# Methotrexate pharmacogenetics in rheumatoid arthritis

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**ABSTRACT**

*Rheumatoid arthritis (RA) is a systemic inflammatory arthritis that can not only result in permanemt joint damage, but is associated with high morbidity and mortality. Disease-modifying anti-rheumatic drugs (DMARDs) are the mainstay of treatment in RA. DMARDs improve the symptoms of joint pain and swelling, but more importantly, prevent the progression of joint damage. Methotrexate (MTX) is the first-line DMARD in RA with over two decades worth of excellent long-term efficacy and safety. However, there is significant variability in patients' response to MTX, both in efficacy and toxicity. Recent advances in genetics, particularly pharmacogenetics, may permit the prediction, a priori, of an individual patient's response to MTX. In this review, we highlight recent published literature on the pharmacogenetics of MTX in RA. Pharmacogenetics may be a useful means of optimising MTX therapy in patients with RA.*

## Introduction

Our understanding of the human genome has improved by leaps and bounds in the last few decades and this has led to an improved understanding of both the genetic susceptibility to rheumatic diseases and the genetic basis of response to therapeutics. Differences in the response of individual patients to the same drug may at least partly be inherited. Such inherited inter-individual differences in drug response may be due to polymorphisms in genes encoding drug-metabolising enzymes, drug transporters, and/or drug targets (1). Pharmacogenetics is the study of genetic polymorphisms in drug-metabolising enzymes, transporters, and targets and the translation of such inherited differences into drug effects (2). A gene is described as "polymorphic" when its allelic variants exist which can alter the activity of the encoded protein compared to the wild type sequence. Pharmacogenetics may help explain the differences in inter-individual response to drug therapy, and more importantly may help optimise drug treatments for individual patients. With currently available molecular sequencing and high-throughput technologies, the human genome can now be scanned rapidly for hundreds of genetic polymorphisms such as single nucleotide polymorphisms (SNPs) that may explain clinically important inter-individual differences in drug response.

Rheumatoid arthritis (RA) is a systemic inflammatory disorder. RA affects approximately 0.5–1% of the western population. When left untreated this disease results in severe joint destruction, deformity, and functional impairment. Corticosteroids, while effective in treating this disease have a large host of undesired systemic side effects. Therefore, disease-modifying antirheumatic drugs (DMARDs) are the treatments of choice to halt the joint damage, radiographic progression, debility, and disability in RA. Methotrexate (MTX), an anti-metabolite, has been widely used to treat RA since the mid-1980s. Even with recent advances in the availability of biologic therapies for RA (3-5), MTX remains a cornerstone in the treatment of this disease. Since this time, it has become apparent that there is great variability not only in the efficacy, but also in the toxicity of this medication. Without the availability of predictive markers, many patients are treated with MTX for prolonged periods with little or no disease response, and many patients experience adverse effects from varying doses of MTX. Thus, MTX, although inexpensive, requires costly and frequent laboratory testing to monitor side effects (6).

In the following article we will review some of the recent, major published articles on the pharmacogenetics of MTX in RA. We realise that it is beyond the scope of this article to review all the published literature in this field to date,

and hence will focus on a few illustrative examples of key genes and SNPs in recent years, to give readers a snapshot of the current status and trends in this field.

## MTX cellular pathway

The exact mechanism of MTX's disease-modifying effects in RA is still not completely understood. However, numerous enzymes are known to be important for MTX's antiproliferative and immunosuppressive effects. The parent drug contains one glutamate moiety ($MTXGlu_1$). Once ingested, $MTXGlu_1$ is absorbed into the cell by active transport through the reduced folate carrier (RFC), also known as solute carrier family 19 member A1 (SLC19A1) (Fig. 1). Once taken intracellularly, up to six additional glutamate residues are added to $MTXGlu_1$ by folylpolyglutamate synthase (FPGS) to create MTX polyglutamates ($MTXPG_{2-7}$). Polyglutamated MTX remains in the cell while $MTXGlu_1$ may be transported out of the cell by ATP-binding cassette (ABC) efflux pumps. The ABC family includes 48 proteins classified into seven distinct subfamilies (A-G) (7). Of these subfamilies, ABCC1-4 and ABCG2 play the largest role in MTX efflux from the cell (8, 9). MTX polyglutamation is not a final process and may be reversed by gamma-glutamyl hydrolase (GGH) to permit efflux of MTX from the cell.

