GENETIC POLYMORPHISMS INFLUENCING EFFICACY AND SAFETY OF METHOTREXATE IN RHEUMATOID ARTHRITIS

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ABSTRACT

Objective: This review will summarize pharmacogenetic studies of single nucleotide polymorphisms in genes coding enzymes of methotrexate (MTX) pathway, related to its response and toxicity in rheumatoid arthritis (RA). In addition, this review focuses on the racial and ethnic differences in distribution of the polymorphisms in genes related to efficacy and toxicity of MTX in RA.

Methods: Articles were searched using PubMed database using the search term "pharmacogenetics and MTX and arthritis." The search revealed 72 articles, of which 27 were given special importance, due to open-access.

Results: Many genes and single nucleotide polymorphisms are investigated in this context, and the highlighting genes are ATP-binding cassette proteins (ABCB1) reduced folate carrier (RFC), methylenetetrahydrofolatereductase (MTHFR), gamma-glutamyl hydrolase (GGH), serine hydroxyl methyltransferase (SHMT), 5-aminomimidazole-4-carboxamide ribonucleotidetransformylase (ATIC), methionine synthase reductase (MTRR), methionine synthase (MS), adenosine monophosphate deaminase1 (AMPD1), inosine triphosphate pyrophosphatase (ITPA). The study highlighted RFC-1 80AA, ITTPA 94CC, and AMPD1 347CC as responders. The allelic types prone to toxicity were MTHFR 677TT, MTRR 2756AA, MS 66GG, SHMT 1420CC, ATIC 347GG, and thymidylate synthase *3/*2. The genotypes reported as non-responders were ABCB1 3434TT, MTHFR 1298AA, DHFR A317G, GGH 160C, and GGH 401TT.

Conclusion: Although these studies highlight inconsistency in results, due to the difference in sample size and assessment parameters and racial and ethnic differences, larger prospective studies are essential to reach the cornerstone of the concept of personalized medicine.

Keywords: Arthritis, Gene, Methotrexate, Pharmacogenetics, Rheumatoid.

INTRODUCTION

Methotrexate (MTX) is the cornerstone for the therapy of rheumatoid arthritis (RA) in spite of the advent of newer biologics. MTX is fast acting and has the best efficacy:toxicity ratio and also cheaper. For the treatment of RA, it was first introduced in 1951, but after 30 years, only widespread use in RA came into force in 1985. It is used at the dose of 5-25 mg/week in the treatment of RA and the dose for its antitumor effect is 5000 mg/week. As a gold standard, it is started as monotherapy, and a low dose is safe and well-tolerated. For patients unresponsive to nonsteroidal anti-inflammatory drugs, it is still the first-line drug for therapy in RA [1].

MTX is taken up by the cells glutamated by poly-poly glutamyl synthase (FFPG) and there is a competition by gamma-glutamyl hydrolase (GGH), which deconjugates the drug and the free drug is effluxed by ATP-binding cassette (ABC) proteins. Polyglutamation up to 7 subunits takes place and MTX-polyglutamates (MTX-PGn) roughly correlate with the therapeutic efficacy of the drug. Free MTX is eliminated within 24 h, and a small portion of it is metabolized in the liver to 7-hydroxy MTX. At cellular level, MTX and MTX-PGs inhibit several enzymes of purine and pyrimidine biosynthesis and also exert an anti-inflammatory effect. Methotrexate inhibits several enzymes of cell proliferation such as dihydrofolate reductase (DHFPR), involved in DNA biosynthesis, thymidylate synthase (TYMS), involved in DNA biosynthesis and repair and 5-aminomimidazole-4-carboxamide ribonucleotide (AICAR), contributing to anti-inflammatory and antiproliferative effects of accrued adenosine. The other enzymes inhibited by the drug are homocysteine pathway enzymes such as methylenetetrahydrofolate reductase (MTHFR), methionine synthase reductase (MTRR), and methionine synthase (MS), which could lead to accumulation of homocysteine and related adverse effects [2]. Due to variation in response and toxicity profile, one-third of the patients discontinue therapy due to its adverse effects. Folate antagonism leads to anemia, stomatitis, oral ulcers, and elevation of transaminases in the liver, which could be alleviated by the administration of folic or folic acid. Accumulation of adenosine also leads to gastrointestinal (GI) adverse events (AEs). The uncommon toxicities are nodulosis, hepatic fibrosis, pulmonary fibrosis, and renal insufficiency [3]. Due to these factors, it is essential to predict the efficacy and adverse effects before administration, to effectively use the drug in the treatment of RA. Since the drug is excreted within 24 h and measurement of MTX-PGs routinely in clinical practice is not feasible, pharmacogenetics could be a useful tool to monitor the treatment outcomes.

