Personalized medicine in rheumatoid arthritis

Eektimmerman, F.

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Chapter 2

Predictive genetic biomarkers for the efficacy of methotrexate in rheumatoid arthritis: a systematic review

Frank Eektimmerman
Jesse J. Swen
Moenira B. Madhar
Cornelia F. Allaart
Henk-Jan Guchelaar

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Abstract

Multiple pharmacogenetic studies investigated the effectiveness of methotrexate. However, due to the use of non-validated outcomes, lack of validation or conflicting results it remains unclear if genetic markers can help to predict response to MTX treatment. Therefore, a systematic review was performed. PubMed was searched for articles reporting potential pharmacogenetic biomarkers associated ($p<0.05$) with MTX efficacy using the validated endpoints DAS(28), EULAR, or ACR response criteria. The PICO method was used for study selection, and PRISMA guidelines to prepare the report. Thirty-five studies met the inclusion criteria, providing 39 potential genetic biomarkers in 19 genes. After Bonferroni correction, six genetic biomarkers were associated with the efficacy of MTX: *ATIC* rs7563206; *SLC19A1* rs1051266; *DHFR* rs836788; *TYMS* rs2244500, rs2847153, and rs3786362 in at least one study. Only *SLC19A1* rs1051266 was replicated in an independent cohort and promising for predicting methotrexate efficacy.

INTRODUCTION

Low-dose methotrexate (MTX) is considered the "anchor drug" for the treatment of rheumatoid arthritis (RA). The precise mechanism of action of MTX remains to be elucidated, but it is known that MTX is transported over the membrane by multiple solute carriers (SLC) and that intracellular MTX has to be bound to polyglutames molecules by folylpolyglutamate synthase (FPGS) to exert its function. As illustrated in Figure 2-1, the polyglutamated MTX affects multiple cellular pathways, e.g., adenosine, *de novo* purine synthesis, folate, methionine, and *de novo* pyrimidine synthesis.

In particular, an essential function of the folate pathway is to provide cofactors for key enzymes, such as dihydrofolate reductase (DHFR) that converts dihydrofolate into the folic acid derivative tetrahydrofolate (THF). THF and other derivatives are required for the purine and pyrimidine synthesis, which are important for cell proliferation and cell growth.[1] The methionine pathway is responsible for the synthesis of adenosine, which is an anti-inflammatory agent, alterated by methionine synthase and methionine synthase reductase (MTRR). Further, methionine is a precursor for S-adenosyl-methionine, which is a methyl donor that serves a variety of cellular functions, including DNA methylation.[2] The ubiquitin pathway is not directly related to the other pathways, but has an essential function in homeostasis and recognition of MHC class 1 for the cytotoxic T cells.[3]

Approximately one-third of RA patients experience insufficient clinical response to MTX. Pharmacogenetics studies the impact of genetic variation to drug response and genetic variants in the MTX pathways described above may affect the potential effects of methotrexate on inflammation in RA. Indeed, multiple studies reported associations between single nucleotide polymorphisms (SNPs) and the efficacy of MTX. However, to date, none of the proposed markers are applied in clinical practice due to lack of validation or conflicting results. In addition, previous systematic reviews[4–10] described the effect of SNPs on the efficacy of MTX, but some included studies with MTX in different diseases such as juvenile idiopathic arthritis[10] or leukemia[5] or applied non-validated endpoints, such as red blood cell MTX polyglutamate concentrations[5,11] or physicians' assessment of patient's response.[9]

The goal of this review is to systematically explore which SNPs related to MTX pharmacology are associated with efficacy in RA by selecting only studies with the validated endpoints DAS(28), European League Against Rheumatism (EULAR), or American College of Rheumatology (ACR) response criteria.[12,13]