In addition to maintaining intracellular MTX levels (10), MTXPG directly inhibits several intracellular enzymes important in purine and pyrimidine synthesis, such as thymidylate synthetase (TYMS) (11) and dihydrofolate reductase (DHFR). TYMS converts deoxyuridylate to deoxythymidylate for the de novo pyrimidine biosynthetic pathway while DHFR reduces dihydrofolate (DHF) to tetrahydrofolate (THF), the precursor of biologically active folates. Another intracellular enzyme, methylenetetrahydofolate reductase (MTHFR) is not directly affected by MTX. However, MTHFR function is impacted by intracellular folate levels. Therefore, MTX's alteration of intracellular folate levels through its effects on DHFR in turn affects MTHFR function. 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (ATIC) is an enzyme responsible for the catalysis of the last two steps of *de novo* purine synthesis. MTX and its polyglutamated forms directly inhibit this enzyme and thereby affect the cellular purine balance and cause accumulation of extracellular adenosine, a potent anti-inflammatory agent (12). Thus, although the exact mechanism of action of MTX is not fully understood, changes in any of the above detailed transmembrane transporters and enzymes in the cellular pathway of MTX, due to polymorphisms in their encoding genes, may influence the efficacy or toxicity of the medication.

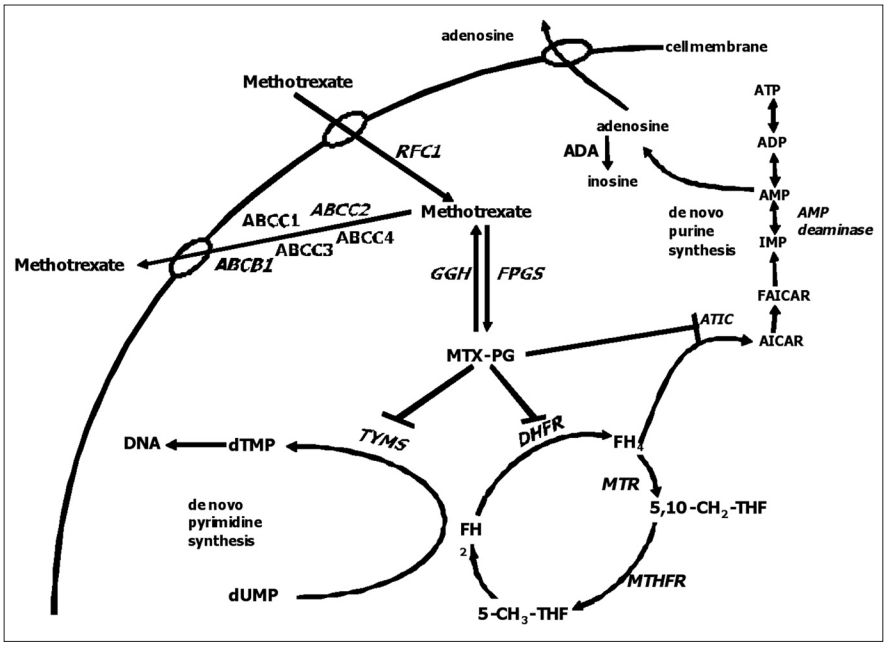


**Fig. 1.** Cellular pathway of methotrexate.
RFC1: reduced folate carrier 1; ABCB1, ABCC1-4: ABC transporters; GGH: γ-glutamyl hydrolase; FPGS: folylpolyglutamate synthase; MTX-PG: methotrexate polyglutmate; TYMS: thymidylate synthase; d UMP: deoxyuridine monophosphate; d TMP: deoxythymidine monophosphate; DHFR: dihydrofolate reductase; $FH_2$: dihydrofolate; 5-$CH_3$-THF: 5-methyl tetrahydrofolate; MTHFR: methylene tetrahydrofolate reductase; 5, 10-$CH_2$-THF: 5, 10-methylene tetrahydrofolate; MTR: methyl tetrahydrofolate reductase; AICAR: aminoimidazole carboxamide ribonucleotide; FAICAR: 10-formyl AICAR; ATIC: AICAR transformylase; IMP: inosine monophosphate; AMP: adenosine monophosphate; ADP: adenosine diphosphate; ATP: adenosine triphosphate; ADA: adenosine deaminase.
Italicised genes have been targets of pharmacogenetic analyses in studies published so far.

