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REVIEW

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Relationship between the efficacy and adverse effects of methotrexate and gene polymorphism

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Abstract

Methotrexate is a widely used drug in clinical practice for the treatment of collagen vascular diseases and malignant tumors. It has good anti-inflammatory and anti-proliferative effects, but the cytotoxicity of methotrexate can cause various adverse reactions in patients. Studies have shown that the sensitivity and tolerance of different individuals to methotrexate is different. There are many reasons for this difference. Among them, genetic polymorphism is one of the main factors that cause individual differences. This article provides an overview of the genetic polymorphisms of key proteins involved in methotrexate metabolism and transport, such as MTHFR, FPGS, γ-GGH, ABC transporter, OATPs, SLC, TS and DHFR, are related to their efficacy and adverse reactions. The aim is to clarify the impact of genetic polymorphisms on the efficacy and adverse effects of methotrexate at the pharmacogenomic level, in order to provide a basis for the clinical application of methotrexate.

Keywords Methotrexate, Gene polymorphism, Adverse effect, Metabolic enzymes, Transporters

Introduction

As a classic immune drug, methotrexate (MTX) is widely used in the treatment of rheumatoid arthritis, acute lymphoblastic leukemia, osteosarcoma, psoriasis, lymphoma, gastric cancer, breast cancer and other diseases. MTX is an analog of dihydrofolate, and its mechanism of action is mainly to play a pharmacological role by inhibiting key enzymes in folate cycle metabolism. The metabolic process of MTX was well clarified in previous study (see it in Fig. 1) [1]. Briefly, MTX is mainly transported from the blood to kinds of cells, such as liver cells, red cells, white cells, synovial cells and so on, through reduced folate carrier-1 (RFC-1). After entering the cell, under the catalysis of polyglutamate synthetase (FPGS), glutamate is connected with MTX to form active methotrexate polyglutamates (MTXPGs) [2]. γ-glutamyl hydrolytic enzyme (GGH) removes polyglutamic acid of MTXPG to form MTX, which was transported out of cells by ATP binding cassette (ABC) [3].

Dihydrofolate reductase (DHFR) and thymidylate synthase (TS), which are involved in folic acid metabolism, can be directly inhibited by MTXPGs, resulting in the reduction of tetrahydrofolate (FH4) and deoxythymidine (dTMP), and the inhibition of protein synthesis, DNA synthesis and repair, thus inhibiting the proliferation of tumor cells. MTXPGs also inhibit the activity of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) and 5-aminoimidazole-4-carboxamide ribonucleotide transformylase/IMP cyclohydrolase (ATIC), resulting in the accumulation of adenosine outside cells. Adenosine has anti-inflammatory activity and can inhibit the production of inflammatory cytokines [2, 4]. Other enzymes related to folic acid metabolism, such as MTHFR, serine hydroxymethyltransferase (SHMT), cannot be directly inhibited by MTX, but their expression

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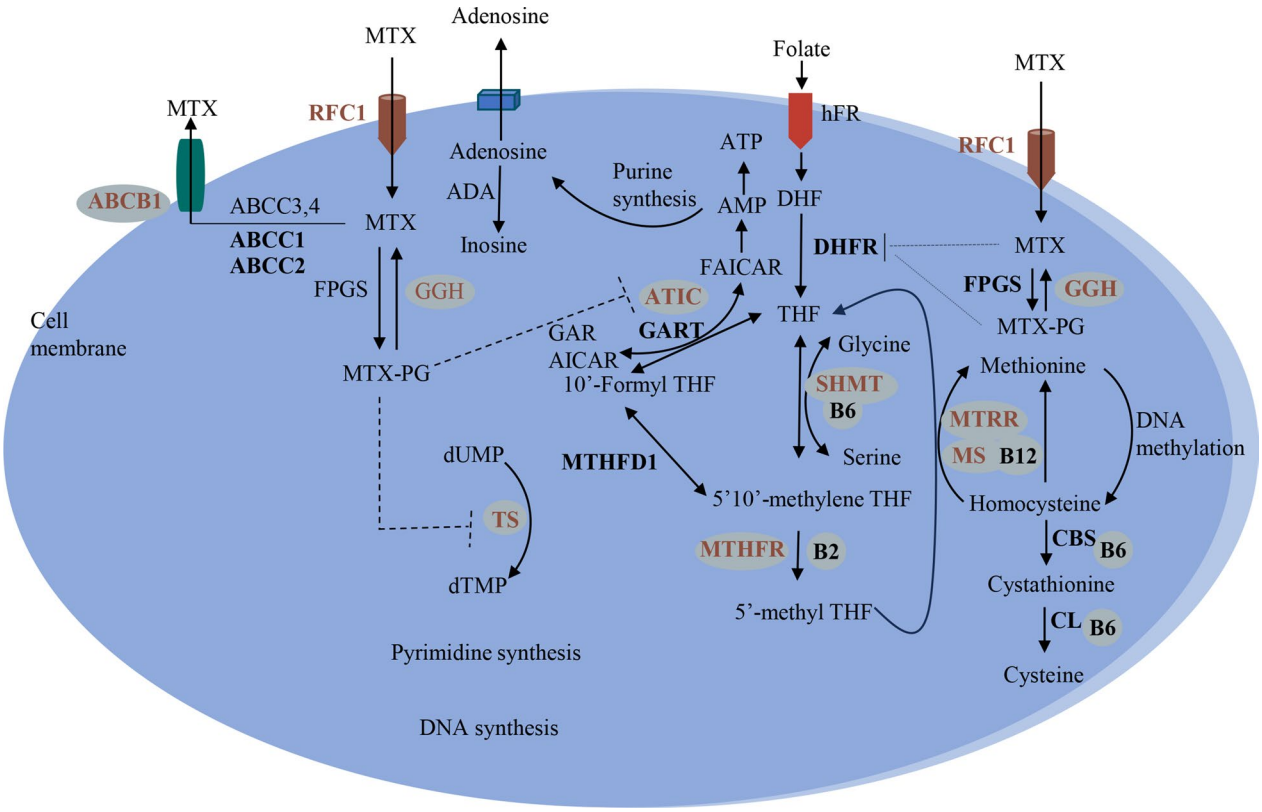