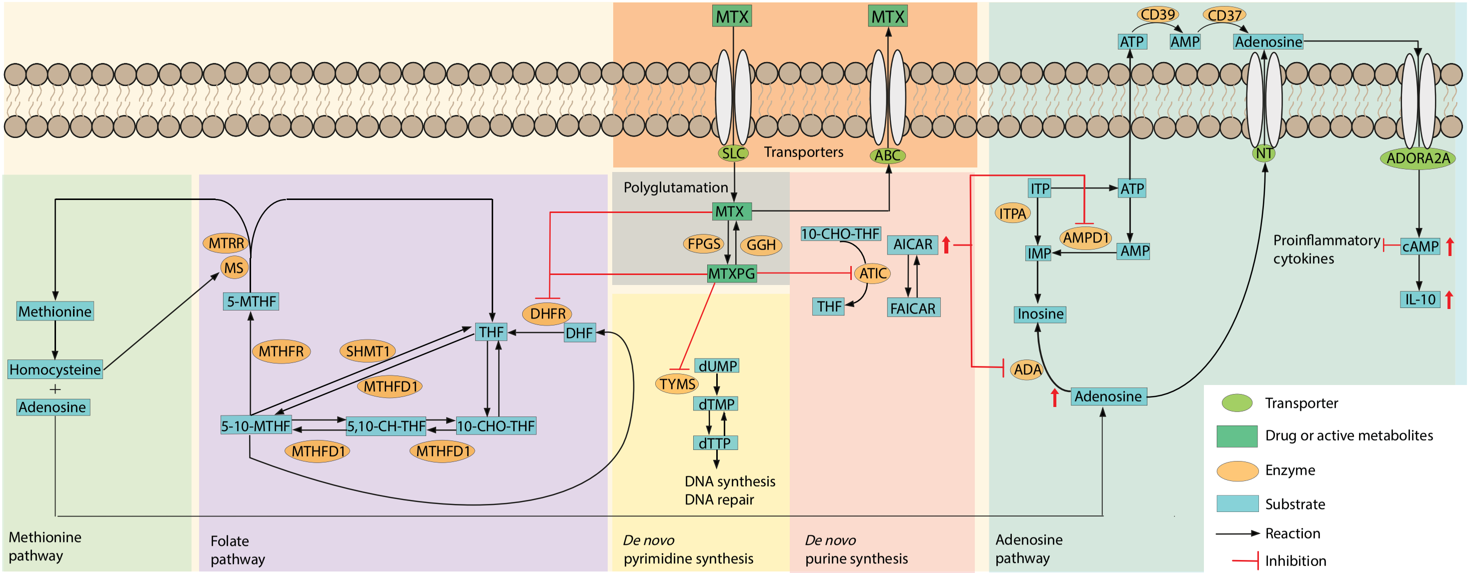

Figure 2-1. Intracellular MTX mechanism pathway, divided into the methionine, folate, *de novo* pyrimidine synthesis, *de novo* purine synthesis, and adenosine pathway.
Abbreviations: 10-CHO-THF: 10-formyltetrahydrofolate, 5,10-CH-THF: 5,10-methylenetetrahydrofolate, 5-MTHF: 5-methyltetrahydrofolate, ABC: ATP-binding cassette transporter, ADA: adenosine deaminase, ADORA2A: adenosine A2A receptor, AICAR: 5-aminoimidazole-4-carboxamide ribonucleotide, AMP: adenosine monophosphate, AMPD1: adenosine monophosphate deaminase 1 ATIC 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase, ATP: adenosine triphosphate, cAMP: cyclic adenosine monophosphate, CD37: transmembrane protein, CD39: transmembrane protein, DHF: dihydrofolate, DHFR: dihydrofolate reductase, dTMP: deoxythymidine monophosphate, dTTP: deoxythymidine triphosphate, dUMP: deoxyuridine monophosphate FAICAR 5-formamidoimidazole-4-carboxamide ribotide, FPGS: folylpolyglutamate synthase, GGH: γ-glutamyl hydrolase, IL-10: interleukin-10, IMP: inosine monophosphate, IPTA: inosine triphosphatase, MS: methionine synthase, MTHFD1: methylenetetrahydrofolate dehydrogenase 1, MTHFR: methylene tetrahydrofolate reductase, MTRR: methionine synthase reductase, MTX: methotrexate, MTXPG: methotrexate polyglutamate, NT: nucleoside transporter, SHMT-1: serine hydroxymethyltransferase 1, SLC: solute carrier, THF: tetrahydrofolate, TYMS: thymidylate synthase.

METHODS

Data extraction and identification of eligible studies Identification and selection of studies were performed according to the PICO method.[14] Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were used to prepare the report.[15] PubMed was used to identify and extract all relevant articles published between April 2002 and March 2017. Search terms consisted of rheumatoid arthritis, methotrexate, pharmacogenetics, and SNP. The full search string is provided in Supplementary File S2-1. Also, we manually checked reference lists from reviews to identify relevant cross-references.

Records were screened on title and abstract. Comments, editorials, narrative reviews, letters (without original data), abstracts, and publications in languages other than English were excluded. Only studies utilizing the DAS(28), the response criteria of the ACR or the EULAR were eligible for inclusion. Included SNPs were analyzed under the additive, allelic, genotypic or haploid genetic model, and had at least one association with either DAS(28), ACR or EULAR response ($p<0.05$, uncorrected for multiple testing). SNPs were divided into MTX-related pathways: adenosine, *de novo* purine synthesis, transporters, polyglutamation, folate, methionine, *de novo* pyrimidine synthesis, and ubiquitin. Results from included studies were summarized, and reported odds ratio (OR) with 95% confidence interval (CI), p-value, type of association and SNP ID were collected. Finally, SNPs were checked on linkage disequilibrium by SNP Annotation and Proxy Search (SNAP, Broad Institute),[16] with the LD threshold of $R^2>0.8$.

To control the risk of false positive findings, Bonferroni correction was applied when no correction for multiple testing was performed in the original study by calculating a significant cutoff p-value at α/n ($p=0.05$ divided by the number of tested SNPs within each study). SNPs were significantly associated if the p-value was <0.05 after Bonferroni correction. Ultimately meta-analyses were used to support our findings of potential significant SNPs.

RESULTS

Study selection

Figure 2-2 shows the results of the study selection. Initially, 115 publications were identified. We excluded 30 comments, editorials, letters, narrative reviews, and seven non-English written publications. Of the remaining 78 studies, 41 were excluded because none of our defined endpoints was reported and one because the report of the study could not be obtained. By cross-references, three more studies were included. In total, 35 original studies were available for analysis in this systematic review and seven meta-analyses were used to support our findings.

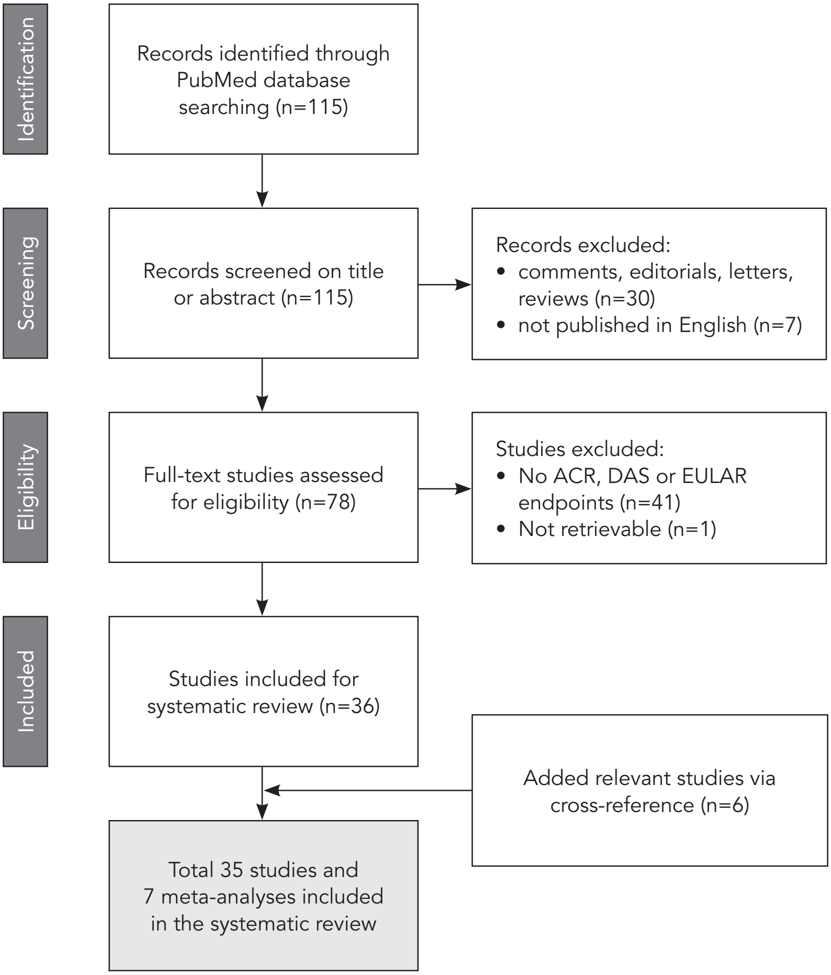

Figure 2-2. Study flow diagram of the systematic review inclusion.[15]
Abbreviations: MTX: methotrexate, MAF: minimum allele frequency, ACR: American College of Rheumatology, DAS: Disease Activity Score, EULAR: European League Against Rheumatism.