## MTX pharmacogenetic studies

### *MTX transporter and glutamation genes*

RFC-1 also known as SLC19A1, is an active transporter of MTX across cellular membranes. Alterations in this transporter may result in increased or decreased levels of intracellular MTX and therefore altered response to MTX treatment. One known RFC polymorphism is the 80G>A polymorphism leading to substitution of arginine for histidine at codon 27 in the first transmembrane domain (TMD1) of the SLC19A1 protein (13, 14). A study by Dervieux *et al*. found that among 105 patients with RA, those homozygous for the RFC SNP 80A/A had a greater response to MTX compared to patients with the wild type (80G/G). Patients homozygous for the A allele were three times more likely to be within the top 25th percentile of MTX responders (confidence interval (CI): 95% 1.3–8.4; $p<0.01$) compared to those with the wild type G allele, suggesting that this SNP is associated with an increased response to the drug (15).

There are SNPs in the GGH promoter that affect GGH expression (16) and MTX polyglutamation (17, 18). A 452C>T SNP causing decreased GGH activity and accumulation of intracellular long chain MTX polyglutamates (18) and a 401C>T promoter polymorphism which also affects intracellular MTXPG levels have been described

**Table I.** Gene polymorphisms in MTX transporter and glutamation pathways.

| Gene | Role of gene product in MTX pathway | Polymorphism | Effects on gene product/enzyme | Clinical effects | Reference |
|---|---|---|---|---|---|
| SCL19A1 / RFC | Active transporter of MTX into the cell | 80 G>A | May affect transcriptional activity of SCL19A1 gene, MTX transport, and MTXPG levels | May affect MTX efficacy | 15,19, 43 |
| ABCB1 | MTX efflux from cell | 3435 C>T | May affect P-gp function and MTX efflux from cell | May affect MTX efficacy and toxicity | 26, 28 |
| ABCC2 | MTX efflux from cell | IVS 23+56 T>C | May affect MTX efflux from cell | May affect MTX toxicity | 27 |
| GGH | Reversal of polyglutamation by removing glutamate moieties | 452C>T<br>16T>C<br>401C>T | May affect GGH activity and MTXPG levels | | 19, 20 |

(17). Another GGH 16T>C SNP has been identified; its functional effects are currently unknown. In a Japanese study of patients with RA, the presence of the SLC19A1 80AA and GGH-401TT genotypes independently predicted $MTXPG_{3-5}$ levels. Patients with the SLC19A1 80AA genotype were 3.4-fold more likely to have $MTXPG_{3-5}$ levels above the group median compared to patients with the SLC19A1 80GG or 80GA genotype (odds ratio (OR) 3.4; 95% confidence interval (CI) 1.4–8.4; $p$=0.007). Also, patients with GGH–401TT genotype were 4.8-fold (OR 4.8; 95%CI 1.8–13.0; $p$=0.002) more likely to have $MTXPG_{3-5}$ below the study median compared to carriers of the GGH-401CC or CT genotype. Thus the GGT 401 C>T also appears to impact intracellular MTXPG levels similar to the RFC 80G>A (19).

Another study by van der Straaten *et al*. evaluated the frequency of GGH 16C>T and 452C>T alleles in RA patients and their correlation with clinical response to MTX therapy. The allele frequency of GGH 16C>T and 452C>T in RA patients was similar to the general population; thus there was not a predisposition for development of RA in carriers of this allele profile. These alleles were also not independently associated with MTX toxicity. However patients with fewer (one or none) copies of the haplotype GGH 452-16T did show better clinical improvement with MTX (OR 2.7) as measured by the Disease Activity Score (DAS) (20).