Pharmacogenetics is advancing area of research, and the data are of value in tailoring therapy according to individual's genotype profile. Single nucleotide polymorphisms (SNPs) are good markers of genetic variation and the frequency of these SNPs among different races and ethnicities. In the case of multiple SNPs in a gene, it is appropriate for haplotype analysis. There are several reports on the association between genetic polymorphisms in metabolic enzymes and efficacy or adverse effects of MTX.

The purpose of this review is to determine the role of polymorphisms influencing therapeutic efficacy and toxicity of MTX in RA, from published studies.
PHARMACOGENETICS OF MTX

Genetic polymorphism related to efflux of MTX

*ABC* proteins (*ABC*1) **C3435T**

This is one of the important genes related to P-gp expression, which is best drug transporters in humans. There are several genes and several polymorphisms related to multiple drug resistance, and *ABC*1 **C3435T** is widely studied in RA patients and this gene is otherwise called as MDR1 gene. This mutation is a synonymous mutation that occurs in chromosome 1, and the reference SNP (rs) assessed is **rs1045642**, which increases the efflux of xenobiotics out of the cells (Table 1).

Four relevant studies were analyzed in relation to response to MTX and susceptibility to its toxicity. Two studies in Japanese RA patients and one Polish study reflected the influence of this SNP to the sensitivity of MTX as therapeutic response. It was found that only one Indian study was performed in this context. In India, the association of this polymorphism was studied in the treatment of lung cancer [4], epilepsy [5], and acute leukemia [6].

In healthy individuals, the distribution of the different alleles of this polymorphism was similar in Indians and Polish but was significantly different when compared to Japanese (Table 2). In Polish RA patients, **C3435TT** genotypes were found to be good responders, whereas contradictory reports were observed in Japanese patients [7-8]. In Japanese studies, one reported them as responders and other reported them as non-responders [8,9]. In Indian study, the **3435CT** genotypes were found to be non-responders [10] and Grabar et al. reported that **3435T** allele carriers have the risk of adverse effects to MTX, in Yugoslavian RA patients [11] (Table 3).

Genetic polymorphism related to influx of MTX

**Reduced folate carrier-1 (RFC-1)** **G80A**

RFC is an anion exchanger, a transmembrane protein comprising 591 amino acids, and transfers folates across the cell membrane. This polymorphism is otherwise called as solute carrier, *SLC19A1* **G80A**. RFC influences the entry of MTX into the cells, and the carriers of **AA** alleles had increased MTX levels than **GG** or **AG** alleles, by increased uptake in **B** and **CD4+** cells, and this polymorphism is relevant for deciding the dosage of MTX in autoimmune disorders [12).

This mutation is a missense mutation that occurs in chromosome 21 and the reference SNP assessed is **rs1051266**, which increases the efflux of xenobiotics out of the cells. The amino acid change that occurs is histidine to arginine, and this leads to decreased influx of MTX into the cells (Table 1).

The allelic distribution of this SNP is significantly different in Indian population when compared to Japanese healthy volunteers (Table 2).

Very few Indian studies were related to this polymorphism, and they were studied in malaria [13], childhood acute lymphoblastic leukemia [14], and breast cancer [15]. The distribution of the allelic

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### Table 1: Genetic polymorphisms of methotrexate in different enzymatic pathways