Study characteristics

Most studies (34 out of 35) were candidate gene studies investigating 1–35 polymorphisms. There was one genome-wide association study (GWAS) investigating 559,007 polymorphisms.[17] The mean study population of the studies was 197 patients (ranging from 48 to 422 patients). Most studies used the EULAR good response criteria (32%), tested <10 SNPs (76%), were conducted in Europe with RA patients of (self-)reported Caucasian origin. The average rate of good EULAR response to MTX monotherapy at t=6 months was 55%, ranging from 23[18] to 85%.[19]

The included studies reported 39 SNPs in 20 genes associated with either DAS(28), EULAR, or ACR response with a $p<0.05$. After Bonferroni correction, 16 SNPs in 10 genes remained significantly associated with MTX efficacy.

Adenosine pathway – *ADA, ADORA2A, AMPD1*, and *ITPA*

AMPD1 rs17602729 (allelic T) showed a significant association with DAS28≤3.2 (OR: 6.73, 95% CI: 1.74–26.01) between t=3 and 6 months.[20] However, this was not confirmed with the genotypic CC model at t=6 months.[21] None of the other SNPs in the adenosine pathways – *ADA* (rs244076), *ADORA2A* (rs5751876), and *ITPA* (rs1127354) – were significantly associated with the MTX response at t=6 months using allelic or genotypic genetic models.

De novo purine synthesis – *ATIC*

Four SNPs in *ATIC* (rs2372536,[22] rs4673993,[23] rs7563206,[1] and rs12995526[1]) had at least one study reporting a significant association with MTX efficacy. *ATIC* rs7563206 (allelic T-carrier) was tested in one study, and showed an association with MTX non-response with the endpoint DAS28≤3.2 at t=6 months (OR: 0.20, 95% CI: 0.09–0.46).[1] At t=6 months, *ATIC* rs4673993 (genotypic TT) showed a significant association with a better response (DAS28≤3.2, OR: 3.86 95% CI:1.50–9.91), while rs12995526 (allelic T-carriers) showed a significant association with a worse response (DAS28≤3.2, OR: 0.23, 95% CI: 0.10–0.53) to MTX.[23]

ATIC (rs2372536, genotype CC) was significantly associated with DAS≤2.4 at t=6 months, with an OR of 2.5 (95% CI: 1.3–4.8).[22] Three other studies – using *ATIC* rs2372536 genotypic CC at t=6 months – reported no significant association, of which one study reported that the CC genotype was related to MTX non-response with a OR below 1.0 (OR: 0.27, 95% CI: 0.08–0.92).[1,20,24]

Transporters – *ABCB1C1*, *ABCC1*, *SLC19A1* (*RFC1*), and *SLC22A11*

None of the SNPs in *ABCB1* (rs1045642), *ABCC1* (rs246240 and rs3784864), and *SLC22A11* (rs11231809) were significantly associated with DAS28≤3.2 or EULAR good response at t=6 months. The most studied genetic *SLC19A1* SNP was rs1051266, which was investigated in 11 studies. Three studies reported a significant association with MTX efficacy at t=6 months using ACR20 or DAS28 and different genetic models (either allelic A-carriers, genotypic GG or genotypic AA). Other studies did not investigate the same genetic models, using the same efficacy endpoints with the same time evaluation point for *SLC19A1* rs1051266.

Polyglutamation – *FPGS* and *GGH*

FPGS rs4451422 (allelic C-carriers) was associated with MTX efficacy using EULAR good response at t=6 months, with an OR of 0.73 (0.54–0.98).[17] *FPGS* SNPs (rs1544105, rs10106, and rs10987742) and *GGH* SNPs (rs2305558 and rs1800909) were not significantly associated with MTX efficacy.

Folate pathway – *DHFR*, *MTHFR*, and *SHMT*

Both *MTHFR* rs1801131 (A1298C) and rs1801133 (C677T) have frequently been studied (>10 studies). One study showed a significant association with *MTHFR* rs1801133 CC genotype with DAS28 ≤.2 at t=6 months, with an OR of 3.4.[25] Three other studies investigated the association of *MTHFR* genotypic CC at t=6 months, and did not find an association using other endpoints (EULAR GR, ΔDAS44 <0.6, and ACR20).[26–28] For two other SNPs in *MTHFR* (rs17421511 and rs1476413) there was no significant association with MTX response. Also, no association was found between *MTHFD1* rs17850560 or *SHMT-1* rs1979277 with MTX response using DAS28 (≤3.2) or EULAR GR. *DHFR* rs836788 was associated in one study with EULAR response at t=6 months, with an OR of 1.44 (95% CI: 1.09–1.93) and 1.47 (95% CI: 1.09–1.96), respectively for the allelic A-carriers and the genotypic AA.[17]

Methionine pathway – *MTR* and *MTRR*

Six studies investigated the role of the *MTR* A2756G (rs1805087), of which one study reported a significant association.[19] Here, *MTR* rs1805087 was associated with MTX efficacy at t=12 month, and the use of the endpoint EULAR good response with the genotypic AA (OR was not available). Other studies could not confirm the association with rs1805087, using the DAS28 with genotypic AA on t=4 months,[29] EULAR GR with the allelic G-carriers on t=4 months,[30] or with the DAS28≤3.2 allelic G-carriers on t=6 months.[31] No significant association was reported with *MTRR* rs162040 and rs1801394.

