	59	No information	No information	6.0 (median)	12
Moya P et al, 2016[27]	Prospective cohort	23	60	33	78	51.6±13.4	65.3±117.0 (mo)	12.33±4.10	24
Plaza-Plaza JC et al, 2012[42]	Prospective cohort	11	15	7	20	54.7±14.37	6.9±3.92 (2–22)	10±2.10 (7.5–15)	52
Drozdzik M et al, 2007[28]	Prospective cohort	1	9	42	122	21–70	7.9	7.5–15	24
Samara SA et al, 2014[10]	Retrospective cohort	35	69	7	9	No information	11.2	16.5 (7.5–25)	32
Stamp LK et al, 2010[25]	Retrospective cohort	45	91	20	31	60.5	10.4	15.0 (median) range 2.5–25.0	52

MTX = methotrexate, RFC = reduced folate carrier.

between these 2 SNPs and MTX toxicity; however, the results were inconsistent. In the last decade, 4 meta-analyses were performed in relatively large samples, and the results suggested that the 2 SNPs were not associated with the toxicity of MTX in RA.[4,13–15] The present study updated the meta-analysis, and a significant association was not observed between either the 677C>T (rs1801133) allele or the 1298A>C (rs1801131) allele and the MTX toxicity (OR=0.75, 95% CI: 0.53–1.06, Z=1.61, P=0.107 and OR=1.02, 95% CI: 0.74–1.39, Z=0.10, P=0.923, respectively). In addition, stratification by ethnicity did not identified a significant association between the MTHFR

A1298C (rs1801131) 3 allele frequency and MTX toxicity in the European (OR=0.86, 95% CI: 0.52–1.42, Z=0.59, P=0.558), and also did not identify a significant association between the MTHFR 677C>T (rs1801133) 3 allele frequency and MTX toxicity in the European (OR=0.76, 95% CI: 0.43–1.34, Z=0.94, P=0.348) or East Asian populations (OR=0.48, 95% CI: 0.21–1.07, Z=1.79, P=0.074).

ATIC is an important gene in the adenosine pathway, and it encodes an enzyme involved in the release of extracellular adenosine, which may have anti-inflammatory properties. Muralidharan et al[22] reported that ATIC 347C>G gene
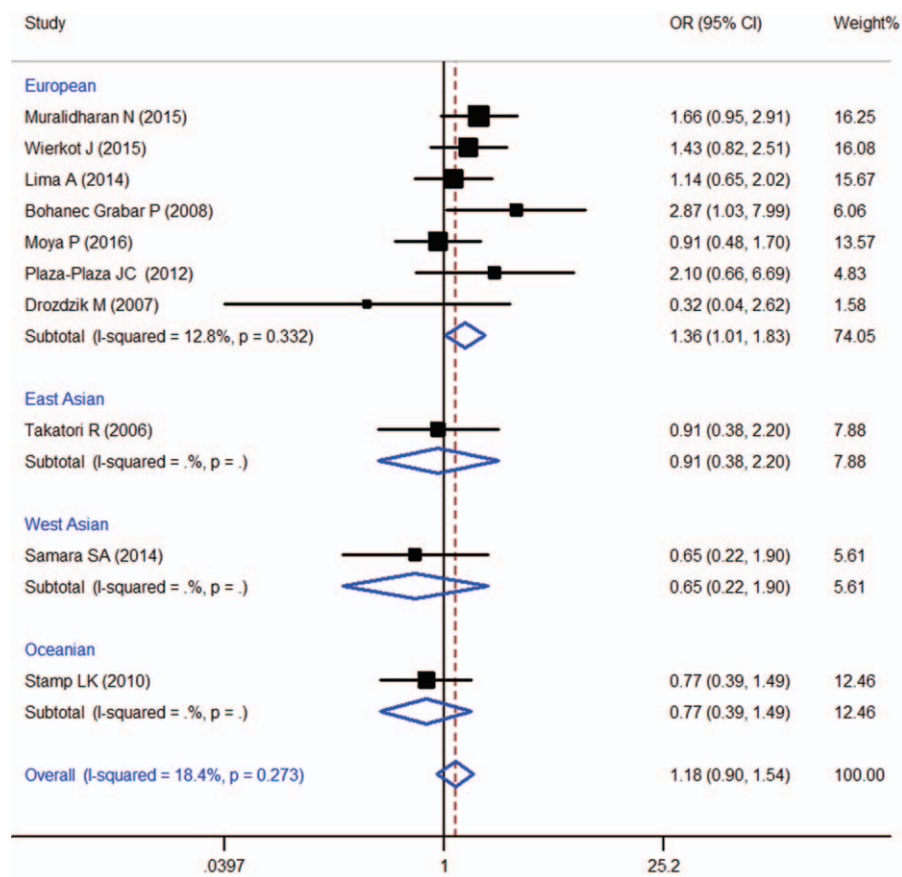
Figure 10. Meta-analysis of RFC-1 80G > A (rs1051266) single-nucleotide polymorphism and associated risk of toxicity of MTX (GG vs GA+AA genotypes). % weight = the percentage weight attributed to each study in the meta-analysis, CI = confidence interval, OR = odds ratio. Squares represent point estimates for effect size expressed as a OR with the size proportional to the inverse variance of the estimate. Lines represent 95% CIs. The diamonds represent the overall pooled estimate.