*ABC family genes*

As stated above, MTX is actively effluxed from the cell by members of the ATP-binding cassette (ABC) family of transporters. P-glycoprotein (P-gp), a product of the ABCB1 (MDR1) gene and a member of the ABC family, can induce drug resistance in RA by increasing cellular efflux of several drugs including MTX. Therefore, P-gp expression and drug resistance is currently an active field of study (21). P-gp expression is increased on activated CD4+ and CD19+ lymphocytes in patients with RA. Patients with active RA and high levels of P-gp may benefit from competitive inhibitors of P-gp (such as tacrolimus) or reduction in P-gp stimulation with tumour necrosis factor (TNF) inhibitors to increase the cellular concentration of multiple DMARDs, including MTX.

SNPs in the ABCB1 gene influence P-gp expression (22). One such SNP is the 3435C>T polymorphism in exon 26 of the gene (23, 24). A retrospective study determined the effects of SLC19A1 80G>A, ABCB1 3435C>T, ATIC 347C>G (a SNP in ATIC), and TYMS 6 base pair (bp) deletion polymorphisms (a 6-bp deletion of the sequence TTAAAG at nucleotide 1494 in the 3'- untranslated region of TYMS) (25) on MTX efficacy and toxicity in 124 RA patients. Patients whose last maintenance dose of MTX was ≤6mg/week were deemed responders and those whose last maintenance dose was >6mg/week or those in whom MTX therapy was changed due to poor response to MTX were deemed non-responders. Significantly more non-responders carried the ABCB1 3435TT genotype compared to the CC genotype by both univariate (crude OR 8.91, $p$=0.001) and multivariate (adjusted OR 8.78, $p$=0.038) analysis; this genotype did not affect MTX toxicity. The SLC19A1, ATIC and TYMS genotypes did not influence MTX efficacy or toxicity (26).

We examined 222 RA patients of Caucasian and African American race in a retrospective, cross-validation design using training and validation cohorts. Of 25 SNPs in 6 genes – ABCB1, ABCC1, ABCC2, FPGS, MTHFR and TYMS, MTHFR 677 C>T SNP was associated with alopecia in African Americans ($p$=0.032) and ABCC2 IVS 23+56 T>C, an intronic SNP correlated with time to MTX discontinuation and/or dose decrease due to toxicity in Caucasian patients with RA ($p$<0.0001) (27).

In a recent Dutch study of 205 patients with RA, patients carrying the ABCB1 3435T-allele and toll-like receptor 4 (TLR4) +896G-allele were 2.5-times more likely to develop adverse drug events due to MTX at 6 months (OR 2.6; 95% CI: 1.1–6.2, and OR 2.5; 95% CI: 1.1–6.1, respectively). This risk increased almost four-fold in patients with the two unfavourable genotypes (OR 3.9; 95% CI: 1.5–10.3). However, none of these associations remained significant after correction for multiple testing ($p$<0.004) indicating that MTX toxicity may potentially be influenced by ABCB1 3435C>T and TLR4 +896A>G SNPs (28).

*Folate pathway genes -MTHFR/DHFR*

MTHFR is the most studied gene with regard to MTX pharmacogenetics. MTHFR is the critical enzyme in the generation of 5-methyl tetrahydrofolate, which is the methyl donor for several important intracellular biochemical reactions including the methylation of

**Table II**. Folate pathway gene polymorphisms.

| Gene | Role of gene product in MTX pathway | Polymorphism | Effects on gene product/enzyme | Clinical effects | Reference |
|---|---|---|---|---|---|
| MTHFR | Generation of 5-methyl tetrahydrofolate | 677C>T 1298A>C | Thermolabile MTHFR variant with decreased activity | May affect MTX efficacy and toxicity | 27, 37, 38 |
| DHFR | Reduction of DHF to THF, direct target of MTX | 473T>C 35289G>A | Important for DNA alignment | No association with MTX efficacy or toxicity | 37 |
| TYMS | Conversion of dUMP to dTMP, direct target of MTX | TSER*2/3 | May alter TYMS enzyme activity | May affect MTX efficacy and toxicity | 43, 45 |
| MTR | Methylation of homocysteine to methionine | 2756 A>G | May decrease MTR activity and increase plasma homocysteine levels | Not associated with MTX efficacy or toxicity | 46 |
| MTRR | Methylation of cobalamin cofactor required for activity of MTR | 66 A>G | May decrease MTRR activity and increase plasma homocysteine levels | Not associated with MTX efficacy or toxicity | 46 |

