Genetic variants contribute to differences in response and toxicity to drugs used in autoimmune diseases: Rheumatoid arthritis and systemic lupus erythematosus

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Chapter 2

Genetic risk factors for drug-induced liver injury in rheumatoid arthritis patients using low-dose methotrexate

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Abstract

Low-dose methotrexate is part of the mainstay of rheumatoid arthritis treatment. Hepatotoxicity is among the most feared side effects of low-dose MTX and is associated with increased morbidity. At present, histological evaluation of liver biopsies is the gold standard to retrospectively diagnose MTX-induced liver damage. Genetic markers present an interesting opportunity to pre-emptively identify patients at risk for MTX-induced hepatotoxicity. Here, we will review the literature on candidate genetic markers for the risk of MTX-induced hepatotoxicity. These candidate genetic markers include polymorphisms in the gene encoding the enzyme MTHFR.

INTRODUCTION

Low-dose methotrexate (MTX) is part of the mainstay of rheumatoid arthritis (RA) treatment (1, 2). MTX shows efficacy in approximately 50% of patients with early RA at doses of 12.5–25 mg/week (1). In this MTX schedule, the drug is thought to act primarily as an anti-inflammatory drug, specifically through the release of adenosine, rather than as an antimetabolite drug (3, 4). Once MTX has entered the cell it is subject to polyglutamation, which inhibits several key enzymes, including MTHFR, an enzyme involved in the folic acid cycle (Figure 2.1).

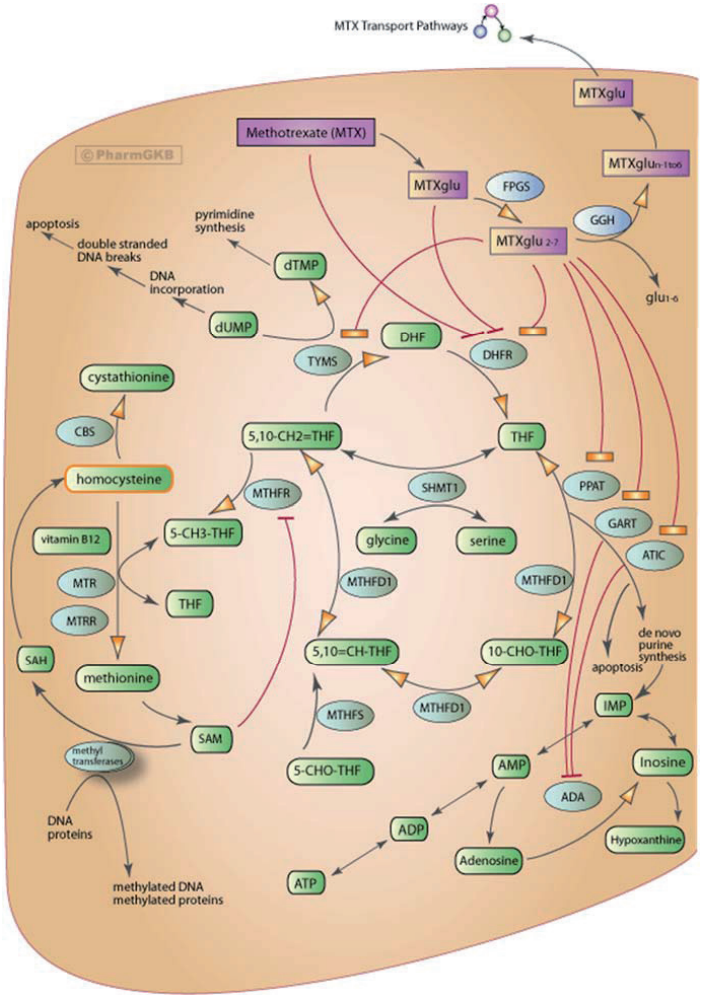

Figure 2.1: Candidate genes in the Methotrexate pathway. Copyright PharmGKB.
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MTX hepatotoxicity

MTX has a well-defined toxicity profile, including bone marrow suppression as well as gastrointestinal, pulmonary and hepatic toxicity. With the low MTX doses used in rheumatology, side effects are frequent and the cause of cessation of MTX therapy in approximately 30% of patients during the first year of treatment (5). Hepatotoxicity is among the most feared side effects of low-dose MTX. Effects of MTX on liver histology during chronic MTX use are fatty infiltration, macrovesicular steatosis, hepatocellular necrosis and fibrosis (6) with predominantly portal and periportal inflammation, which may herald the development of cirrhosis (7). However, clinically, or even biochemically, these findings may be silent for years (6).

Different mechanisms for MTX-induced hepatotoxicity have been proposed (4), including a depletion of hepatic folate stores and the accumulation of toxic MTX polyglutamates in the liver. Also, MTX has been shown to enhance the release of adenosine from hepatic slices *ex vivo*.

This event, in turn, activates and stimulates matrix protein production by fibrogenic stellate cells in the liver (Figure 2.1) (4).