Table 8

Summary of the analyzed studies and the distribution of methylenetetrahydrofolate reductase ABCB1 3435 C > T (rs1045642) genotypes.

		Genotype counts							
		With AE		Without AE					
		(Case)		(Control)			**Mean disease**	**MTX dose (mg per week)**	**Date of end**
Study	**Study design**	**TT**	**CC+CT**	**TT**	**CC+CT**	**Mean age, y**	**duration, y**	**(range or mean ± SD)**	**point, wk**
Muralidharan N et al, 2015 [31]	prospective cohort	13	54	100	169	42.6 ± 0.5	3.8 ± 0.2	16.8 ± 0.2	16
Bohanec GP et al, 2008 [38]	Retrospective cohort	28	85	5	32	Median age: 54.2	Responders: 7.3 (6.8–8.4); Inefficacy: 6.4 (5.8–10.4)	>15	24
Takatori R et al, 2006 [24]	Retrospective cohort	12	36	11	65	61 (51–69)	69.0 (40.5–137.5)mo	10.0 (10.0–12.5)	104
Plaza-Plaza JC et al, 2012 [42]	prospective cohort	5	20	7	20	54.7 ± 14.37	6.9 ± 3.92 (2–22)	10 ± 2.10 (7.5–15)	52
Stamp LK et al, 2010 [25]	Retrospective cohort	43	95	5	46	60.5	10.4	15.0 (median) range 2.5–25.0	52

MTX = methotrexate.

polymorphism may be associated with the development of MTX induced gastrointestinal adverse events. Wessels et al[23] also found that ATIC G allele carriers experienced a greater frequency of adverse events. However, a lack of association has been reported between the ATIC 347C > G gene polymorphism and the MTX toxicity.[24,25] One meta-analysis found that the significant association between the ATIC 347 GG + GC genotype and MTX toxicity in Caucasians (OR = 1.741, 95% CI 1.080–2.806, P = 0.023), but not in Asian patients.[16] In the present meta-analysis, when all of the samples were included, the association between the ATIC 347C > G (rs2372536) and MTX toxicity was not significant (OR = 0.71, 95% CI: 0.50–1.01, Z = 1.88, P = 0.060).