Fig. 1 Intracellular methotrexate metabolic pathway. Figure illustrates schematic representation of the intracellular folate biosynthetic pathway and related pathways. Enzymes involved in different pathways are denoted in bold. Transporters: ABCB1 and ABCC1–4: Adenosine triphosphate–binding cassette (ABC) transporters; hFR: Human folate carrier; RFC-1: Reduced folate carrier1. Enzymes: ADA: Adenosine deaminase; ATIC: 5-aminoimidazole-4-carboxamide ribonucleotide transformylase/IMP cyclohydrolase; CBS: Cystathionine-β-synthase; CL: Cystathionine lyase; DHFR: Dihydrofolate reductase; FPGS: Folylpolyglutamyl synthase; GART: Glycinamide ribonucleotide formyltransferase; GGH_γ: Glutamyl hydrolase; MS: Methionine synthase; MTHFR: Methylenetetrahydrofolate reductase; MTHFD1: Methylenetetrahydrofolate dehydrogenase; MTRR: Methionine synthase reductase; SHMT: Serine hydroxymethyltransferase; TS: Thymidylate synthase. ADP: Adenosine diphosphate; AICAR: 5′-aminoimidazole-4′-carboxamide ribonucleotide; AMP: Adenosine monophosphate; ATP: Adenosine triphosphate; CH3: Methyl group; DHF: Dihydrofolate; dTMP: Deoxythymidine-5′-monophospate; dUMP: Deoxyuridine-5′-monophospate; FAICAR: 10-formyl-AICAR; IMp: Inosine monophosphate; GAR: Glycinamide ribonucleotide; MTX: Methotrexate; MTXPG: methotrexate polyglutamates; THF: tetrahydrofolate

can induce MTX to change the normal metabolism of folic acid [5]. Intracellular methotrexate metabolic pathway shows as Fig. 1 [5].

However, in clinical, MTX often causes serious adverse effects due to cytotoxicity, such as liver and kidney injury, anemia, neurotoxicity, mucositis, gastrointestinal reactions, etc. Therefore, it is necessary to monitor the blood concentration of MTX to reduce the occurrence of these adverse effects. Because the efficacy and adverse effects of different individuals to the same dose of MTX are quite different, more accurate detection indicators are needed in clinical practice to predict the efficacy and adverse effects of MTX. In recent years, studies have shown that individual genetic differences are one of the important factors affecting drug efficacy and adverse effects [6]. Therefore, by studying the influence of gene polymorphism on the sensitivity and tolerance of individuals to methotrexate, we can provide guidance and theoretical basis for clinical individualized precise drug use.

This article will explore the impact of gene polymorphism on MTX's efficacy and adverse effects from the perspective of MTX's key metabolic enzymes and their transporters, which may provide a basis for the clinical practice of MTX.

Relationship between methylenetetrahydrofolate reductase and MTX

The main function of MTHFR is to catalyze the reduction of substrate 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate (5-MTHF). The latter, as a methyl donor, enters the blood and participates in multiple biological metabolic processes, which

is crucial for the conversion of toxic homocysteine into methionine and purine in body [7]. The polymorphism of MTHFR gene may reduce the activity of MTHFR enzyme, thus preventing the reduction of 5, l0-MTHF to 5-MTHF. The decrease of folic acid level and the increase of toxic homocysteine led to a series of adverse effects [8]. Among them, *MTHFR C677T* (*rs1801133*) and *MTHFR A1298C* (*rs1801131*) are the most deeply studied polymorphic loci.