Early prospective studies of MTX in patients with RA showed that its use is associated with an increase of hepatic transaminase enzymes such as ALT and AST in some patients (8). In addition, studies showed that liver enzyme abnormalities correlated with actual biopsy samples of hepatic tissue (9, 10). The incidence of MTX-induced increase of transaminases varies according to different definitions. Some investigators have defined it as elevated liver enzymes two- to three-times greater than the upper limit of the normal (ULN) range. These studies have estimated the frequency of increase of transaminases to be 7.5–26% of all patients treated with MTX. Others have shown that the incidence of abnormal ALT/AST is 48.9% with cutoff transaminase values of above ULN and 16.8% with cutoff transaminase values of >two- to three-times ULN level (11, 12).

Studies on the incidence of liver injury after long-term MTX therapy in RA patients showed that the risk of developing cirrhosis or fibrosis is less than 2% (13–16). Thus, liver enzyme elevations in RA patients on MTX are frequent but often transient and MTX-induced fibrosis/cirrhosis is rare. Whiting-O´Keefe reported a prevalence of advanced histological changes (Grade IIIb/IV) of 2.7% after 4 years on MTX in a meta-analysis of 334 RA patients (17).

The impact of MTX-induced hepatotoxicity can be serious. Two surveys, from Sweden and Spain, suggest that drug-induced liver injury (DILI) with jaundice is associated with greater mortality or the need for liver transplantation than is hepatocellular and/or mixed injury

(18, 19). Interestingly, it was shown that elevated AST and bilirubin levels were independent predictors of death and liver transplantation in patients with hepatocellular injury (18).

Assessing MTX hepatotoxicity

At present, histological evaluation of liver biopsies is the gold standard to diagnose MTX-induced liver damage (6, 20, 21). However, this procedure is invasive and uncomfortable for patients and serious complications (e.g., hemorrhage and pneumothorax) may occur incidentally (22). In addition, many trials show the incoherence of liver enzymes and histological findings (23). Therefore, noninvasive methods for detecting and monitoring liver fibrosis are highly desirable (24). Imaging methods of the liver have been evaluated but ultrasound and magnetic resonance imaging are both inadequate for detecting fibrosis since they yield morphological rather than dynamic and functional information regarding the liver (24-26). The amino terminal peptide of type III collagen in serum correlates directly with the amount of ongoing hepatic fibrogenic activity. However, the amino terminal peptide of type III collagen is not organ specific and may be raised in children and in various other pathologies, including arthritis, scleroderma and hyperthyroidism (22, 24).

The use of a standardized severity and causality score for MTX-induced hepatotoxicity is of importance as to objectively register events in patients and to compare incidences across studies. A clinical diagnostic scale was developed for the diagnosis of DILI and considered to be suitable for use in routine clinical practice (27, 28, 88). In a multinational evidence-based guideline for the use of MTX in RA (29) it is recommended that when initiating MTX treatment or increasing the dose, ALT with or without AST, creatinine and complete blood count measurements should be performed every 1–1.5 months until a stable dose is reached and every 1–3 months thereafter. MTX treatment should be stopped if there is a confirmed increase in ALT/AST > 3 ULN, but may be reinstituted at a lower dose following normalization of the liver enzymes. If ALT/AST levels are persistently elevated up to 3 ULN, the dose of MTX should be adjusted and diagnostic procedures should be considered in the case of persistently elevated ALT/AST > 3 ULN after discontinuation (29). It has been observed that cessation of MTX therapy does not always result in immediate improvement in abnormal liver enzyme values but may persist for several days or even weeks after discontinuation. Obviously, other risk indicators such as the use of nonsteroidal anti-inflammatory drugs, obesity and the use of alcohol contribute to the risk of MTX-induced hepatotoxicity (29). More recently, studies have reported that pharmacogenetic variants in genes encoding proteins involved in the mechanism of action of MTX are associated with MTX-induced hepatotoxicity. These genetic and non-genetic determinants may be useful

to predict the individual patients' risk for MTX-induced hepatotoxicity and could help to reduce the incidence and morbidity. This study aims to review the literature on potential risk factors for MTX-induced hepatotoxicity in RA patients, including the role of genetic markers that contribute to this important clinical side effect.

METHODS

In general, DILI is defined as a rise in either ALT level above ULN/more than two- to three-times of ULN, or alkaline phosphatase level more than twice ULN, or total bilirubin level more than twice ULN when associated with increased ALT or AST (30, 89). A systematic literature search of PubMed was performed in September 2012 using MESH terms 'Methotrexate', 'Rheumatoid arthritis', 'DILI and/or Risk factors and/or SNP'. Individual abstracts were reviewed for relevance related to determinants of low-dose MTX-induced hepatotoxicity. Selected papers were studied comprehensively and summarized. Cross references were screened for relevance and included when useful.

RESULTS

Our initial literature search identified 230 publications (Figure 2.2). In total, 30 of these references were excluded; eight because they were not published in English, ten because they did not include RA and 12 because they did not include MTX.