| Gene   | Nucleotide sequence | Consequence | Amino acid substitution | Location | dbSNP  | Effect                                  |
|--------|---------------------|-------------|-------------------------|----------|--------|-----------------------------------------|
| ABCB1  | **C3435T**          | Synonymous  | No amino acid substitution | Chr7     | **rs1045642** | Increased MTX efflux from the cells |
| RFC1   | **G80A**            | Missense    | Histidine to arginine    | Chr21    | **rs1051266** | Decreased MTX entry into cells      |
| MTHFR  | **C677T**           | Intergenic  | Alanine to valine        | Chr1     | **rs1801133** | Decreased enzyme activity            |
| MTHFR  | A1298C              | Missense    | Glutamine to alanine     | Chr1     | **rs1801131** | Decreased enzyme activity            |
| GGH    | C401T               | Missense    | Threonine to isoleucine  | Chr8     | **rs11545078** | Increased deconjugation of MTX      |
| SHMT   | C1420T              | Intrinsic   | No amino acid substitution | Chr17    | **rs9901160** | Decreased enzyme activity           |
| ATIC   | C347G               | Missense    | Threonine to serine      | Chr2     | **rs2372536** | Decreased enzyme activity           |
| MTRR   | A2756G              | Missense    | Aspartate to glycine     | Chr1     | **rs1805087** | Decreased enzyme activity            |
| MTRR   | A666G               | Missense    | Isoleucine to methionine | Chr1     | **rs1801394** | Decreased enzyme activity            |
| AMPD1  | C34T                | Stop gained | Glutamate to lysine      | Chr1     | **rs17602729** | Decreased enzyme activity            |
| RFC1   | **G80A**            | Missense    | Proline to threonine     | Chr1     | **rs11273554** | Decreased enzyme activity           |

### Table 2: Allelic distribution of different SNPs of methotrexate genes in different population

| Gene   | Healthy individuals | Chi-square | p value | Rheumatoid arthritis | PMID |
|--------|---------------------|------------|---------|----------------------|------|
|        | CC                  | CT         | TT      | CC                   | TT   |
| **ABCB1 C3435T** |             |            |         |                      |      |
| Indians | 249                | 19.7       | 51.4    | 28.9                 |      |
| Japanese | 188                | 31.4       | 52.2    | 16.4                 |      |
| Polish  | 97                 | 26.9       | 53.8    | 18.3                 |      |
| RFC-1 G80A | 173               | 50.6       | 60.6    | 33.5                 |      |
| Indians | 299                | 17.4       | 51.8    | 30.8                 |      |
| MTHFR C677T | 173            | 73.4       | 24.8    | 0.02                 |      |
| Indians | 173                | 53         | 73      | 23                   |      |
| Caucasian | 50                | 50         | 44      | 6                    |      |
| MTHFR A1298C | 50            | 50         | 44      | 6                    |      |
| Indians | 140                | 45.7       | 52.9    | 1.40                 |      |
| African-Americans | 53       | 38         | 30      | 2                    |      |
| Caucasians | 50                | 50         | 38      | 12                   |      |
| Indians | 299                | 68.9       | 28.1    | 3                    |      |

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**ABCB1**: ATP-binding cassette proteins, MTX: Methotrexate, Chr: Chromosome number, dbSNP: Database of single nucleotide polymorphisms, RFC: Reduced folate carrier, MTHFR: Methyleneetetrahydrofolate reductase, GGH: Gamma-glutamyl hydrolase, SHMT: Serine hydroxyl methyltransferase, ATIC: 5-aminoimidazole-4-carboxamide ribonucleotide transformylase, MTRR: Methionine synthase reductase, MS: Methionine synthase, AMPD1: Adenosine monophosphate deaminase1, ITPA: Inosine triphosphate pyrophosphatase

**MTHFR**: Methylenetetrahydrofolate reductase, SNPs: Single nucleotide polymorphisms

**RFC-1**: Reduced folate carrier, **G80A**: Missense mutation that occurs in chromosome 21, and the reference SNP (rs) assessed is **rs1051266**, which increases the efflux of xenobiotics out of the cells. The amino acid change that occurs is histidine to arginine, and this leads to decreased influx of MTX into the cells (Table 1).

The allelic distribution of this SNP is significantly different in Indian population when compared to Japanese healthy volunteers (Table 2).
variants significantly differed when compared to Japanese healthy individuals [13,16].

Dervieux et al. investigated the effects of this polymorphism in American RA patients and reported that carriers of 80AA genotype have higher MTX-PG levels and thus influence polyglutamation and found to be good responders to MTX therapy [2]. Drozdzik et al. also reported that the remission in Polish RA patients is increased when they have the 80AA genotype [17]. Hayashi et al. also reported that the same and AA allele genotypes had increased intracellular MTX uptake and increased efficacy and the need for a combination with biologics is less and insisted that genotypes had increased intracellular MTX uptake and increased efficacy [17]. Hayashi et al. also reported that the AA allele carriers of 80AA genotype have higher MTX-PG levels and thus influence polyglutamation and found to be good responders to MTX therapy [2].