At present, some research results show that MTHFR gene polymorphism is related to MTX efficacy. The mean disease activity score (DAS) of RA patients with *MTHFR 1298AA* genotype was 28, which was significantly lower than that of patients with *MTHFR 1298AC/CC* genotype ($p=0.04$). Therefore, *MTHFR A1298C* polymorphism may affect the efficacy of MTX [9]. Salazar et al. showed that *C677T*, *A1298C* were related to the decrease of MTHFR enzyme activity, thrombocytopenia, and the increase of serum creatinine level. This finding plays an important role in optimizing the treatment of high-dose MTX.C.M. Ulrich et al. found that 220 patients with chronic myeloid leukemia were treated with methotrexate for a short period of time after receiving bone marrow transplantation. The enzyme activity of patients with *MTHFR 677 TT* genotype was 30% of that of patients with CC genotype, the tolerance to MTX was reduced, and the oral mucositis index (OMI) was increased, suggesting that this genotype may affect the dosage of MTX [10]. However, some studies reported that MTHFR gene polymorphism and MTX efficacy were not related [11, 12]. For example, an analysis of MTHFR gene polymorphism in 110 Chinese patients with rheumatoid arthritis (RA) showed that *C677T* and *A1298C* were not related to MTX efficacy [13].

The results of the study on the relationship between MTHFR gene polymorphism and MTX adverse effects are different. Meta analysis of Huang et al. and retrospective cohort study of 162 RA patients in China found that *C677T* gene polymorphism was associated with MTX induced adverse effects [14]. A meta-analysis of 50 literatures drew similar conclusions: *C677T* gene polymorphism was statistically associated with the increased risk of MTX toxicity including liver injury, kidney injury and mucositis. whereas a tendency toward the decreased risk of nephrotoxicity of A1298C gene polymorphism [15]. Erculj et al. found that *MTHFR G1958A* and *A1298C* were not related to adverse effects of MTX in the analysis of patients with non-Hodgkin's lymphoma (NHL) after receiving high-dose MTX (HD-MTX) treatment. The probability of thrombocytopenia (OR = 1.14; 95% CI 1.11–112.01; $p=0.041$) and leukopenia (OR = 1.86; 95% CI 1.12–3.07; $p=0.006$) increased in carriers with *C677T* allele [16]. On the contrary, Lu et al. conducted a retrospective study on 93 children with NHL in China, and confirmed the relationship between the *A1298C* and *C677T* gene polymorphisms and HD-MTX toxicity. *MTHFR 677 CT/TT* genotype carriers are more prone to oral mucositis, leukopenia, anemia and other adverse effects. *MTHFR A1298C* mutant plays a protective role in patients with adverse vomiting effects, but increases the risk of anemia and leukopenia [17]. A study on children with ALL and NHL treated with HD-MTX (5 g/m^2) has similar results. Patients with MTHFR A1298C polymorphism have significantly increased MTX blood concentration within 48 h of administration, and show more blood toxicity symptoms, such as thrombocytopenia [18]. Other studies reported that the MTX clearance rate of ALL patients with *677 TT* genotype decreased, and the probability of mucositis after MTX treatment increased (OR = 23, 95% CI 2.1–240); In addition, patients with homozygous *A1298C* mutation have a lower risk of leukopenia [19]. Kyvsgaard et al. performed SNP analysis on 119 patients with juvenile idiopathic arthritis (JIA). The results showed that *MTHFR C677T* was associated with MTX adverse effects, and CC type was significantly associated with MTX intolerance compared with CT/TT type ($p=0.02$) [20]. In addition, some scholars found that *677 TT* genotype was associated with an increased risk of recurrence. Compared with CC/CT genotype, the 7-year disease-free survival rate and overall survival rate of TT genotype carriers were lower [21]. Lambrecht et al. also showed that although 677 TT, CT, CC genotype osteosarcoma patients had no significant difference in survival rate. However, patients with TT genotype osteosarcoma have a higher risk of recurrence [22]. Contrary to the above results, *C677T* or *A1298C* has not been reported to be associated with MTX adverse effects [23–25].

There are reasons for inconsistency in the research results. Scholars generally believe that the number of samples included in the study, the patient's race, age, differences in dietary habits, their own underlying diseases, MTX dose and drug differences in combination, folic acid supplementation and so on, will affect the research results. Subsequently, the research scheme can be improved by expanding the number of samples and adding other genotype polymorphisms into the analysis model.

Polyglutamine synthetase and γ-Glutamyl hydrolase are involved in the efficacy of MTX

FPGS and γ-GGH is a key enzyme for transforming MTX into MTXPGs after entering cells and transporting MTX out of cells for metabolism. After MTX enters the cell, FPGS catalyzes the formation of MTX's active form—MTXPGs. MTXPGs promotes cell apoptosis by inhibiting DHFR, TS, DNA synthesis, protein synthesis and

other related enzymes, which is responsible for hydrolyzing long chain MTXPGs, transforming them into short chain MTXPGs and further MTX, so as to combine with transporters to transport them out of cells [7]. The dynamic balance of MTX in cells is maintained by FPGS and GGH. The gene polymorphism of FPGS and GGH may change the enzyme activity, regulate the concentration of MTXPGs in cells, and lead to differences in individual sensitivity to MTX and drug resistance.