De novo pyrimidine pathway – *TYMS*

TYMS rs2244500, rs2847153, and rs3786362 were all significantly associated with EULAR good response at t=6 months and had OR of resp. 1.48, 0.68, and 0.51.[17] No other studies investigated the effect of *TYMS* with MTX response.

Ubiquitin pathway – *CUL1*

Negi *et al*. investigated the association of *CUL1* haplotypes with MTX efficacy using the DAS28≤3.2 at t=6 months.[32] Here, *CUL1* rs122571 haplotype A-T-T (OR: 2.83, 95% CI: 1.33–6.04) and rs243480 haplotype G-T-T (OR: 0.16, 95% CI 0.04–0.67) were significant.

KIR – gene

One study tested multiple length variants of the KIR gene and showed that the full-length *KIR2DS4* gene was significantly associated with DAS28≤2.5 (OR: 0.4344, 95% CI: 0.215, 0.987) at t=6 months.[33] Here, possessing the KIRSDS4 gene had a lower chance of responding to MTX treatment.

Most promising genetic variants related to MTX efficacy

Table 2-1 lists the most promising SNPs that were significantly associated with MTX efficacy after Bonferroni correction without having conflicting results from other studies. For instance, it is ATIC rs467393 genotypic TT with better response, while allelic T-carriers results in worse response or lacks validation.

The most promising SNPs were derived from the pathways *de novo* purine (*ATIC*), *de novo* pyrimidine (TYMS), and transporters (SLC19A1). The SNPs have a minor allele frequency >0.2, except TYMS rs3786362 (MAF<0.2 for all races). ATIC rs7563206 and TYMS rs2244500 were found significantly associated with an OR below 1.0, while the other eight SNPs had an OR between 1.42 and 2.83. The used genetic models were with either allelic, genotypic or haplotype. No linkage disequilibrium (R^2>0.8) was observed for any of the SNPs in Table 2-2. SLC19A1 rs1051266 was tested in multiple studies and positively associated in three studies.

Of the six promising SNPs, ATIC rs7563206, TYMS rs2847153, and rs3786362 were associated with non-response to MTX, while SLC19A1 rs1051266, DHFR rs836788, and TYMS rs2244500 were associated with response to MTX. ORs range from 0.2 to 0.68 for MTX non-response and 1.42–2.76 for MTX response. The six SNPs had a MAF of >0.2 in all races except for TYMS rs3786362 which is sparse and even does not occurred in the European population.

Despite the findings of one significant association of ATIC rs473993 and rs12995526, AMPD1 rs17602729, MTHFR rs1801133, and MTR rs180508, and FPGS rs4451422, we did not mark those as promising genetic variants due to conflicting results. Also, we did not include the full-length KIR2DS4 gene as a promising genetic marker for the response to MTX, due to the complexity of the determination of the whole KIR2DS4 gene (with 15,894 bases) and the fact that it is not one SNP. This was also the case of CUL1 that was significantly associated with MTX response for two haplotypes; A-T-T (rs122571) and G-T-T (rs243480).

Table 2-1. Genetic biomarkers related to MTX efficacy

Gene	SNPs	Genetic model	Endpoint	Time of response evaluation (months)	N	Reported P-value	OR (95%CI)	Study
Adenosine pathway								
ADA	rs244076	Allelic A carriers	EULAR GR	6	281	**0.02**	1.66 (1.01–2.75)	Sharma (2009)[21]
		Genotypic AA	EULAR GR	6	281	0.17	-	Sharma (2009)[21]
ADORA2A	rs5751876	Allelic C carriers	EULAR GR	6	281	**0.04**	1.55 (1.01–2.37)	Sharma (2009)[21]
		Genotypic TT	EULAR GR	6	281	0.12	-	Sharma (2009)[21]
AMPD1	rs17602729	Allelic T carriers	DAS≤2.4	6	204	**<0.05**	2.1 (1.0–4.5)	Wessels (2006-2)[22]
	(C34T)	Allelic T carriers	DAS28≤3.2	3–6	205	**0.006***	6.73 (1.74–26.01)	Grabar (2010)[20]
		Allelic C carriers	EULAR GR	6	281	0.39	-	Sharma (2009)[21]
		Genotypic CC	EULAR GR	6	281	0.38	-	Sharma (2009)[21]
ITPA	rs1127354	Genotypic CC	DAS≤2.4	6	204	**<0.05**	2.7 (1.1–8.1)	Wessels 2006-2[22]
	(C94A)	Allelic A carriers	EULAR GR	4	255	**0.006**	2.95 (1.36–6.38)	Dervieux (2009)[30]

De novo purine synthesis pathway								
ATIC	rs2372536 (C347G)	Allelic C carriers	DAS28≤3.2	6	233	0.57	0.83 (0.43–1.69)	Lima (2016)[1]
		Allelic C carriers	EULAR GR	6	281	0.96	-	Sharma (2009)[21]
		Allelic C carriers	EULAR GR	12	98	0.56	-	James (2008)[19]
		Allelic C carriers	EULAR GR	6	319	0.94	0.98 (0.67–1.43)	Muralidharan (2016-1)[36]
		Allelic C carriers	ACR 20 & 50	12	217	NS	-	Ghodke-Puranik (2015)[18]
		Allelic C carriers	DAS28≤2.4	6	422	0.23	1.29 (0.87–1.91)	Kurzawski (2016)[24]
		Allelic G carriers	EULAR GR	4	255	0.71	1.09 (0.66–1.80)	Dervieux (2009)[30]
		Genotypic GG	DAS28	6	170	NS	-	Hayashi (2013)[37]
		Genotypic GG	DAS28≤2.4	6	422	**0.005**	2.40 (1.30–4.42)	Kurzawski (2016)[24]
		Genotypic CC	EULAR GR	6	281	0.17	-	Sharma (2009)[21]
		Genotypic CC	EULAR GR	12	98	0.85	-	James (2008)[19]
		Genotypic CC	DAS28≤3.2	6	233	**0.036**	0.27 (0.08–0.92)	Lima (2016)[1]
		Genotypic CC	DAS28≤3.2	3–6	208	NS	-	Grabar (2010)[20]
		Genotypic CC	DAS≤2.4	6	205	**0.007***	2.5 (1.3–4.8)	Wessels 2006-2[22]
		Genotypic CC	EULAR GR	6	61	0.12	1.95 (0.83–4.56)	Salazar (2014)[38]
	rs4673993	Allelic C carriers	DAS28≤3.2	6	233	**0.036**	0.27 (0.08–0.92)	Lima (2016)[1]
		Genotypic TT	DAS28≤3.2	6	120	**0.006***	3.86 (1.50–9.91)	Lee (2009)[23]
		Genotypic TT	DAS28≤3.2	6	233	0.95	0.98 (0.51–1.89)	Lima (2016)[1]
	rs7563206	Allelic T carriers	DAS28≤3.2	6	233	**<0.001***	0.20 (0.09–0.46)	Lima (2016)[1]
		Genotypic TT	DAS28≤3.2	6	233	0.56	0.81 (0.40–1.65)	Lima (2016)[1]
	rs12995526	Allelic T carriers	EULAR GR	6	233	**0.001***	0.23 (0.10–0.53)	Lima (2016)[1]
		Allelic T carriers	DAS28≤2.4	6	422	0.11	0.71 (0.47–1.07)	Kurzawski (2016)[24]
		Genotypic TT	DAS28≤2.4	6	422	0.14	0.65 (0.38–1.10)	Kurzawski (2016)[24]
		Genotypic TT	EULAR GR	6	233	0.41	0.74 (0.37–1.51)	Lima (2016)[1]
		Genotypic CC	EULAR GR	6	61	0.22	1.78 (0.70–4.52)	Salazar (2014)[38]