Table 3: Genetic polymorphisms of methotrexate and their relation to its efficacy and toxicity in rheumatoid arthritis

| Genetic studies in different population | MTX efficacy | MTX toxicity | Reference |
|----------------------------------------|--------------|--------------|-----------|
| ABCB1 C3435T                            |              |              |           |
| Japanese                               | 124          | 3435TT nonresponders | [8]       |
| Japanese                               | 55           | 3435TT responders   | [9]       |
| Polish                                 | 255          | 3435TT responders   | [7]       |
| Indians                                | NA           | 3435CT nonresponders| [10]      |
| Yugoslavia                             | 213          | 3435TT is a risk genotype |       |
| RFC-1 G80A                             | N            |              |           |
| Americans                              | 226          | 80AA responders   | [2]       |
| Polish                                 | 174          | 80AA responders   | [17]      |
| Japanese                               | 81           | 80AA responders   | [18]      |
| Indians                                | 322          | 80AA responders   | [19]      |
| Yugoslavia                             | 213          | 80G allele is a risk genotype | [11] |
| MTHFR C677T                            |              |              |           |
| Dutch                                  | 236          | 677TT hepatic AE  | [23]      |
| Japanese                               | 106          | 677TT hepatic AE  | [24]      |
| Japanese                               | 186          | 677TT hepatic AE  | [25]      |
| Americans                              | 214          | 677TT CNS adverse effects | [26] |
| Americans                              | 71           | 677TT risk genotypes for osteoporosis/osteopenia | [27] |
| Indians                                | 322          | 677CC responders  | [19]      |
| MTHFR A1298C                           |              |              |           |
| Dutch                                  | 205          | 1298AA responders | [29]      |
| Japanese                               | 106          | 1298CC responders | [24]      |
| Japanese                               | 186          | 1298CC responders | [25]      |
| Japanese                               | 55           | 1298AA responders | [9]       |
| Americans                              | 223          | 1298AA (Indigestion) | [30]   |
| Americans                              | 48           | 1298CC (GI AE, Neurologic AE) | [29] |
| Americans                              | 319          | 1298AC/CC risk genotypes | [31] |
| Israelis                               | 93           | 1298CC responders | [32]      |
| Indians                                | 322          | 1298AA responders | [19]      |
| Yugoslavia                             | 213          | 1298CC alleles had lesser adverse effects | [11] |
| Koreans                                | 167          | 1298CC allele is a risk genotype | [33] |
| GGH C401T                              | 322          | 401TT hepatic AE  | [19]      |
| Americans                              | 214          | 1420CC alopecia AE | [26]      |
| Indians                                | 322          | 1420CC GI AE      | [19]      |

ABCBI: ATP-binding cassette proteins, MTX: Methotrexate, RFC: Reduced folate carrier, MTHFR: Methylene tetrahydrofolate reductase, GGH: Gamma-glutamyl hydrolase, SHMT: Serine hydroxyl methyl transferase

Genes related to metabolic pathway of MTX

**MTHFR**

This gene encodes an enzyme that catalyzes the reduction of 5, 10-methylene tetrahydrofolate to 5-methyltetrahydrofolate, a carbon donor in the metabolism of folate to methionine, and the polymorphism led to a reduction in enzyme activity and associated with hyperhomocysteinemia. There were about dozen polymorphisms in this gene; it was identified that MTHFR C677T and A1298C were the widely studied gene polymorphisms.

This polymorphism C677T was first reported by Frostrate et al. in 1995 [21] and the second polymorphism A1298C was reported by Weisberg et al. in 1998 [22]. In C677T, heterozygous mutants have ~40% reduction in enzyme activity and homozygous mutants have ~70% reduction, and this leads to thermostable variant of the enzyme. In A1298C polymorphism, the homozygous mutants have about ~40% reduction in enzyme activity.

The first article related to this polymorphism C677T was published by van Ede et al. which assessed discontinuation of MTX due to an elevation of transaminases. In this study, it was concluded that TT genotypes were found to be the risk genotypes. The elevation of liver enzymes is due to increased homocysteine levels, and supplementation with folic acid or folinic acid reduced the toxicity-related discontinuation rates [23].

**MTHFR C677T**

This mutation is a missense mutation located in chromosome 1 and the reference SNP (rs) assessed is rs1801133, and the change in amino acid sequence occurs as valine instead of alanine and leads to defective enzyme activity (Table 1).