It has been found that the mutation of *FPGS rs35789560* is related to the decrease of FPGS enzyme activity, resulting in the decrease of MTXPG content in cells. In addition, this mutation is significantly associated with an increased risk of relapse in ALL patients [26]. Huang et al. reported that compared with *rs1544105* GG/GA genotype, patients with *rs1544105* AA genotype had significantly higher MTX blood drug level, longer median survival time and significant difference in overall survival. Hence, polymorphism of FPGS *rs1544105* might be used as an effective approach for prediction of the treatment outcome of MTX [27]. Other studies have confirmed that carrying FPGS *rs1544105* AG and rs10106 AG in RA patients is related to MTX induced adverse effects, which may regulate MTXPGs level by changing enzyme activity [28]. For the study of GGH gene polymorphism, the current focus is mainly on *C401T*. Wierkot et al. found that the frequency of adverse effects of *GGH 401 CC* type was higher than that of CT/TT type in white RA patients. T allele may have protective effect on MTX induced adverse effects [29]. Kalantari et al. reported that 401 CC/CT genotype is related to thrombocytopenia (95% CI 0.009–0.019, OR = 0.265) and leukopenia (95% CI 0.021–0.042, OR = 2.182) in ALL patients after receiving MTX treatment. C allele may be an important factor leading to leukopenia and thrombocytopenia, while T allele may play a role in preventing thrombocytopenia [30]. However, Koomdee et al. conducted research on children with ALL who received HD-MTX (2.5 or 5 g/m^2) chemotherapy, and confirmed that GGH 401 CT/TT genotype was related to blood toxicity, and the carrier's risk of grade 2–4 and grade 3–4 thrombocytopenia and grade 3–4 leucopenia increased [31]. In addition, Jekic et al. [32] included 184 RA patients receiving MTX treatment in the study, and found that GGH-G354T mutation was significantly related to bone marrow suppression. Another study found that *GGH T16C* (*rs1800909*) was related to hepatotoxicity. After single nucleotide polymorphism (SNP) analysis of 92 Japanese JIA patients, compared with TT genotype, patients with CC/CT genotype were more likely to have liver dysfunction. It is speculated that the cause of adverse reaction is that the mutation of this allele may be related to the decrease of GGH activity, leading to the increase of MTXPG content in liver cells and the influence of MTX metabolism [33]. At present, there are few studies on the correlation between the gene polymorphism and the efficacy of MTX, and the study on the correlation with adverse effects has problems such as small sample size, unclear mechanism of gene polymorphism, etc., which need further research and data support.

Relationship between adenosine triphosphate binding transporter and MTX efficacy

ABC transporter family is a kind of transmembrane proteins. After binding with drug molecules and ATP, it pumps drugs out of cells with ATP hydrolysis. Primary drug resistance of malignant tumors is closely related to drug metabolism mediated by ABC transporter. The ABC transporter family includes seven subfamilies, namely, ABCA-ABCG, and each subfamily contains multiple members. Among them, ABC transporters involved in MTX metabolism are multidrug resistance 1 (MDRl/ABCBl), multidrug resistance associated protein 2 (MRP2/ABCC2), and breast cancer resistance protein (BCRP/ABCG2) [3]. MTX is mainly metabolized in kidney and liver. The reduction of MTX clearance rate will lead to the accumulation of MTX, which will increase the risk of tissue cell damage [7]. Most MTX in hepatocytes is pumped into the blood stream through ABCC3 and ABCC4, and only a small part is discharged from the bile duct through ABCC2, ABCB1 and ABCG2. In addition, ABCB1, ABCC2, ABCC4 and ABCG2 can mediate MTX excretion through the intestine and urethra [34]. The gene polymorphism of ABC transporter family mainly affects the clearance rate of MTX and changes its blood concentration, leading to the difference of MTX efficacy and adverse effects among individuals.

The results of the study on the relationship between gene polymorphism of ABC transporter family and MTX efficacy are inconsistent. Chuan Xiang Ma and others suggested that ABCB1 gene polymorphism can be used as an important marker to predict the efficacy of MTX in treating ALL. Patients with *C3435T* site CT/TT genotype and *G2677T/A* site TT/TA genotype had higher MTX blood concentration; The complete remission (CR) rate of ALL patients with *G2677T* genotype was significantly lower than that of other genotype carriers [35]. Jannie Gregers also analyzed 522 children with ALL in Denmark and found that *ABCB1 G1199A* and *C3435T* were related to the efficacy of children with ALL after receiving MTX monotherapy. In addition, she found that the recurrence risk of patients with ABCB1 *G1199A/C3435C* was almost twice that of other patients with ABCB1 mutation [36]. ABCB1 gene polymorphism is also associated with prognosis. Studies have shown that the CT genotype and TT genotype of ABCB1 rs1045642 are significantly

associated with event free survival (EFS) [37]. Some studies have also shown that ABC family gene polymorphism has no effect on the efficacy of MTX [38].

