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Research Article

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-aminoimidazole-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, ITPA 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 16CC, 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.

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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 anticancer 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 foly-poly glutamyl synthase (FPGS) 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-PG$_{5\text{-}7}$) 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 (DHFR), involved in DNA biosynthesis, thymidylate synthase (TYMS), involved in DNA biosynthesis and repair and 5-aminoimidazole-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 folinic 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 (ABCB1) C3435T

This is one of the important genes related to P-gp expression, which are best drug transporters in humans. There are several genes and several polymorphisms related to multiple drug resistance, and ABCB1 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, 3435TT 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

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	Chr.7	rs1045642	Increased MTX efflux from the cells
RFC1	G80A	Missense	Histidine to arginine	Chr.21	rs1051266	Decreased MTX entry into cells
MTHFR	C677T	Intergenic	Alanine to valine	Chr.1	rs1801133	Decreased enzyme activity
MTHFR	A1298C	Missense	Glutamine to alanine	Chr.1	rs1801131	Decreased enzyme activity
GGH	C401T	Missense	Threonine to isoleucine	Chr.8	rs11545078	Increased deconjugation of MTX
SHMT	C1420T	Intronic	No amino acid substitution	Chr.17	rs9901160	Decreased enzyme activity
ATIC	C347G	Missense	Threonine to serine	Chr.2	rs2372536	Decreased enzyme activity
MTRR	A2756G	Missense	Aspartate to glycine	Chr.1	rs1805087	Decreased enzyme activity
MS	A66G	Missense	Isoleucine to methionine	Chr.1	rs1801394	Decreased enzyme activity
AMPD1	C34T	Stop gained	Glutamine to lysine	Chr.1	rs17602729	Decreased enzyme activity
ITPA	C94A	Missense	Proline to threonine	Chr.1	rs1127354	Decreased enzyme activity

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

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

Gene	Healthy individuals				Chi-square	p value	Rheumatoid arthritis				PMID
ABCB1 C3435T	**N**	**CC**	**CT**	**TT**				**CC**	**CT**	**TT**	
Indians	249	19.7	51.4	28.9			No relevant data				[6]
Japanese	188	31.4	52.2	16.4	6.1329	<0.046	159	34	51.6	14.4	[16]
Polish	97	26.9	53.8	18.3	3.6978	NS	No relevant data				[7]
RFC-1 G80A		GG	GA	AA				GG	GA	AA	
Indians	173	0.06	60.6	33.5			No relevant data				[13]
Japanese	299	17.4	51.8	30.8	15.0021	<0.0005	159	21.4	46.5	32.1	[16]
MTHFR C677T		CC	CT	TT				CC	CT	TT	
Indians	173	73.4	24.8	0.02			No relevant data				[13]
African-Americans	53	75	23	2	0.4387	NS	138	79	20	1	[30]
Caucasians	50	50	44	6	13.0994	<0.001	393	50	40	10	[30]
MTHFR A1298C		AA	AC	CC				AA	AC	CC	
Indians	140	45.7	52.9	1.40			No relevant data				[47]
African-Americans	53	38	30	2	10.9524	<0.004	138	74	25	1	[30]
Caucasians	50	50	38	12	11.9469	<0.002	393	45	42	13	[30]
Japanese	299	68.9	28.1	3	13.316	<0.001	159	68.6	26.4	5	[16]

ABCB1: ATP-binding cassette proteins, MTX: Methotrexate, Chr: Chromosome number, dbSNP: Databse of single nucleotide polymorphisms, RFC: Reduced folate carrier, MTHFR: Methylenetetrahydrofolate reductase, SNPs: Single nucleotide polymorphisms

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		3435T 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				[11]
Dutch	205	1298AA responders	1298CC (GI AE)	[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	1298AA higher homocysteine levels	[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				
Indians	322		401TT hepatic AE	[19]
SHMT C1420T				
Americans	214		1420CC alopecia AE	[26]
Indians	322		1420CC GI AE	[19]

ABCB1: ATP-binding cassette proteins, MTX: Methotrexate, RFC: Reduced folate carrier, MTHFR: Methylenetetrahydrofolate reductase, GGH: Gamma-glutamyl hydrolase, SHMT: Serine hydroxyl methyl transferase

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 this polymorphism influences MTX efficacy in Japanese RA patients [18], and Ghodke *et al*. also reported the same in Indian RA patients [19] and Grabar *et al*. reported that 80 G allele carriers have the risk of adverse effects to MTX, in Yugoslavian RA patients [20] (Table 3).