The allelic distribution of the different genotypes of this polymorphism is similar in Indians and Afro-Americans but significantly different from Caucasian healthy population (Table 2).

van Ede et al. reported that the presence of 677CT or 677TT genotypes in Dutch RA patients has led to discontinuation of MTX therapy due to the risk of adverse effects and the presence of this mutation increased the liver toxicity. The presence of defective enzyme leads to...
hyperhomocysteinemia, and elevation of alanine aminotransferase and supplementation of folic or folinic acid could prevent these adverse effects [23].

Urano et al. reported that, in Japanese RA patients, the presence of 677C allele leads to good response and presence of 677T allele increases the adverse effects [24]. Taniguchi et al. also proved that 677TT genotypes were prone to adverse effects of MTX [25].

Weisman et al. studied the effect of this polymorphism in American RA patients and reported that 677TT genotypes are prone to the risk of central nervous system (CNS) adverse effects [26]. Brambila-Tapia et al. studied this MTHFR C677T in American RA patients and carriers of T alleles (TT homozygotes) had lower BMD and reported to have increased risk of osteoprosis, and folic acid supplementation is suggested as a prophylactic measure [27].

Ghodke et al. reported that 677TT genotypes are susceptible to adverse effects of MTX [19]. In another Indian study by Aggarwal et al., it was concluded that this polymorphism was not related to efficacy or toxicity of MTX [28].

MTHFR A1298C

This mutation is a missense mutation located in chromosome 1 and the reference SNP (rs) assessed is rs1801131; the change in amino acid sequence occurs as glutamine instead of alanine and leads to defective enzyme activity (Table 1).

This mutation is a missense mutation located in chromosome 1 and the reference SNP (rs) assessed is rs1801131; the change in amino acid sequence occurs as alanine instead of glutamine and leads to defective enzyme activity (Table 1).

The allelic distribution of the different genotypes of this polymorphism is significantly different in Afro-Americans, Caucasians, and Japanese healthy population when compared to Indian healthy population (Table 2).

Wessels et al. reported that MTHFR 1298AA was found to be responders and has improvement in the clinical indices and also concluded that C allele is also susceptible to adverse effects of MTX [29].

Urano et al. reported that, in Japanese RA patients, 1298C allele carriers were found to be good responders and they received lower doses of MTX to attain remission [24]. Similar result was reported by Taniguchi et al. in Japanese RA patients, the 1298GC genotypes were found to be good responders to MTX, and they were found to receive lower doses of MTX in a year of its therapy [25]. Kato et al. studied the effect of A1298C in Japanese RA patients and reported that AA genotypes were associated with good response, which is contradictory to previous studies in Japanese RA patients [9].

Hughes et al. found that, in American RA patients, the carriers of 1298A allele were susceptible to adverse effects of MTX that too in Caucasians. The reported adverse effects were indigestion due to GI disturbances caused by MTX [30].

Dervieux et al. reported that the carriers of C allele in American RA patients and adverse effects of GI and CNS were probable and 1298AC/CC genotypes were risk genotypes [2].

Davis et al. found that A1298C polymorphism was associated with adverse effects and increased copies of this lead to a higher incidence of adverse effects in American RA patients [31].

Berkun et al. reported that the allele frequency of 1298CC was higher in Israeli RA population and the carriers of 1298AA allele had a higher frequency of adverse effects, such as hyperhomocysteinemia in spite of higher folic acid supplementation, and 1298CC may protect against MTX-related adverse effects [32].

Grabar et al. reported that MTHFR A1298C polymorphism is protective related to adverse effects of MTX in Yugoslavian RA patients [20].

Choe et al. investigated the C677T polymorphism in Korean RA patients and found 1298AC/CC genotypes experienced at least one adverse effect of MTX when compared to 1298AA genotypes [33].

Haplotype analysis

The MTHFR is a gene with two SNPs related to RA, and some studies were performed based on haplotype analysis of MTHFR C677T and A1298C. In Japanese RA patients, it was reported that carriers of 677T-1298A were found to have a higher frequency of adverse effects and carriers of 677C-1298C were found to receive lower MTX doses and are good responders [24].

Serine hydroxyl methyltransferase (SHMT C1240T)

It is an important enzyme in one-carbon pathway of the purine and thymidylate biosynthesis, leading to DNA synthesis.