The study found that the relationship between the polymorphism of ABC transporter family gene and MTX adverse effects was complex. Studies find that MTX has nothing to do with toxicity in the treatment of ALL with MTX. Other studies have shown that ABCG2 C376T, C421A, G34A are not related to the recurrence of central nervous system after HD-MTX treatment in children with ALL in Iran [39]. It has also been shown that the polymorphism of ABC transporter family genes is associated with adverse effects. ABCB1 is expressed in liver, kidney and gastrointestinal tract, and the most studied site is *rs1045642*. Samara et al. found that *ABCB1 3435TT/CT* was significantly related to the hepatotoxicity after HD-MTX treatment [40]. However, some studies have shown that *ABCB1 3435CC* will increase the risk of hepatotoxicity; TT patients with *ABCB1 G2677T/A* are more prone to thrombocytopenia and neutropenia than other genotypes [36]. The incidence of hepatotoxicity and infection in patients with GG ALL carrying *G2677T/A* was lower than that of other genotypes [35]. ABCC2 is highly expressed in liver and kidney, and the gene polymorphism of *rs717620*, *rs3740065* and *rs3740066* is a research hotspot. The study reported that the wild type of *ABCC2 rs717620* homozygote carried by osteosarcoma patients had a high statistical correlation with high-level bilirubinemia (OR = 2.05, 95% CI 1.05–4.01, $p = 0.037$). In addition, the homozygous mutation and heterozygous genotype of *ABCC3 rs4793665* were significantly associated with severe renal impairment (OR = 0.34, 95% CI 1.6–0.72, $p = 0.005$) [41]. Another researcher conducted serum level measurement and gene polymorphism analysis on 38 children with ALL in Malaysia, and found that the CT/TT genotype of *ABCC2 rs717620* was significantly higher than other genotypes 48 h after treatment ($p = 0.017$), which was significantly related to the adverse reaction of leukopenia [42]. Patients with primary central nervous system lymphoma carrying *ABCC2 rs3740065* gene 29 GA+GG have an increased risk of hepatotoxicity after HDMTX treatment [43]. ABCG2 is mostly expressed in gastrointestinal cells, and the most studied site is *rs2231142*. Some scholars have also studied that *ABCG2C421A* polymorphism is not related to adverse effects such as mucositis [24, 44].

Relationship between organic anion transport polypeptide 1B1 (SLCO1B1) and MTX efficacy

OATPs belong to solute carriers. They are a large family of transmembrane transporters, including OATP1-6, with 6 subfamilies. They are widely distributed in liver, kidney, gastrointestinal tract, heart and other tissues and cells, and can mediate the transport of a variety of endogenous substances and exogenous drugs [45]. SLCO1B1, also known as organic anion transporting polypeptides 1B1 (OATP1B1), is mainly distributed in the basal side of liver cells and is an important carrier for MTX absorption by blood through the liver. A key factor determining the MTX clearance rate is the drug uptake rate mediated by this transporter [46]. The SLCO1B1 gene polymorphism may lead to the change of its transport function, affect the uptake of MTX by the liver, increase the blood drug concentration, and cause the difference of drug action among individuals. The genetic variation affecting OATP1B1 activity was more deeply studied in *rs4149056* (*T521C*).

It is reported that the variation of *rs4149056* reduces the transport capacity of OATP1B1, leading to a significant increase in drug concentration in plasma, and increasing the risk of MTX induced toxicity [47]. Aurea Lima's study confirmed that 521T allele carriers in RA patients are associated with MTX cytotoxicity. This mutation causes an increase in SLCO1B1 expression, and its mRNA is also detected in gastrointestinal cells, which may lead to the accumulation of a large amount of MTX in liver and gastrointestinal cells, leading to cytotoxicity [48]. In other types of diseases, a scholar analyzed the correlation between SLCO1B1 single nucleotide polymorphism (*rs4149056*, *rs2306283*) and MTX treatment adverse effects in 100 patients with juvenile idiopathic arthritis (JIA). The results showed that *SLCO1B1 521 CT/CC* genotype was significantly related to MTX gastrointestinal side effects, and TT mutation was more likely to have adverse liver reactions than CT/CC genotype [49]. Some studies have yielded inconsistent results. The risk of liver injury in NHL patients with *SLCO1B1 521 CT/CC* genotype is higher than that in patients with TT genotype [50]. Other studies have shown that *rs4149056* gene polymorphism has a low correlation with oral mucositis in ALL patients, and is significantly related to poor prognosis. Patients with CC genotype did not have oral mucositis, while the incidence of TC and TT genotype was 8.47% and 13.25% respectively. However, the long-term prognosis of patients with CC genotype is worse than that of patients with TT/TC genotype [51].