Table 2-1 continues on next page.

Table 2-1. ***Continued***

Gene	SNPs	Genetic model	Endpoint	Time of response evaluation (months)	N	Reported P-value	OR (95%CI)	Study
Transporters								
ABCB1	rs1045642 (C3435T)	Genotypic CT	DAS28≤3.2	6	281	**0.01**	1.97 (1.13–3.42)	Sharma (2008)[39]
		Genotypic CC	DAS28≤3.2	6	281	**0.01**	0.32 (0.13–0.80)	Sharma (2008)[39]
		Genotypic CC	DAS<2.4	6	186	0.77	-	Kooloos (2010)[40]
		Allelic C carriers	DAS<2.4	6	186	0.082	-	Kooloos (2010)[40]
ABCC1	rs246240	Allelic G carriers	DAS28≤3.2	6	233	**0.008**	5.47 (1.56–19.25)	Lima (2015)[31]
		Genotypic GG	DAS28≤3.2	6	233	0.85	0.76 (0.05–11.46)	Lima (2015)[31]
	rs3784864	Allelic A carriers	EULAR GR	6	233	0.40	0.64 (0.23–1.80)	Lima (2015)[31]
		Genotypic AA	EULAR GR	6	233	**0.015**	4.24 (1.32–13.65)	Lima (2015)[31]
SLC19A1 / RFC1	rs1051266 (G80A)	Allelic A carriers	ACR 20 & 50	12	217	**0.030**	2.20 (1.1–4.4)	Ghodke-Puranik (2015)[18]
		Allelic A carriers	EULAR GR	12	98	**0.009**	-	James (2008)[19]
		Allelic A carriers	ACR 20	6	174	**0.021***	3.32 (1.26–8.79)	Drozdzik (2007)[41]
		Allelic A carriers	DAS28≤3.2	6	233	0.67	1.23 (0.47–3.18)	Lima (2015)[31]
		Allelic A carriers	DAS28≤3.2	6	281	NS	-	Sharma (2008)[39]
		Allelic A carriers	EULAR GR	6	225	0.28	1.24 (0.85–1.81)	Muralidharan (2016-2)[42]
		Allelic A carriers	EULAR GR	4	255	0.07	1.63 (0.95–2.79)	Dervieux (2009)[30]
		Genotypic AA	EULAR GR	12	98	**0.036**	-	James (2008)[19]
		Genotypic AA	ACR 20%	6	174	**0.013***	1.78 (1.13–2.81)	Drozdzik (2007)[41]
		Genotypic AA	DAS28≤3.2	6	233	0.92	1.05 (0.36–3.09)	Lima (2015)[31]
		Genotypic AA	DAS28≤3.2	6	281	NS	-	Sharma (2008)[39]
		Genotypic GG	DAS28	4	255	0.27	-	Dervieux (2009)[30]
		Genotypic GG	DAS28	6	170	**0.0018***	2.27 (1.36–3.80)	Hayashi (2013)[37]
		Genotypic GG	EULAR GR	6	76	0.60	-	Moya (2016)[43]

		Genotypic GG	EULAR GR	6	54	NS	-	Chatzikyriakidou (2007)[44]
		Genotypic GG	DAS28≤3.2	6	240	NS	-	Świerkot (2015)[25]
		Genotypic GG	EULAR GR	6	225	0.56	0.81 (0.46–1.43)	Muralidharan (2016-2)[42]
SLC22A11	rs11231809	Genotypic AA	DAS28≤3.2	6	233	**0.031**	0.19 (0.04–0.86)	Lima (2015)[31]
		Allelic A carriers	DAS28≤3.2	6	233	0.12	0.44 (0.16–1.22)	Lima (2015)[31]
Polyglutamation								
FPGS	rs4451422	Allelic A carriers	DAS28≤3.2	6	232	0.077	0.52 (0.025–1.07)	Lima (2016)[1]
		Allelic C carriers	EULAR GR	6	457	**0.035*#**	0.73 (0.54–0.98)	Senapati (2014)[17]
		Genotypic AA	DAS28≤3.2	6	232	0.27	1.57 (0.70–3.49)	Lima (2016)[1]
		Genotypic CC	EULAR GR	6	457	**0.05#**	0.72 (0.52–1.00)	Senapati (2014)[17]
	rs1544105	Allelic A carriers	EULAR GR	6	281	**0.008**	3.47 (1.19–10.12)	Sharma (2008)[21]
		Allelic G carriers	DAS28≤3.2	6	233	0.32	1.53 (0.68–3.60)	Lima (2016)[1]
		Allelic G carriers	DAS28≤3.2	6	281	**0.043**	1.55 (1.01–2.37)	Sharma (2008)[39]
		Allelic A carriers	DAS28≤2.4	6	422	0.92	0.96 (0.65–1.43)	Kurzawski (2016)[24]
		Genotypic GG	DAS28≤3.2	6	233	0.12	0.56 (0.27–1.15)	Lima (2016)[1]
		Genotypic AA	DAS28≤2.4	6	422	0.40	0.77 (0.44–1.36)	Kurzawski (2016)[24]
	rs10106 (A1994G)	Allelic C carriers	DAS28≤2.4	6	422	0.84	0.94 (0.64–1.40)	Kurzawski (2016)[24]
		Allelic C carriers	DAS<2.4	6	352	0.9	2.90 (1.50–5.40)	van der Straaten (2007)[45]
		Allelic A carriers	DAS28≤3.2	6	233	0.32	1.50 (0.68–3.29)	Lima (2016)[1]
		Allelic A carriers	DAS≤2.4	6	186	0.64	-	Wessels (2007)[46]
		Allelic A carriers	DAS≤2.4	6	352	NS	-	van der Straaten (2007)[45]
		Genotypic AA	DAS≤2.4	6	186	0.13	-	Wessels (2007)[46]
		Genotypic AA	DAS28≤3.2	6	233	0.07	0.51 (0.25–1.06)	Lima (2016)[1]
		Genotypic TT	EULAR GR	6	76	**0.041**	-	Moya (2016)[43]
		Genotypic CC	DAS28<2.4	6	422	0.25	0.70 (0.69–1.24)	Kurzawski (2016)[24]
	rs10987742	Genotypic GG	EULAR GR	6	76	**0.033**	-	Moya (2016)[43]