Genes related to metabolic pathway of MTX

MTHFR

This gene encodes an enzyme that catalyzes the reduction of 5, 10-methylenetetrahydrofolate 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 Frosst *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 thermolabile 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 osteoporosis, 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]. A similar result was reported by Taniguchi *et al*. in Japanese RA patients, the 1298CC 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 C1420T)

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

This mutation is a intronic 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 allelic 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 rs1643657, 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 deconjugation of MTX, which is effluxed out of the cells. The amino acid change that occurs is threonine to isoleucine, and this leads to increased efflux of MTX into the cells (Table 1).

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 3).

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 homocysteine 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].

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 (rs2853539)

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-aminoimidazole-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 $5HT_3$ 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].

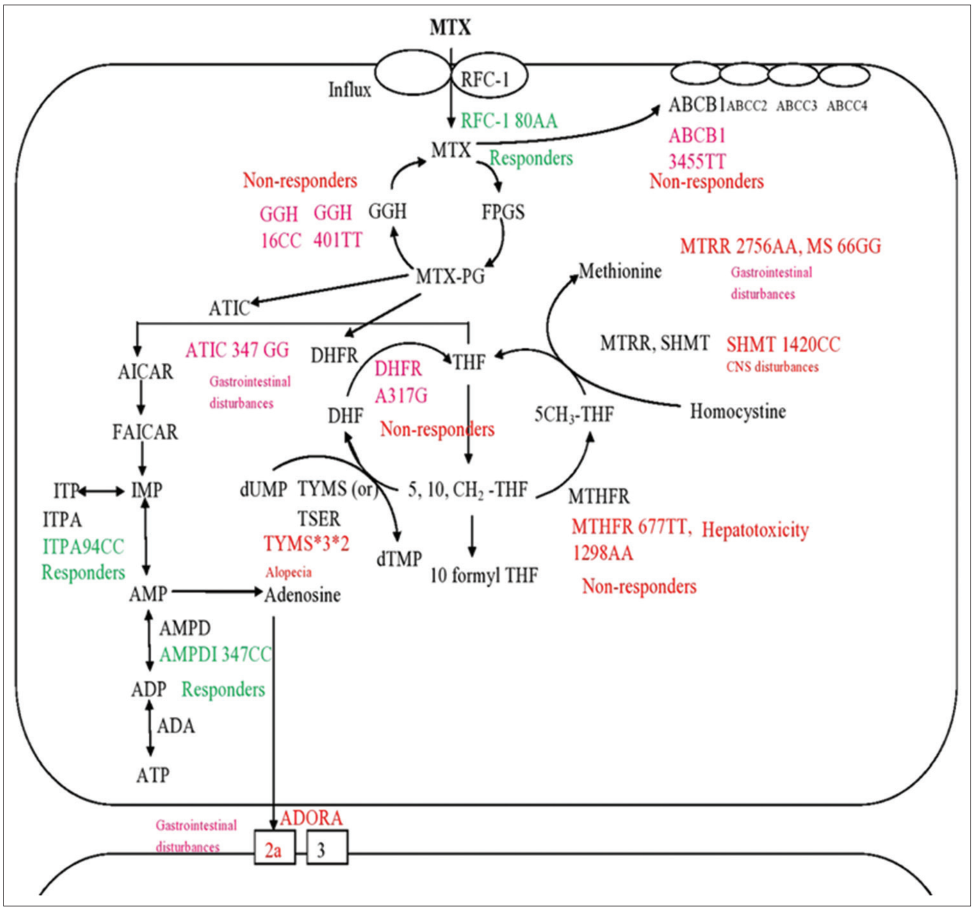

Fig. 1: Genetic polymorphic alleles of different genes in methotrexate pathway with respect to its safety and efficacy. ABCB1: ATP-binding cassette proteins, MTX: Methotrexate, Chr: Chromosome number, RFC: Reduced folate carrier, MTHFR: Methylenetetrahydrofolate reductase, GGH: Gamma-glutamyl hydrolase, SHMT: Serine hydroxyl methyltransferase, ATIC: 5-aminoimidazole-4-carboxamide ribonucleotidetransformylase, MTRR: Methionine synthase reductase, MS: Methionine synthase, AMPD1: Adenosine monophosphate deaminase1, ITPA: Inosine triphosphate pyrophosphatase, FPGS: Foly-poly glutamyl synthase, AICAR: aminoimidazole-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

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 C347G, and TSER 2*/3* polymorphisms in 108 RA patients in relation with MTX-PG and efficacy. Favorable alleles were reported to be homozygotes of these polymorphisms (RFC-1 AA, ATIC 347GG, and TSER *2/*2) and a pharmacogenetic 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 1298 AC/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 C347G, and CC allele of ITPA C94A are good responders [44].

Weisman *et al*. studied the polygenic effects of MTHFR C677T, TYMS *2/*3, ATIC C34TG, and serine hydroxyl methyltransferase (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 C34TG (GI adverse effects), and *2/*2 alleles 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 folic 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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