In addition to rs4149056, studies have shown that *rs11045879* (C > T), *rs4149081* (A > G) and *rs2306283* (A > G) gene polymorphisms also affect MTX clearance, which is related to gastrointestinal adverse effects. RR Schulte found that rs4149056 and *rs2306283* interacted to affect the clearance rate of high-dose MTX drugs. The MTX clearance rate of patients carrying only T521C genotype was 4% lower than that of patients carrying wild type. The MTX clearance rate of patients with one or more T521 C and 388 AA wild-type decreased

the most. The MTX clearance rate of patients with only T521C mutation or only A388G mutation decreased slightly [52]. The *rs11045879 T* allele (OR = 16.4, 95% CI 8.7–26.7) and the r*s4149081 G* allele (OR = 15.3, 95% CI 7.9–24.6) were both associated with gastrointestinal mucositis (grade 3–4) and infection [53]. Yu Cheng and others have similar findings. ALL patients with rs2306283 AG and GG are more prone to oral mucositis, liver injury and bone marrow suppression [54]. The research results of some scholars also confirmed the correlation between *rs4149056*, *rs11045879*, *rs2306283* and MTX clearance and hepatotoxicity [55–57].

Relationship between reducing folate carrier and MTX efficacy

SLC family is a family of membrane transporters, including more than 400 members. RFC, also known as solute carrier family member 1 (SLC19A1), is responsible for the transmembrane transport of MTX. The RFC-1 encodes RFC. The functional change of MTX transporter in folic acid metabolism pathway will not only affect MTX plasma concentration, but also break the stability of MTX concentration in cells even if MTX can be excreted out of cells normally. Therefore, the genetic polymorphism of RFC-1 may affect the entry of MTX into cells, leading to MTX drug resistance and adverse effects [58]. At present, the research focus of RFC-1 gene polymorphism is *rs1051266* (G > A), and the research results of different scholars are inconsistent.

Laverdiere et al. analyzed the correlation between G80A mutation, MTX blood concentration and prognosis in 204 children with ALL, and found that the prognosis of children with AA genotype was worse than that the serum MTX concentration of children with AA genotype was also higher than that of children with GG genotype. It is speculated that the existence of A allele may lead to the decrease of the affinity between RFC-1 and MTX, which may weaken its transport capacity [59]. Shimasaki retrospectively analyzed the clinical data of 20 patients with ALL who received continuous chemotherapy, and found that G80A may be an important marker for monitoring chemotherapy responses such as bone marrow suppression [60]. *SLC19A1 rs1051266* mutation is also significantly associated with hepatotoxicity [37, 61]. Gregers et al. speculated that the G80A polymorphism might affect the prognosis of childhood ALL and be related to chemotherapy response (such as bone marrow suppression). The study involving 500 children with ALL found that AA mutation was 50% more likely to be in remission than GG/GA genotype ($p = 0.046$). For children receiving high-dose MTX course of treatment, the bone marrow suppression degree of AA genotype patients is higher than that of GA/GG genotype patients (platelet 73 vs. 99/105 × 109/L, hemoglobin 5.6 vs. 5.9/6.0 nmol/L), and it is found that GG genotype patients have a higher probability of hepatotoxicity than other genotypes, and the MTX blood concentration is low 20–24 h after administration. This study suggests that the reason for the low MTX clearance rate and high blood drug concentration of patients with A allele is that the number of A alleles may be related to the increase of folate polyglutamic acid content in liver cells, so as to avoid the damage of MTX toxicity to liver cells, which may also reduce MTX entering liver cells [62]. Some studies have also confirmed that AA genotype is related to the clearance rate of MTX, especially after patients receive high-dose MTX treatment, the initial clearance rate and the total clearance rate are significantly reduced. The steady-state MTX concentration of ALL patients with AA genotype was significantly higher than that of other genotypes, and the risk of hepatotoxicity was increased [63]. In RA patients, carriers of SLC19A1 G allele have a significantly increased risk of gastrointestinal adverse effects compared with homozygous carriers of AA gene. The main reason may be that in gastrointestinal cells, the presence of G allele makes RFC vector preferentially combine with MTX, hinders the entry of folic acid, reduces the content of folic acid in cells, and exposes tissues to high concentration of MTX, causing damage [64]. However, some scholars carried out relevant meta-analysis and concluded that G80A mutation was not related to MTX toxicity [65, 66].

In addition to *RFC-1 rs1051266*, other SNPs have also been shown to be associated with MTX adverse effects. *SLC19A1 rs7499* (G > A) G allele carriers and G homozygotes ($p = 0.012$, OR = 5.64; $p = 0.045$, OR = 2.39), *SLC19A1 rs1051266* (G > A) G allele carriers ($p = 0.034$, OR = 3.07), *SLC19A1 rs2838956* (A > G) ($p = 0.049$, OR = 3.21) were all associated with adverse gastrointestinal effects. Some scholars speculate that mutations in the G allele of *rs1051266* and *rs7499* and the A allele of *rs2838956* enhance the transport activity of RFC, and MTX flows more into tissues and cells, especially those with high SLC19A1 expression (such as gastrointestinal tract), leading to increased cytotoxicity [48]. In RA patients with *SLC19A1 rs4149081* (G > A) GA genotype, the mean plasma concentrations of MTX and MTX-7-OH metabolites were higher ($p < 0.05$), resulting in a decrease in patients' sensitivity to MTX [67].