Table 2-1 continues on next page.

Table 2-1. ***Continued***

Gene	SNPs	Genetic model	Endpoint	Time of response evaluation (months)	N	Reported P-value	OR (95%CI)	Study
GGH	rs2305558	Allelic A carriers	EULAR GR	6	457	**0.05**#	1.46 (0.98–2.17)	Senapati (2014)[17]
		Genotypic AA	EULAR GR	6	457	0.23#	1.51 (0.74–3.08)	Senapati (2014)[17]
	rs1800909 (C16T)	Allelic C carriers	DAS≤2.4	3	352	**0.036**	2.1 (1.0–4.7)	van der Straaten (2007)[45]
		Allelic C carriers	DAS≤2.4	6	352	NS	-	van der Straaten (2007)[45]
		Allelic C carriers	EULAR GR	4	255	0.66	1.11 (0.68–1.83)	Dervieux (2009)[30]
		Allelic T carriers	DAS≤2.4	6	186	0.71	-	Wessels (2007)[46]
		Genotypic TT	DAS≤2.4	6	186	0.31	-	Wessels (2007)[46]
Folate pathway								
DHFR	rs836788	Allelic A carriers	EULAR GR	6	457	**0.014*#**	1.44 (1.08–1.93)	Senapati (2014)[17]
		Genotypic AA	EULAR GR	6	457	**0.011*#**	1.47 (1.09–1.96)	Senapati (2014)[17]
	rs12517451	Allelic A carriers	EULAR GR	6	457	**0.05**#	1.35 (0.99–1.85)	Senapati (2014)[17]
		Genotypic AA	EULAR GR	6	457	**0.016**#	1.56 (1.07–2.26)	Senapati (2014)[17]
	rs408626 (-317)	Genotypic AA	ΔDAS	6	125	**0.050**	-	Milic (2012)[47]
		Genotypic AA	EULAR GR	6	125	0.2	-	Milic (2012)[47]
	rs1643650	Additive	EULAR GR	6	61	**0.026**	0.31 (0.10–0.96)	Salazar (2014)[38]

MTHFR	rs17421511	Additive	EULAR GR	6	61	**0.024**	3.35 (1.10–10.24)	Salazar (2014)[38]
	rs1801131 (A1298C)	Additive	EULAR GR	6	61	**0.08**	2.19 (0.89–5.37)	Salazar (2014)[38]
		Allelic A carriers	ACR 20 & 50	12	217	**0.020**	2.6 (1.1–5.8)	Ghodke-Puranik (2015)[18]
		Allelic A carriers	EULAR GR	12	98	1.00	-	James (2008)[19]
		Allelic A carriers	ACR20	6	69	0.56	-	Taraborelli (2009)[28]
		Allelic C carriers	DAS28≤3.2	6	233	**0.045**	0.51 (0.26–0.98)	Lima (2015)[31]
		Allelic C carriers	EULAR GR	4	255	0.66	0.89 (0.54–1.46)	Dervieux (2009)[30]
		Genotypic AA	ΔDAS44<1.2	6	186	**0.014**	2.30 (1.18–4.41)	Wessels (2006-1)[27]
		Genotypic AA	ACR20	6	69	0.35	-	Taraborelli (2009)[28]
		Genotypic AA	EULAR GR	12	98	0.92	-	James (2008)[19]
		Genotypic AA	DAS28≤3.2	6	240	NS	-	Świerkot (2015)[25]
		Genotypic AA	DAS28	4	48	NS	-	Dervieux (2006)[29]
		Genotypic CC	DAS28≤3.2	6	120	0.84	0.90 (0.40–2.02)	Lee (2009)[23]
		Genotypic CC	DAS28≤3.2	6	233	0.91	1.07 (0.35–3.28)	Lima (2015)[31]
		Genotypic AA	EULAR GR	6	120	0.23	-	Soukup (2015)[48]
	rs1476413	Additive	EULAR GR	6	61	**0.0086**	3.56 (1.28–9.91)	Salazar (2014)[38]

Table 2-1 continues on next page.