MTR and MTRR participate in folate metabolism and are also involved in the metabolism of adenosine. MTRR is an auxiliary factor of MTR and catalyzes the regeneration of the methylco-amine, maintains sufficient activation of MTR, and is indirectly involved in the process of in vivo methylation. The MTRR 66A >
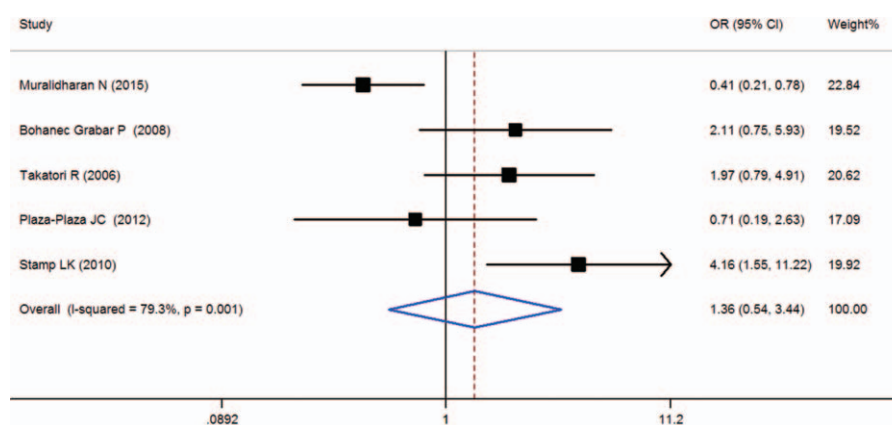
Figure 11. Meta-analysis of ABCB1 3435C > T (rs1045642) single-nucleotide polymorphism and associated risk of toxicity of MTX (TT vs CC + CT genotypes). % weight = the percentage weight attributed to each study in the meta-analysis, CI = confidence interval, OR = odds ratio. Squares represent point estimates for effect size expressed as a OR with the size proportional to the inverse variance of the estimate. Lines represent 95% CIs. Diamond represents the overall pooled estimate.

G gene polymorphism might affect the activity of the enzyme and the pharmacological effects of MTX. Dervieux et al[26] observed that patients with A/A genotype at MTR 2756 and patients with G/G genotype at MTRR 66 had a significantly higher risk for gastrointestinal ADR than patients with MTR 2756G and MTRR 66A alleles. For the MTR A2756G (rs1805087) and MTRR 66A > G (rs1801394), 3 and 2 studies were included respectively in the present meta-analysis, but no significant association was observed between the 2 genotype and MTX toxicity.

Solute carriers, especially SLC19A1/RFC-1 and ABCs (ABCC1–4, ABCB1, and ABCG2) are 2 groups of MTX transporters that influence cellular MTX uptake and efflux. The RFC-1 80G > A (rs1051266), and ABCB1 3435C > T (rs1045642) polymorphisms were included in the present meta-analysis.

For RFC-1 80G > A (rs1051266), 10 studies with a total of 791 patients with AE and 1008 patients without AE were included in the present meta-analysis. When all of the samples were included, no significant association was found between the RFC-1 80G > A (rs1051266) 3 allele frequency and MTX toxicity was identified (OR = 1.18, 95% CI: 0.90–1.54, Z = 1.21, P = 0.225). Moreover, the stratification by ethnicity identified a significant association between the RFC -1 80G > A (rs1051266) 3 allele frequency and MTX toxicity in Europeans (OR = 1.36, 95% CI 1.01–1.83, Z = 2.05, P = 0.041). This result was inconsistent with a previous meta-analyses, which found that the RFC-1 80G > A polymorphism was not associated with toxicity to MTX therapy,[1] and differences in the inclusion and exclusion criteria are the main reasons for these inconsistent conclusions. In the present study, we only focused on the association between gene polymorphisms and the toxicity to MTX monotherapy in RA patients and did not investigate gene–gene interactions.[65] In addition, combined MTX and biologic disease-modifying anti-rheumatic drug (bDMARD) treatment[66] were excluded from the meta-analysis of the RFC1 80G > A (rs1051266) polymorphism. Remarkably, the research from Lima et al[67] was included in the present research but not in a previous meta-analysis because the same SNP (rs1051266) was identified by a different name (SLC19A1 G > A).

For the ABCB1 3435C > T (rs1045642) polymorphism, a previous meta-analysis that included 2 studies founded that MTX treatment toxicity was associated with the ABCB1 C3435T polymorphism in RA when an over-dominant model (TC vs TT + CC) was used (OR 0.483, 95% CI 0.259–0.900, P = 0.022), indicating that heterozygotes (TC) for the polymorphism had a lower risk for developing MTX toxicity than homozygotes (TT and CC).[12] The present meta-analyses included 5 studies with 391 patients with AE and 460 patients without AE. When all of the samples were included, no significant association between the ABCB1 3435C > T (rs1045642) 3 allele frequency and MTX toxicity was found (OR = 1.36, 95% CI: 0.54–3.44, Z = 0.65, P = 0.518).