This mutation is an intrinsic mutation located in chromosome 17 and the reference SNP assessed is rs9901160; no amino acid substitution takes place but could decrease the enzyme activity (Table 1).

Very few studies were performed in RA patients, and an Indian study [19] highlighted that GI adverse effects are common in CC allele genotypes and the same was reported in American patients [26]. Other Indian studies reflected investigation of this polymorphism in breast cancer [34] and autism [35] (Table 3).

DHFR

This is a key enzyme inhibited by MTX and polymorphisms in it are less studied (Fig. 1). It has reported polymorphisms such as rs12517451, rs10072026, and rs1643567, associated with adverse effects [36]. DHFR A317G was studied by Milic et al. and found that 317AA genotypes were associated with poor response [37].

Genetic polymorphism related to polyglutamation

GGH

GGH is a lysosomal peptidase that catalyzes elimination of gamma-linked polyglutamates. Long-chain MTX-PGs are converted to short-chain MTX-PGs and further converted back to MTX and efflux from the cell. Since the MTX-PGs are associated with disease activity in RA, GGH polymorphisms could influence the therapeutic outcome. Three polymorphisms had been widely studied, and they are GGH C401T, GGH C452T, and GGH T16C.

This mutation is a missense mutation located in chromosome 8 and the reference SNP assessed is rs11545078, which increases the expression could progress as resistance to MTX [38]. Ghodke et al. reported that TT allelic genotypes in Indian RA patients were found to be more susceptible to hepatic adverse effects of MTX [19] (Table 5).

Chave et al. studied the expression of this polymorphisms and its functional activity in MCF-7 cells and reported that increased expression could progress as resistance to MTX [38]. Ghodke et al. reported that TT allelic genotypes in Indian RA patients were found to be more susceptible to hepatic adverse effects of MTX [19] (Table 5).

FPGS

This gene is related to polyglutamation of MTX and is important in one-carbon metabolism. In a study by Oppeneer et al., they found that this gene is not associated with homocystine metabolism [39]. Sharma et al. reported that a polymorphism in this gene (rs1544105) is associated with poor response to MTX therapy in RA [40]. In UK rheumatoid cohort study by Owen et al., they found that FPGS polymorphism was associated with adverse effects [36].
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Genes related to pyrimidine pathway

TYMS or TSER *2/*3

Dervieux et al. assessed this polymorphism and concluded that patients having two tandem repeats had better clinical response than triple repeat [2]. Kumagai et al. assessed the impact of this polymorphism in Japanese RA patients and found that triple-repeat allele of the polymorphism (*3/*3) received a higher dose of MTX than double repeat allele [41].

TYMS or TSER 3’UTR 6 bp deletion TTAAAG

Kumagai et al. studied this polymorphism in Japanese RA patients and had shown that this 6 bp deletion leads to decrease in CRP levels and improvement in response [36]. This deletion polymorphism is associated with decreased expression of mRNA and could increase the drug response in RA patients [37].

TYMS or TSER (rs28535329)

Sharma et al. studied the effect of this polymorphism in RA patients and found that carriers of AA genotype are non-responders [40].

Genes related to adenosine pathway

Blockade of AICAR affects purine synthesis and leads to accumulation of adenosine, and the anti-inflammatory effects of MTX are mediated through this pathway. The polymorphisms in genes influencing anti-inflammatory adenosine release are 5-aminomimidazole-4-carboxamide ribonucleotide transformylase (ATIC) C347G, inosine triphosphate pyrophosphatase (ITPA) C94A, and AMPD1 C34T and adenosine receptors (ADORA) 2a. MTX inhibits the deamination of adenosine and modulates its pharmacokinetics and pharmacodynamics. Adenosine exhibits its anti-inflammatory effect through modulation of inflammatory cells.

Adenosine binds to several receptors such as A1, A2a, A2b, and A. ADORA 2a is highly expressed in synovium of RA patients receiving MTX, and SNPs in this gene are reported to influence adverse effect profile of MTX [42].

Hider et al. studied five SNPs in ADORA 2a in 309 RA patients (rs5760410, rs2298383, rs 3761422, rs2267076, and rs2236624) and found an association with GI adverse effects. The possible explanations were anti-proliferative effects in the gut and sensitization of chemoreceptors in the brain due to this polymorphism and could be alleviated by administration of folic acid and 5HT3 antagonists [43].