Table 2-1. ***Continued***

Gene	SNPs	Genetic model	Endpoint	Time of response evaluation (months)	N	Reported P-value	OR (95%CI)	Study
	rs1801133 (C677T)	Additive	EULAR GR	6	61	0.53	0.73 (0.27–1.98)	Salazar (2014)[38]
		Allelic T carriers	ACR 20 & 50	12	217	NS	-	Ghodke-Puranik (2015)[18]
		Allelic T carriers	EULAR GR	4	255	0.86	1.04 (0.63–1.72)	Dervieux (2009)[30]
		Allelic C carriers	EULAR GR	12	98	0.39	-	James (2008)[19]
		Allelic C carriers	ACR20	6	69	0.34	-	Taraborelli (2009)[29]
		Allelic C carriers	DAS28≤3.2	6	233	**0.019**	3.86 (1.25–11.89)	Lima (2016)[1]
		Genotypic CC	EULAR GR	6	113	NS	-	Aggarwal (2006)[26]
		Genotypic CC	ΔDAS44<0.6	6	186	**0.044**	2.73 (1.03–7.26)	Wessels (2006-1)[27]
		Genotypic CC	ACR20	6	69	0.26	-	Taraborelli (2009)[28]
		Genotypic CC	EULAR GR	12	98	0.64	-	James (2008)[19]
		Genotypic CC	DAS28≤3.2	6	240	**0.001***	3.4	Świerkot (2015)[25]
		Genotypic TT	DAS28	4	48	NS	-	Dervieux (2006)[29]
		Genotypic TT	EULAR GR	4	48	**<0.05**	22.2 (1.2–42.2)	Dervieux (2006)[29]
		Genotypic TT	EULAR GR	6	120	0.43	1.41 (0.51–4.55)	Soukup (2015)[48]
MTHFD1	rs17850560 (G1958A)	Genotypic GG	DAS28≤3.2	3–6	208	**0.021**	4.67 (1.27–17.26)	Grabar (2010)[20]
		Genotypic GG	DAS≤2.4	6	186	0.10	-	Wessels (2006-2)[22]
		Allelic A carriers	EULAR GR	4	255	0.11	1.62 (0.90–2.92)	Dervieux (2009)[30]
SHMT-1	rs1979277 (C1420T)	Genotypic TT	DAS28	4	48	**<0.05**	7.4 (1.0–56.4)	Dervieux (2006)[29]
		Allelic T carrier	EULAR GR	4	255	0.53	0.85 (0.52–1.40)	Dervieux (2009)[30]

Methionine pathway								
MTR (MS)	rs1805087 (A2756G)	Allelic A carriers	EULAR GR	12	98	0.06	-	James (2008)[19]
		Allelic A carriers	ACR 20 & 50	12	217	NS	-	Ghodke-Puranik (2015)[18]
		Allelic G carriers	EULAR GR	4	255	0.41	1.23 (0.73–2.10)	Dervieux (2009)[30]
		Allelic G carriers	DAS28≤3.2	6	233	**0.017**	0.42 (0.20–0.86)	Lima (2015)[31]
		Genotypic AA	EULAR GR	12	98	**0.003***	-	James (2008)[19]
		Genotypic AA	DAS28	4	255	NS	-	Dervieux (2006)[29]
		Genotypic GG	DAS28≤3.2	6	233	0.25	0.27 (0.03–2.51)	Lima (2015)[31]
MTRR	rs162040	Allelic C carriers	EULAR GR	6	457	**0.04**	1.45 (1.00–2.10)	Senapati (2014)[17]
		Genotypic CC	EULAR GR	6	457	**0.02**	2.22 (1.11–4.43)	Senapati (2014)[17]
	rs1801394 (A66G)	Allelic A carriers	DAS28≤3.2	6	233	**0.041**	2.16 (1.03–4.53)	Lima (2015)[31]
		Allelic A carriers	ACR 20 & 50	12	217	NS	-	Ghodke-Puranik (2015)[18]
		Genotypic AA	DAS28	4	48	NS	-	Dervieux (2006)[29]
		Genotypic AA	DAS28≤3.2	6	233	**0.046**	2.36 (1.01–5.52)	Lima (2015)[31]
De novo pyrimidine pathway								
TYMS	rs2244500	Allelic A carriers	EULAR GR	6	457	**0.005*#**	1.48 (1.12–1.94)	Senapati (2014)[17]
		Genotypic AA	EULAR GR	6	457	**0.004*#**	1.48 (1.13–1.94)	Senapati (2014)[17]
	rs2847153	Genotypic AA	EULAR GR	6	61	0.26	1.92 (0.62–5.97)	Salazar (2014)[38]
		Allelic A carriers	EULAR GR	6	457	**0.009*#**	0.68 (0.51–0.91)	Senapati (2014)[17]
		Genotypic AA	EULAR GR	6	457	**0.04#**	0.71 (0.52–0.98)	Senapati (2014)[17]
	rs3786362	Allelic G carriers	EULAR GR	6	457	**0.011*#**	0.51 (0.30–0.86)	Senapati (2014)[17]
		Genotypic GG	EULAR GR	6	457	0.99#	-	Senapati (2014)[17]

Table 2-1 continues on next page.

Table 2-1. ***Continued***

Gene	SNPs	Genetic model	Endpoint	Time of response evaluation (months)	N	Reported P-value	OR (95%CI)	Study
Ubiquitin pathway								
CUL1	rs122571	Haplotype A-T-T	DAS28≤3.2	6	29	**0.0051***	2.83 (1.33–6.04)	Negi (2011)[32]
	rs243481	Haplotype G-C-T	DAS28≤3.2	6	74	**0.05**	1.42 (1.0–2.02)	Negi (2011)[32]
	rs243480	Haplotype G-T-T	DAS28≤3.2	6	25	**0.0045***	2.83 (1.33–6.04)	Negi (2011)[32]
Other								
KIR	2DS4 gene	Full-length	DAS28≤2.5	6	312	**0.0334***	0.43 (0.215–0.987)	Majorczyk (2014)[33]

P-values marked in bold p-values have a reported p-value below 0.05. P-values marked with an asterisk (*) were significantly associated after multiple testing correction (Bonferroni correction, p<0.05).

P-values marked with # have a reported p-values that was already corrected by multiple testing.

Abbreviations: BF: Bonferroni EULAR GR: European league against rheumatism good response criteria. ACR: American College of Rheumatology. OR: Odds Ratio. CI: Confidence Interval, SNPs: single nucleotide polymorphisms, NS: Not significant.

Table 2-2. Most promising SNPs that were significantly associated with MTX efficacy.