In addition to the above MTX transporter genes, an increased likelihood of toxicity has been reported to be associated with ABCB1 SNPs, rs868755, rs10280623, and rs1858923.[27] Stamp et al[25] reported that there were weak associations between central nervous system adverse effects and AMPD1 34C > T (P = 0.04) and between gastrointestinal adverse effects and MTHFD1 1958G > A (P = 0.03) and ABCC2 IVS23 + 56T > C (P = 0.045), and there was a stronger association between any adverse effect and ABCG2 914C > A (P = 0.004). Lima et al demonstrated that SLC19A1, SLC46A1, and SLCO1B1 genotypes may help to identify patients with increased risk of MTX-related overall toxicity and that SLC19A1 and SLCO1B1 genotypes, and SLC19A1 haplotypes may help to identify patients with increased risk of MTX-related gastrointestinal toxicity.[68]

Certain limitations of our meta-analysis warrant consideration. First, the possibility of publication bias is always a concern. Although our analysis did not observe clear evidence of such a bias, it should be recognized that publication bias is difficult to exclude with certainty, especially when the number of incorporated studies is small. Second, publication bias could have distorted our meta-analysis because of the small number of included studies. We included 20, 16, 4, 3, 2, 10, and 5 studies in the meta-analysis of the MTHFR (677C > T (rs1801133) and 1298A > C (rs1801131)), ATIC 347C > G (rs2372536), MTR A2756G (rs1805087), MTRR 66A > G (rs1801394), RFC-1 80G > A (rs1051266), and ABCB1 C3435T (rs1045642) polymorphisms, respectively. Third, heterogeneity and confounding factors may have affected the meta-analysis. Variables such as sex, rheumatoid factor status, disease duration, and even patient's reports all have the potential to influence this analysis.

Taken together, this SR and meta-analysis demonstrated an association between MTX toxicity in RA patients and the RFC -1 80G > A (rs1051266) allele in European patients. Significant associations were not observed between the MTHFR (677C > T (rs1801133) and 1298A > C (rs1801131)), ATIC 347C > G (rs2372536), MTR 2756A > G (rs1805087), MTRR 66A > G (rs1801394), ABCB1 3435C > T (rs1045642), and RFC-1 80G > A (rs1051266, when all the patients were included) and the toxicity of MTX in RA patients. However, larger and more stringent study designs may provide more accurate results for the effect of these SNPs on the MTX treatment response.

References

[1] 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;66:1111–20.

[2] 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.

[3] 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;13:137–47.

[4] Song GG, Bae SC, Lee YH. Association of the MTHFR C677T and A1298C polymorphisms with methotrexate toxicity in rheumatoid arthritis: a meta-analysis. Clin Rheumatol 2014;33:1715–24.

[5] Lee YH, Bae SC, Song GG. Association of the ABCB1 C3435T polymorphism with responsiveness to and toxicity of DMARDs in rheumatoid arthritis: a meta-analysis. Z Rheumatol 2016; 75:707–15.

[6] Kyburz D, Gabay C, Michel BA, et al. The long-term impact of early treatment of rheumatoid arthritis on radiographic progression: a population-based cohort study. Rheumatology (Oxford) 2011;50: 1106–10.

[7] Finckh A, Liang MH, van Herckenrode CM, et al. Long-term impact of early treatment on radiographic progression in rheumatoid arthritis: a meta-analysis. Arthritis Rheum 2006;55:864–72.

[8] Jekic B, Lukovic L, Bunjevacki V, et al. Association of the TYMS 3G/3G genotype with poor response and GGH 354GG genotype with the bone marrow toxicity of the methotrexate in RA patients. Eur J Clin Pharmacol 2013;69:377–83.

[9] Świerkot J, Ślęzak R, Karpiński 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: 152–61.

[10] Samara SA, Irshaid YM, 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;52:746–55.

[11] Saleh MM, Irshaid YM, Mustafa KN. Methylene tetrahydrofolate reductase genotypes frequencies: association with toxicity and response to methotrexate in rheumatoid arthritis patients. Int J Clin Pharmacol Ther 2015;53:154–62.