Relationship between thymidylate synthase and the efficacy of MTX

TS is one of the key enzymes in folic acid metabolism, which can catalyze the methylation of 2′-deoxyuridine-5′-phosphate (dUMP) to synthesize deoxythymidine (dTMP). The methyl donor is 5,10-MTHF. DTMP can be further metabolized into dTTP, which is involved in

DNA synthesis [68]. MTXPG directly inhibits the activity of TS, thus hindering the synthesis of dTMP, leading to the reduction of DNA synthesis materials, thus preventing the proliferation of tumor cells [69]. TS gene polymorphism mainly occurs in 5′-untranslated region (5′-UTR) and 3 'untranslated region (3′-UTR). 5′-UTR has 28 bp variable number of tandem repeats (VNTR), and the most common sequence is the repeat sequence of 2 fragments (2R) or the repeat sequence of 3 fragments (3R), which is related to the transcription and translation of TS [70, 71]. On the 3R allele, there is also a substitution of G > C, producing 3RC and 3RG. The production of C allele may lead to the change of amino acid residues at the upstream stimulatory factor (USF) binding site, which enhances TS activity [72]. Another important gene polymorphism is the deletion of 6 bp nucleic acid fragment at 3′-UTR 1494 bp. The deletion of this sequence may affect the AU rich elements (AREs) on TS messenger ribonucleic acid (mRNA), which preferentially bind to the RNA binding factor 1 (AUF1) in the AU rich area, accelerating the degradation of mRNA, and thus reducing TS activity [73, 74]. Therefore, changes in TS enzyme activity or function may affect the efficacy or toxicity of MTX.

In recent years, there are different views on the study of the correlation between TYMS and MTX efficacy and adverse effects. Some studies believe that TYMS has nothing to do with adverse effects of MTX [75, 76]. For example, studies by Natanja Oosterom and others showed that after MTX treatment, TYMS 1494 del6 and TYMS 2R > 3R were not related to MTX induced oral mucositis in 117 children with acute lymphoblastic leukemia in the Netherlands. Although patients with low expression of TYMS 2R > 3R, 2R/2R, 3R/3R increased the probability of oral mucositis, there was no statistical significance [77]. Owen et al. analyzed 129 kinds of SNPs in 309 RA patients receiving MTX treatment in the UK, and the results showed that TYMS was not related to the side effects of MTX [78]. However, some studies have confirmed that TYMS is helpful to predict the therapeutic effect and adverse effects of patients with MTX. TYMS can be used as an important pharmacogenomic marker for the response of ALL children to MTX therapy. Sheikh et al. studied the relationship between the genetic polymorphism of TYMS and MTX hepatotoxicity in children with ALL. They found that TYMS 1494 del6 was associated with neutropenia and leukopenia. Dominant gene carriers were six times more likely to develop neutropenia than recessive gene carriers [79]. Nikola Kotur also found that patients carrying TYMS 1494 del6 are more likely to have gastrointestinal reactions [80]. Other studies have shown that in RA patients, patients carrying TYMS 3R/3R, 3RG/3RG, 3RC/3RG may be more prone to adverse effects, and the increased expression of 3R related genotypes in TS may reduce the inhibition of TMXPG on TS [32, 81]. Kumagai et al. found that the dosage of MTX for RA patients with 3R allele needs to be higher than that for RA patients with at least one 2R allele [82]. Similarly, in children with ALL, homozygous 3R allele carriers need higher dosage of MTX, and 5-year event free survival rate is lower than homozygous 2R allele carriers, but homozygous 2R allele carriers are more likely to have side effects of drugs [83]. However, some scholars found that the 3R allele and the 6 bp deletion allele may be helpful to reduce the adverse reaction of MTX in South Indian Tamils after analyzing the TYMS polymorphism of 254 patients with rheumatoid arthritis in South Indian Tamils [84]. Other studies have shown that TYMS 3R/3R may be related to reducing the incidence of leukopenia and thrombocytopenia; TYMS 2R/2R may be related to hepatotoxicity in patients [16]. However, some scholars hold the opposite opinion. Dervieux, T showed that patients with 2R/2R genotype had better drug sensitivity and tolerance to MTX [58]. The main reasons for these inconsistent research results are: (1) The sample size of the study patient population is low; (2) Racial differences of patients; (3) MTX combined with other chemotherapy drugs, resulting in other gene polymorphisms; (4) The regulation mechanism of TS expression needs to be further clarified, etc. [16].

Relationship between dihydrofolate reductase and MTX efficacy

DHFR is one of the target enzymes of MTX. It can catalyze the reduction of dihydrofolate to tetrahydrofolate. As an essential coenzyme in DNA synthesis, tetrahydrofolate transfers a carbon unit, which plays an important role in cell proliferation. The inhibition of DHFR will lead to the consumption of tetrahydrofolate without supplementation, break the homeostasis of folate cycle in the body, and then affect the synthesis of DNA, protein, etc. In recent years, many DHFR gene polymorphic loci have been studied, including *rs408626* (A317G), *rs442767* (C680A), *rs34764978* (C829T), *rs70991108* (19 bp ins/del).