**Table III.** Adenosine pathway gene polymorphisms.

| Gene | Role of gene product in MTX pathway | Polymorphism | Effect of polymorphism on gene product | Clinical effects | References |
|---|---|---|---|---|---|
| ATIC | Catalysis of the last two steps of de novo purine synthesis | 347C>G | May alter enzyme activity | May affect MTX efficacy and toxicity | 43, 45, 46 |
| AMPD1 | Converts AMP to IMP | 34 C>T | May alter enzyme activity | May affect MTX efficacy | 46 |
| ITPA | Converts ITP to IMP | 94 C>A | May alter enzyme activity | May affect MTX efficacy | 46 |
| Adenosine receptor 2a | Receptors for adenosine, an anti-inflammatory purine | 5 SNPs | May alter adenosine's effects | May affect MTX toxicity | 47 |

homocysteine to methionine by methionine synthase (MS). Over a dozen MTHFR gene polymorphisms have been described. Two functional polymorphisms, 1298A>C and 677C>T, have been examined in numerous studies to determine whether these polymorphisms are predictive of patient response to MTX. The 677C>T polymorphism causes an alanine to valine substitution at codon 222 of the MTHFR gene which encodes a thermolabile variant of MTHFR with decreased enzyme activity and increased plasma homocysteine levels (29). The homozygous 677C>T variant, with about 30% of wild-type activity, has a prevalence of about 8–10% in the general population. Heterozygotes have about 60% activity with a prevalence of 40% in the population.

The MTHFR 1298A>C polymorphism with a glutamine to alanine substitution at codon 222 (30) leads to reduced activity of the MTHFR enzyme, although not a thermolabile variant. The homozygous genotype with ~60% of enzyme activity in lymphocytes is prevalent in ~10% of the Canadian population (prevalence worldwide unknown) (31). Persons heterozygous for the 677C>T and 1298A>C polymorphisms have diminished MTHFR enzyme activity and elevated plasma homocysteine levels comparable to individuals homozygous for the 677C>T polymorphism (30).

S-adenosyl homocysteine (SAH) is derived from homocysteine, which is, in turn, generated by the remethylation of methionine. 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR) methylates homocysteine to methionine in the presence of a cobalamin cofactor. A polymorphism in the MTR gene, 2756A>G, resulting in an aspartic acid to glycine change at codon 919 (D919G) (32) may alter the activity of the MTR enzyme, as individuals homozygous for the SNP (DD genotype) have high homocysteine levels compared to individuals with the wild type (GG) genotype (33, 34). 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (MTRR) methylates the cobalamin cofactor of MTR. A 66A>G SNP in MTRR with substitution of methionine for isoleucine at codon 22 also leads to elevated homocysteine levels (35, 36). SNPs in MTR and MTRR have been studied in MTX pharmacogenetics and are described in the following section (47).

DHFR is a direct target of MTX. It is an essential enzyme for the reduction of folate and the continuous reduction of DHF to THF. Two DHFR polymorphisms 473G>A and 35289G>A (important in DNA alignment), MTHFR 677C>T and 1298 A>C, and SLC19A1 80G>A were analysed for their effects on MTX response in 205 Dutch RA patients on MTX at doses ranging from 15 to 25mg/week. MTX efficacy was measured using the DAS 44 and adverse events (AEs) (pneumonitis, gastrointestinal effects, skin and mucosal effects, and elevated liver enzymes) were monitored at 3 and 6 months after initiation of MTX. At 6 months, MTHFR 1298AA was associated with efficacy compared to 1298CC (OR 2.3, 95% CI 1.18-4.41) and this association

was strengthened by the presence of the 677CC haplotype ($p$=0.02) and the number of copies of the 677CC haplotype (OR 3.0, 95% CI 1.4-6.4; $p$=0.021). SLC19A1 and DHFR SNPs had no effects on MTX efficacy. Patients carrying MTHFR 1298AC and CC genotypes had more overall AEs compared to those with the 1298AA genotype ($p$=0.015 at 3 months and $p$=0.005 at 6 months). MTHFR 677C>T, SLC19A1 80G>A and DHFR SNPs did not affect MTX toxicity (37).