[12] Lee YH, Bae SC, Song GG. Association of the ABCB1 C3435T polymorphism with responsiveness to and toxicity of DMARDs in rheumatoid arthritis: a meta-analysis. Z Rheumatol 2016;75:707–15.

[13] 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;22:117–33.

[14] Lee YH, 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;30:101–8.

[15] Mark C, Fisher BNC. Meta-analysis of methylenetetrahydrofolate reductase (MTHFR) polymorphisms affecting methotrexate toxicity. J Rheumatol 2009;36:539–45.

[16] Lee YH, 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;36:1591–9.

[17] Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ 2009;339:b2535.

[18] Zheng F, Zhang Y, Xie W, et al. Further evidence for genetic association of CACNA1C and schizophrenia: new risk loci in a Han Chinese population and a meta-analysis. Schizophr Res 2014;152:105–10.

[19] Liu O, Jia L, Liu X, et al. Clopidogrel, a platelet P2Y12 receptor inhibitor, reduces vascular inflammation and angiotensin II induced-abdominal aortic aneurysm progression. PLoS One 2012;7:e51707.

[20] Jia LX, Qi GM, Liu O, et al. Inhibition of platelet activation by clopidogrel prevents hypertension-induced cardiac inflammation and fibrosis. Cardiovasc Drugs Ther 2013;27:521–30.

[21] Ghodke-Puranik Y, Puranik AS, Shintre P, et al. Folate metabolic pathway single nucleotide polymorphisms: a predictive pharmacogenetic marker of methotrexate response in Indian (Asian) patients with rheumatoid arthritis. Pharmacogenomics 2015;16:2019–34.

[22] 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;17:241–8.

[23] 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;54:2830–9.

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

[25] 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;20:367–76.

[26] Dervieux T, Greenstein N, Kremer J. Pharmacogenomic and metabolic biomarkers in the folate pathway and their association with methotrexate effects during dosage escalation in rheumatoid arthritis. Arthritis Rheum 2006;54:3095–103.

[27] Moya P, Salazar J, Arranz MJ, et al. Methotrexate pharmacokinetic genetic variants are associated with outcome in rheumatoid arthritis patients. Pharmacogenomics 2016;17:25–9.

[28] 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;35:879–85.

[29] Lima A, Bernardes M, Sousa H, et al. SLC19A1 80G allele as a biomarker of methotrexate-related gastrointestinal toxicity in Portuguese rheumatoid arthritis patients. Pharmacogenomics 2014;15: 807–20.

[30] 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;41:385–93.

[31] 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;71:959–65.

[32] 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.

[33] Milic V, Jekic B, Lukovic L, et al. Association of dihydrofolate reductase (DHFR) -317AA genotype with poor response to methotrexate in patients with rheumatoid arthritis. Clin Exp Rheumatol 2012;30: 178–83.

[34] Tasbas Ö, Borman P, Karabulut HG, 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;5:30–5.

[35] Cáliz R, del AJ, 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;41:10–4.

[36] 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;28:728–33.

[37] Mena JP, Salazar-Páramo M, González-López L, et al. Polymorphisms C677T and A1298C in the MTHFR gene in Mexican patients with rheumatoid arthritis treated with methotrexate: implication of elevation of transaminases. Pharmacogenomics J 2011;11:287–91.

[38] Bohanec GP, 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;64:1057–68.

[39] Berkun Y, Abou AI, 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;34:1664–9.

[40] 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;63:1227–31.

[41] 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;35:1149–61.

[42] Plaza-Plaza JC, Aguilera M, Cañadas-Garre M, et al. Pharmacogenetic polymorphisms contributing to toxicity induced by methotrexate in the southern Spanish population with rheumatoid arthritis. OMICS 2012;16:589–95.

[43] Borman P, Tasbas Ö, Karabulut H, et al. Thymidylate synthase genetic polymorphism and plasma total homocysteine level in a group of Turkish patients with rheumatoid arthritis: relationship with disease activity and methotrexate toxicity. Rev Bras Reumatol 2015;55:485–92.