It has been reported that thrombocytopenia in children with ALL is related to the DHFR 19 bp ins/ins genotype. It is speculated that MTXPGs can not only promote cell apoptosis, but also affect the DNA synthesis in red blood cells, resulting in a slower growth rate of red blood cells and an increase in the number of abnormal red blood cells, leading to anemia [85]. Ongaro et al. analyzed the DHFR 19 bp ins/del of ALL patients after receiving MTX treatment, and found that compared with wild type (WT) patients, the probability of liver injury in heterozygous (WD) patients increased by 2.07 times, that in

homozygous (DD) patients increased by 4.57 times, and that in WD+DD patients increased by 2.42 times. The possible reason is that the mutation causes the imbalance of folate cycle in cells, the increase of homocysteine and the increase of liver enzyme expression [86]. Dulucq et al. [87] also confirmed that the deletion of DHFR 19 bp was significantly related to MTX hepatotoxicity. Milic et al. [31] found that RA patients carrying 317AA genotype had poor drug sensitivity to MTX. In addition, the 317GG genotype of DHFR gene was associated with poor prognosis. Sunitha et al. showed that DHFR 317GG genotype was associated with an increased risk of recurrence and a reduced overall survival rate in ALL patients, while 317AA and 680CA genotypes might lead to severe leukopenia [88]. A study of 70 Mexican ALL patients found that patients with 317GG had a higher risk of relapse than those with 317 AA genotype (OR = 8.55, 95% CI 1.84–39.70). Similarly, patients with 829TT genotype had a higher risk of relapse than those with 829 AA genotype (OR = 14, 95% CI 1.13–172.63) [89]. It has also been reported that the presence of DHFR 829TT allele may mitigate MTX hepatotoxicity [90].

Conclusion and prospect

In recent years, the relationship between gene polymorphisms of key proteins and enzymes of MTX metabolism and transport process and their efficacy and adverse effects has been a research hotspot, but the specific relationship between them has not reached consensus. The possible reasons are as follows: (1) The sample sources of different studies are different, and the physiological and environmental factors such as disease type, region, race of the study population are different; (2) The difference of dosage, time and concomitant use of MTX in different treatment schemes; (3) The number of research samples needs to be expanded; (4) The evaluation criteria for efficacy and adverse effects of MTX were not uniform; (5) The level of folic acid in patients before treatment [37, 91]. In addition to the above reasons, the dietary structure, the degree of hydration and alkalization during treatment, disease characteristics, single nucleotide polymorphisms at other sites and other influences may also cause inconsistent research results. Therefore, further research to clarify the mechanism of metabolism, transport, excretion and drug resistance of MTX in vivo, improve research methods, and unify disease risk assessment standards will help to achieve individualized drug use of MTX [92].

To sum up, gene polymorphism has the potential to become a genetic marker that can effectively predict the sensitivity, efficacy, adverse effects and prognosis of MTX and guide the individualized treatment of related diseases. However, the efficacy and adverse effects of MTX are significantly different from each other, and are affected by many factors. The pharmacogenomic analysis of a single enzyme or transporter cannot completely solve the problem of individualized treatment of MTX. Future research needs to further expand the inclusion of clinical samples in order to provide more pharmacogenomics support for clinical drug use of MTX.

Abbreviations

MTX	Methotrexate
RFC-1	Reduced folate carrier-1
MTXPGs	Methotrexate polyglutamates
FH4	Tetrahydrofolate
dTMP	Deoxytothymidine
AICAR	5-Aminoimidazole-4-carboxamide ribonucleotide
ATIC	5-Aminoimidazole-4-carboxamide ribonucleotide transformylase/IMP cyclohydrolase
SHMT	Serine hydroxymethyltransferase
MTHFR	Methylenetetrahydrofolate reductase
FPGS	Polyglutamine synthase
GGH	γ-Glutamyl hydrolase
ABC	Adenosine triphosphate binding transporter
OATPs	Organic anion transporting polypeptides
SLC	Solute carrier
TS	Thymidylate synthase
DHFR	Dihydrofolate reductase
5,10-MTHF	5,10-Methylenetetrahydrofolate
5-MTHF	5-Methyltetrahydrofolate
OMI	Oral mucositis index
NHL	Non-Hodgkin's lymphoma
HD-MTX	High-dose MTX
JIA	Juvenile idiopathic arthritis
MDRI/ABCBI	Multidrug resistance 1
MRP2/ABCC2	Multidrug resistance associated protein 2
EFS	Event free survival
BCRP/ABCG2	Breast cancer resistance protein
OATP1B1	Organic anion transporting polypeptides 1B1
SLC19A1	Solute carrier family member 1
dUMP	2′-Deoxyuridine-5′-phosphate
dTMP	Synthesize deoxythymidine
5′-UTR	5′-Untranslated region
3′-UTR	3′-Untranslated region
VNTR	Variable number of tandem repeats
USF	Upstream stimulatory factor
AREs	AU rich elements
mRNA	Messenger ribonucleic acid
AUF1	RNA binding factor 1
WT	Wild type
WD	Heterozygous
DD	Homozygous

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Declarations

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