Other studies on the two MTHFR polymorphisms have often yielded conflicting results. A recent meta-analysis on these two MTHFR SNPs illustrated the contradictory findings of the studies published to date best. This meta-analysis included 8 studies with a total of 1514 patients with RA and concluded that there were no associations between the MTHFR C677T and A1298C polymorphisms and MTX efficacy and toxicity. The OR for adverse effects in patients with 677CC versus those with 677CT and 677TT was 0.633(95% CI 0.325, 1.234; $p$=0.180) and 0.621 (95% CI 0.233, 1.655; $p$=0.341), respectively. The ORs for adverse effects in patients with 1298AA versus those with 1298AC and 1298CC was 0.942 (95% CI 0.479, 1.851; $p$=0.861) and 0.978 (95% CI 0.569, 1.681; $p$=0.936), respectively. Similarly the meta-analysis failed to show any associations between these MTHFR polymorphisms and MTX efficacy (38). Thus, despite these two MTHFR SNPs being the focus of several pharmacogenetic studies in RA, there is no clear-cut evidence on the utility of either of these polymorphisms as predictive markers of MTX response in RA.

*-TYMS*

TYMS is a direct enzyme target of MTX. TYMS converts deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), a key step in de novo pyrimidine synthesis. Previous studies in the oncology literature have demonstrated that an increased number of 28-bp tandem repeats (thymidylate synthase enhancer repeat (TSER)*2 or *3 with two or three 28-bp tandem repeats in the 5-untranslated region of TYMS) is associated with increased TYMS expression and decreased response to MTX (39-42).

Several studies in RA have examined the TSER polymorphism, of which two representative studies are described below. In a cross-sectional study in which 108 RA patients were categorised as responders to MTX (VAS score <2 cm) versus non-responders to MTX (VAS score >2 cm), and variants in RFC1, ATIC, and TYMS assessed for effects on MTX efficacy, patients with the TSER*2/*2 genotype displayed lower scores for physician's global assessment of disease activity (2.9±0.5 *vs*. 3.6±0.2 ($p$=0.049)), physician's assessment of patient's response to MTX (2.1±0.4 *vs*. 2.8±0.2 ($p$=0.06)), and M-HAQ scores (0.38±0.08 *vs*. 0.60±0.05 ($p$=0.05)) compared with carriers of TSER*3/*3 and TSER*2/*3. Although the effect of the TSER polymorphism by itself on MTX efficacy was not impressive as evident by the $p$-values above, a pharmacogenetic index composed of the sum of homozygous variant genotypes in RFC-1, ATIC, and TYMS, and RBC long chain MTXPG concentrations were excellent predictors of MTX efficacy (OR 14.0, 95% CI 3.6–53.8, and 3.7, 95% CI 1.7–9.1, respectively) (43).

Another study by Weisman *et al*. of 214 RA patients on MTX examined MTHFR 677C>T, TSER *2/*2, ATIC 347C>G, and serine hydroxymethyltransferase (SHMT) 1420C>T polymorphisms. SHMT encodes a vitamin B6-dependent enzyme crucial for the synthesis of 5,10-methylene THF. A 1420C>T polymorphism in this gene that alters red blood cell folate levels and hence can potentially have effects on MTX toxicity, has been described (44). Information on side effects from MTX at the time of a single study visit was collected. A sum of the homozygous variants for each gene was calculated to generate the toxicogenetic index for each patient. Among the individual genotypes, TSER *2/*2 and SHMT 1420CC were associated with alopecia ($p$<0.01, OR 5.6; CI 3.0, 9.5 and p<0.01, OR 3.2; CI 2.2, 4.5, respectively). The toxicogenetic index (MTHFR 677TT + SHMT 1420CC + TSER *2/*2 + ATIC 347GG) ranged from 0 to 3. There was a 1.9-fold increase in the likelihood of MTX side effects with each unit increase in the toxicogenetic index ($p$=0.004, CI 1.1, 3.1). Thus homozygosity for one or more of these alleles appeared to confer a "MTX toxicity phenotype"(45).