Wessels et al. reported favorable alleles for response as T allele of AMPD1, CC allele of ATIC C347G, and CC allele of ITPA C94A. Regarding toxicity, G allele of ATIC C347G was associated with GI AEs [44]. ATIC rs4673993 was assessed and reported as associated with low disease activity in the study [45].

Fig. 1: Genetic polymorphic alleles of different genes in methotrexate pathway with respect to its safety and efficacy. ABCB1: ATP-binding cassette proteins, MTX: Methotrexate, Chr: Chromosome number, RFC: Reduced folate carrier, MTHFR: Methylenetetrahydrofolate reductase, GGH: Gamma-glutamyl hydrolase, SHMT: Serine hydroxyl methyltransferase, ATIC: 5-aminomimidazole-4-carboxamide ribonucleotide transformylase, MTRR: Methionine synthase reductase, MS: Methionine synthase, AMPD1: Adenosine monophosphate deaminase1, ITPA: Inosine triphosphate pyrophosphatase, FPGS: Foly-poly glutamyl synthase, AICAR: aminomimidazole-4-carboxamide ribonucleotide, TYMS or TSER: Thymidylate synthase, IMP: Inositol mono phosphate, ITP: Inositol triphosphate, DHFR: Dihydrofolate reductase, ADP: Adenosine diphosphate, ADA: Adenosine deaminase, SHMT: Serine hydroxyl methyltransferase

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Polygenetic analysis
Since several genes are associated with the absorption, distribution, metabolism, and elimination pathways of a drug and several genes contribute to its pharmacokinetics and pharmacodynamics. Some of the studies were reported with polygenetic analysis, and some are highlighted in this regard.

Dervieux et al. studied the combined effect of RFC G80A, ATIC C347T, and TSER 2*3 polymorphisms in 108 RA patients in relation with MTX-PG and efficacy. Favorable alleles were reported to be homoyzogotes of these polymorphisms (RFC-1 AA, ATIC 347GG, and TSER *2/*2) and a pharmacogenenic index was calculated. Patients having all the favorable alleles were reported to have increased RBC-MTX PG levels and improvement in disease activity (reduction in pain, tender joint count, swollen joint count, and physician’s global assessment) compared to noncarriers of all the genotypes. Pharmacogenetic and metabolite measurements are valuable tools in designing optimal drug therapy or individualized therapeutic strategies [2]. The same author in another study reported that the effect of GGH C401T, ATIC C347T, MTHFR A1298C, MTRR A2756G, and MS A66G combinations was studied by Dervieux et al. The risk alleles were identified as GGH 401CC, ATIC 347GG, MTHFR 1298AC/CC, MTRR 2756AA, and MS 66GG. These genotypes were found to be associated with CNS and GI adverse effects [46].

Wessels et al. studied the polygenetic effect of genes related to adenosine release and reported that the carriers of T allele of AMPD1, CC allele of ATIC C347T, and CC allele of ITPA C94A are good responders [44].

Weisman et al. studied the polygenic effects of MTHFR G677T, TYMS *2/*3, ATIC C347T, and serine hydroxymethyltransferase (SHMT) C1420T and found that the risk genotypes were TT alleles of MTHFR C677T (CNS adverse effects), CC alleles of SHMT C1420T (CNS adverse effects and alopecia), GG alleles of ATIC C347T (GI adverse effects), and *2/*2 allele of TYMS *2/*3 (alopecia) [26].

As a snapshot of the above-mentioned studies, the alleles of different genes and their relation to efficacy and susceptibility to toxicities of MTX are depicted in Fig. 1.

CONCLUSION
The application of pharmacogenetics from laboratory to personalized medicine is a tough task. The hurdles include identification of a subset of patients, economic constraints, and development of rapid techniques, ethical, legal, and moral implications. The potential genes influencing MTX efficacy or toxicity highlighted in this review insists the importance of pharmacogenetics. Identification of patients as good responders and segregating those susceptible to adverse effects could improve patient compliance. Although the studies have different results, the contributing reasons include lack of replication sets, inconsistent sample size, difference in parameters of measurement of drug toxicity, and efficacy. In addition, the genetic markers could be influenced by environmental factors such as diet-related folate acid intake. Application of pharmacogenetics as a right drug to the right person at the right time still has to go a long way to hit the nail of personalized medicine.

In Indian scenario, only few studies are performed in relation to MTX in RA, and more prospective studies are essential to improve the therapeutic outcome.

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