			MAF							
Gene	SNP	Location	AF	AFR	AMR	EUR	SAS	Association	OR [95% CI]	Study
SLC19A1	rs1051266*	21:45537880	0.4886	0.3268	0.5821	0.5487	0.5941	Genotypic AA	1.78 [1.13–2.81]	Drozdzik (2007)[41]
ATIC	rs7563206	2:215325931	0.4018	0.5129	0.4280	0.4871	0.3292	Allelic T carriers	0.20 [0.09–0.46]	Lima (2016)[1]
DHFR	rs836788	5:80616225	0.4235	0.5106	0.3631	0.3807	0.4335	Allelic A carriers	1.44 [1.08–1.93]	Senapati (2014)[17]
								Genotypic AA	1.47 [1.09–1.96]	Senapati (2014)[17]
TYMS	rs2244500	18:661005	0.6160	0.8101	0.4251	0.4612	0.5706	Allelic A carriers	1.48 [1.12–1.94]	Senapati (2014)[17]
								Genotypic AA	1.48 [1.13–1.94]	Senapati (2014)[17]
TYMS	rs2847153	18:661647	0.2901	0.2428	0.2305	0.2097	0.3865	Allelic A carriers	0.68 [0.51–0.91]	Senapati (2014)[17]
TYMS	rs3786362	18:662247	0.0623	0.0015	0.0490	0.0000	0.1063	Allelic G carriers	0.51 [0.30–0.86]	Senapati (2014)[17]

* Confirmed by the meta-analyses of Kung *et al.* (2014)[52] and Li *et al.* (2016)[53]

Abbreviations: AFR: African population, AMR: American population, EAS: East Asian population, EUR: European population, SAS: South Asian population, derived from the HapMap project.

DISCUSSION

This systematic review assesses the effect of genetic variation on the efficacy of MTX in RA using the validated endpoints DAS, EULAR, or ACR response criteria. After Bonferroni correction for multiple testing, we identified six genetic biomarkers related to MTX efficacy. Of these, *SLC19A1* rs1051266 had the most convincing evidence with two independent studies showing significant associations. Other potentially promising SNPs are *ATIC* rs7563206, *DHFR* rs836788, *TYMS* rs2244500, rs2847153, and rs3786362, but these lack replication studies. The six genetic biomarkers could have clinical implications for the disease outcome of RA. In fact, *SLC19A* rs1051266, *DHFR* rs836788, and *TYMS* rs2244500 showed a 40% or more increased chance of the effectiveness of MTX, and *ATIC* rs7563206 and rs378636, and *TYMS* rs2847153 showed 45% or more chance of the reduced effectiveness of MTX. Still we believe that additional studies are necessary before implementing pharmacogenetic testing for these SNPs in the treatment of RA.

A limitation of the investigated studies in this systematic review is the difference in the evaluation time points for measuring MTX efficacy. MTX is a slow-acting prodrug that becomes active when polyglutamated in the cells. The process of polyglutamation is slow and takes up to 27.5 weeks (range 6.6–62.0 weeks) to reach steady state.[34] This delay in steady-state polyglutamation explains the relatively long time to clinical response, and therefore most studies had the endpoint set to 6 months after the start of MTX therapy. However, some studies evaluated response earlier than t=6 months, while MTX may not yet have exerted its full potential. Furthermore, the genotypic or allelic genetic models were often used, when in fact the hypothesis-free driven additive genetic model seems more appropriate because the underlying genetic model is unknown.

Another limitation is that most studies tested with univariate analysis, without taking into account baseline variables (multivariate testing), such as gender, smoking status, disease severity which are known to influence response to MTX. Most drug-gene interaction studies were explorative, with the use of retrospective data and lack validation. Pharmacogenetic testing in RA remains limited mainly because the evidence for drug-gene interactions are marginal. MTX is involved in multiple pathways with different genes. Yet, most pharmacogenetic studies were candidate studies that tested only a single or a small number of SNPs, but not a combination of multiple genes or pathways.[35] To get clear evidence, additional studies with the use of a combination of multiple genes are needed. This review can show a basis, to test all suggestive SNPs together in association with the efficacy of MTX.

The strength of our study is that a systematic approach was used to identify SNPs and the selection of the articles was performed according to the PRISMA guidelines. Another strength is that only validated outcome criteria were used and that adjustment for multiple testing by Bonferroni correction was applied for the included studies. A potential weakness of this review is that only English publications were included. This results in the exclusion of seven non-English studies, and important findings could have been missed. Another weakness was the limited sample size of some studies and the lack of power analysis to check the validity of the outcomes. Finally, a common limitation of systematic reviews is publication bias. Meaning that important – albeit negative – results were never published, which could lead to misinterpretation of the actual findings. Another limitation was that not all studies were performed with MTX monotherapy, and therefore the effect on response could be influenced by other DMARDs. Several meta-analyses have been performed on pharmacogenetics biomarkers for the efficacy or toxicity of MTX in RA. Of our promising SNPs, SLC19A1 rs1051266 with the genotypic AA (vs AG/AG) was tested in MTX efficacy in three meta-analyses. Two meta-analyses, conducted by Li *et al.*[50] and Chen *et al.*,[51] confirmed the significant association with an OR of 1.42 (95% CI: 1.04–1.93) and 1.49 (CI: 1.17–1.90), respectively. However, the third meta-analysis by Chen *et al.*[51] showed substantial heterogeneity (I^2) of 72% for the allelic model and thus represented inconsistencies of the pooled studies and affects the validity of the results. None of the other variants was evaluated in meta-analysis.

In summary, through the use of a systematic review and inclusion of studies with validated RA efficacy endpoints, we identified six SNPs for which there is substantial evidence for an association with MTX response in RA patients. For clinical application more evidence from prospective studies with multivariate testing is needed.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Supplementary File S2-1. Full search string.

((“Arthritis, Rheumatoid”[Majr:NoExp] OR “Rheumatoid Arthritis”[ti]) AND (“Methotrexate”[Majr] OR methotrexat*[ti] OR “Amethopterin”[ti]) AND (“Pharmacogenetics”[Mesh] OR pharmacogenet*[tw] OR pharmacogenom*[tw] OR “Epigenomics”[Mesh] OR epigenet*[tw] OR epigenom*[tw] OR “Polymorphism, Single Nucleotide”[Mesh] OR “SNPs”[tw] OR “Single Nucleotide Polymorphism”[tw] OR “Single Nucleotide Polymorphisms”[tw]))