*- Adenosine pathway genes*

5-aminoimidazole-4-carboxamide ribonucleotide transformylase (ATIC) is the enzyme responsible for the catalysis of the last two steps of *de novo* purine synthesis. Polyglutamated MTX directly inhibits this enzyme and therefore alters the cellular purine balance and causes accumulation of adenosine, a potent anti-inflammatory agent (12). Inosine monophosphate (IMP) in the purine synthetic pathway is derived from two sources; inosine triphosphate pyrophosphatase (ITPA) converts inosine triphosphate (ITP) to IMP, while adenosine monophosphate deaminase (AMPD) converts adenosine monophosphate (AMP) to IMP (Fig. 1).

Several studies have examined adenosine pathway polymorphisms as potential markers of MTX response. Some of these studies are described above (26, 43, 45). Two other examples are described here. Polymorphisms in three genes encoding enzymes in the adenosine pathway and two in the folate pathway were examined in the same Dutch cohort of RA patients described in the section under MTFHR/DHFR. MTX efficacy was measured by DAS and MTX-related AEs were assessed and their associations with genotypes examined. Genotyping for the following polymorphisms was performed: AMPD1 34C>T, ATIC 347C>G, ITPA 94C>A, MTR 2756A>G and MTRR 66A>G. Patients carrying the AMPD1 34T allele, ATIC 347CC, or ITPA 94CC were more likely to have a good clinical response to MTX as measured by DAS (OR 2.1(95% CI 1.0–4.5); OR 2.5 (95% CI 1.3–4.7) and OR 2.7 (95% CI 1.1–8.1)), respectively with the likelihood of a good clinical response further increased if patients carried all 3 favourable genotypes (OR 27.8). Of all the SNPs, only the ATIC G allele was associated with MTX-related AEs (OR 2.0 (95% CI 1.1–3.7)). Thus, SNPs in 3 adenosine pathway genes AMPD1,

ATIC, and ITPA, were associated with a good clinical response to MTX, while the ATIC 347C>G SNP was a marker for MTX toxicity (46).

More recently, SNPs in the adenosine receptor 2a gene were analysed for their effects on MTX response. MTX efficacy was assessed by erythrocyte sedimentation rate (ESR), and physician assessment, and information on MTX adverse events collected. Five SNPs in the adenosine receptor 2a gene were associated with discontinuation of MTX due to toxicity, specifically gastrointestinal toxicity (OR 2.1–3.07; $p<0.05$). None of the SNPs was associated with MTX efficacy (47).

## Conclusions and future directions

Studies published to date on the pharmacogenetics of MTX in RA offer conflicting results. What emerges is a confusing picture, with some studies suggesting that polymorphisms in genes controlling the MTX cellular pathway correlate with response to MTX, while others contradict this finding. There are several caveats to consider while interpreting the results of these studies. One possible explanation for the inconsistency in results among the studies may be that almost all these studies were underpowered, because of small sample sizes. For the same reason, genotype-(response) phenotype associations as determined in some of these studies, although statistically significant, may be spurious. These issues can be remedied only by the conduct of large, well-powered multi-centre studies. Further complicating the issue is the fact most of these studies except a few have examined racially homogenous populations. Allele frequencies vary significantly between races and affect pharmacogenetic associations as we and other investigators have shown (27, 48).

More importantly, unless the functional significance of a polymorphism and its association with a specific phenotype is unequivocally established, replication studies are crucial to confirm associations seen in prior studies, and are sadly lacking in the field at the present time. Hence, although some of these pharmacogenetic studies have shown the way forward, they have also underlined the difficulty of ascribing causality to a variant when it is found associated with efficacy or toxicity. Also, in order for these approaches to be cost-effective, in addition to a well-established association between genotype and clinical phenotype such as toxicity, the frequency of the variant allele should be high. For example, if the frequency of a variant homozygous allele is low (<1%), several hundred patients will have to be tested to identify one patient with the variant allele. Despite these caveats, personalised medicine is a rapidly advancing field, and is the way of the future. Given the increasing interest and commitment of major funding agencies to pharmacogenetics research as evident by the establishment of the International HapMap Consortium (www.hapmap.org), and the Pharmacogenetics Research Network (http://www.nigms.nih.gov/pharmacogenetics/) by the National Institutes of Health, these wrinkles in pharmacogenetic research should be ironed out soon, and personalised medicine should become a reality in the not too distant future.

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