[44] 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;124:521–6.

[45] 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.

[46] 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;27:787–9.

[47] van Ede AE, Laan RF, Blom HJ, et al. The C677T mutation in the methylenetetrahydrofolate reductase gene. Arthritis Rheum 2001;44:2525–30.

[48] 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;32:324–32.

[49] 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;54:1087–95.

[50] Choe JY, Park SH, Kim SK. MTHFR polymorphisms, C677T and A1298C, are not a genetic risk factor for liver stiffness index in methotrexate-treated rheumatoid arthritis patients. Clin Exp Rheumatol 2011;29:144–5.

[51] 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;17:383–90.

[52] 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;33:1266–74.

[53] Urano W, Taniguchi A, Yamanaka H, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene were associated with both the ef®cacy and the toxicity of methotrexate used for the treatment of rheumatoid arthritis, as evidenced by single locus and haplotype analyses. Pharmacogenetics 2002;12:183–90.

[54] Owen SA, Hider SL, Martin P, et al. Genetic polymorphisms in key methotrexate pathway genes are associated with response to treatment in rheumatoid arthritis patients. Pharmacogenomics J 2013;13:227–34.

[55] Kooloos WM, Wessels JA, van der Straaten T, et al. Functional polymorphisms and methotrexate treatment outcome in recent-onset rheumatoid arthritis. Pharmacogenomics 2010;11:163–75.

[56] Salazar J, Moya P, Altés A, et al. Polymorphisms in genes involved in the mechanism of action of methotrexate: are they associated with outcome in rheumatoid arthritis patients. Pharmacogenomics 2014;15:1079–90.

[57] Zhu H, Deng FY, Mo XB, et al. Pharmacogenetics and pharmacogenomics for rheumatoid arthritis responsiveness to methotrexate treatment: the 2013 update. Pharmacogenomics 2014;15:551–66.

[58] Malik F, Ranganathan P. Methotrexate pharmacogenetics in rheumatoid arthritis: a status report. Pharmacogenomics 2013;14:305–14.

[59] 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;32:1837–42.

[60] Ranganathan P. An update on methotrexate pharmacogenetics in rheumatoid arthritis. Pharmacogenomics 2008;9:439–51.

[61] Liu O, Li JR, Gong M, et al. Genetic analysis of six SNPs in candidate genes associated with high cross-race risk of development of thoracic aortic aneurysms and dissections in Chinese Han population. Acta Pharmacol Sin 2010;31:1376–80.

[62] Liu O, Li J, Xin Y, et al. Association of MMP-2 gene haplotypes with thoracic aortic dissection in chinese han population. BMC Cardiovasc Disord 2016;16:11.

[63] Wang XL, Liu O, Qin YW, et al. Association of the polymorphisms of MMP-9 and TIMP-3 genes with thoracic aortic dissection in Chinese Han population. Acta Pharmacol Sin 2014;35:351–5.

[64] Liu O, Xie W, Qin Y, et al. MMP-2 gene polymorphisms are associated with type A aortic dissection and aortic diameters in patients. Medicine (Baltimore) 2016;95:e5175.

[65] Dervieux T, Wessels JA, van der Straaten T, et al. Gene-gene interactions in folate and adenosine biosynthesis pathways affect methotrexate efficacy and tolerability in rheumatoid arthritis. Pharmacogenet Genomics 2009;19:935–44.

[66] James HM, Gillis D, Hissaria P, et al. Common polymorphisms in the folate pathway predict efficacy of combination regimens containing methotrexate and sulfasalazine in early rheumatoid arthritis. J Rheumatol 2008;35:562–71.

[67] Aurea Lima MB, Rita Azevedo RM, Seabra V. Pharmacogenomics of methotrexate membrane transport pathway: can clinical response to methotrexate in rheumatoid arthritis be predicted. Int J Mol Sci 2015;16:13760–80.

[68] Lima A, Bernardes M, Azevedo R, et al. SLC19A1, SLC46A1 and SLCO1B1 polymorphisms as predictors of methotrexate-related toxicity in Portuguese rheumatoid arthritis patients. Toxicol Sci 2014;142:196–209.