Non-genetic risk factors

An overview of the identified nongenetic risk factors is presented in Table 2.1. The cumulative MTX dose and duration of treatment play an important role in the evolution of MTX-induced hepatotoxicity (15, 31, 32). A cumulative dose of > 1.5 g and a duration of therapy exceeding 2 years in RA patients are considered risk factors for hepatotoxicity. However, long-term treatment, as long as 10 years, of weekly oral low-dose MTX, did not result in cirrhosis or severe fibrosis in RA patients who did not abuse alcohol (33) suggesting that the relationship is probabilistic rather than absolute and is only one of the potential risk factors that contributes to MTX-induced hepatotoxicity (34). Patients on average had a 6.7% (95% CI: 2.1–11.4) chance of progressing at least one histologic grade on liver biopsy for each gram of MTX taken (17).

Another possible nongenetic risk factor is the use of other hepatotoxic drugs or chemicals (35) such as alcohol (17, 34, 36-38). Patients who are heavy drinkers (at least 100 g of

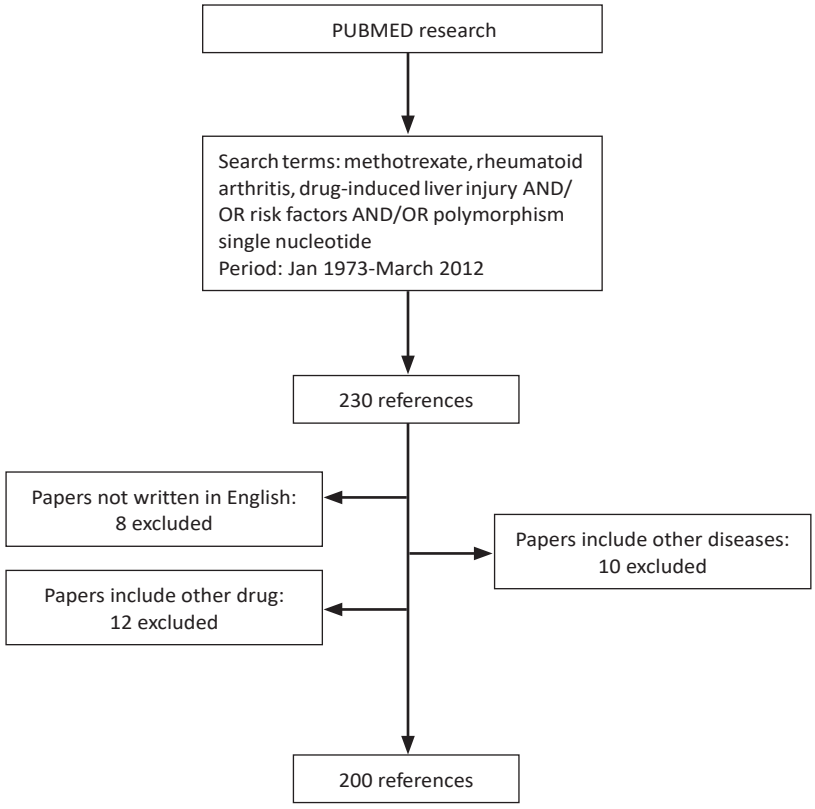

Figure 2.2: Results of the literature search.

alcohol/week) were more likely to have advanced changes on liver biopsy (17.8 vs 4.5%, p = 0.0003) (17). However, in the study by Malatjalian et al. no statistically significant correlation between alcohol consumption and MTX-induced hepatotoxicity could be demonstrated (39). Furthermore, it has been suggested that impaired renal function and concomitant use of drugs that decrease the elimination of MTX or facilitate tissue uptake by displacing MTX from plasma protein-binding sites, such as aspirin and probenecid, may pose a risk for MTX-induced hepatotoxicity (40). Curtis et al. showed that risk of developing abnormal ALT/AST levels was incrementally greater in those receiving MTX (≥ 10 mg/day) in addition to leflunomide compared with those who receive MTX only (41).

Other obvious risk factors for MTX-induced liver disease include persistent abnormal liver functions, history of liver disease (including infection with hepatitis B and C virus), and a family history of liver disease (29, 34).

Table 2.1: Non-genetic risk factors for methotrexate-induced liver injury

Study	n	Mean dose (mg/week)	Duration (weeks)	Definition of hepatotoxicity	Patients with hepatotoxicity, n (%)	Risk factors	Ref.
Kremer et al.	719	7.5–25	18–36	Transaminases level > ULN	111 (15.43)	Obesity and alcohol use	(37)
Hoekstra et al.	411 (137 non-folate and 274 use folate)	7.5–25	48	ALT > 3 × ULN	36 (26) non-folate and 11 (4) use folate	Obesity and alcohol use	(38)
van Ede et al.	236	7.5–25	48	ALT > 3 × ULN	30 (12.71)	Non-folate supplementation	(46)
Philips et al.	134	7.5–25	DNS	DNS	3 (2.24)	Obesity, diabetes and viral/ alcoholic hepatitis	(87)
Whiting O'Keefe et al.	636	7.5–25	18–48	Worsening of at least one grade on classification of Roenigk	177 (27.9)	Alcohol and cumulative dose MTX	(17)
Tilling et al.	550 RA 69 PsA	1.25–30	144	3 × ULN	41 in RA (7.5) and 10 in PsA (14.5)	Male PsA and alcohol	(82)
Morgan et al.	32	7.5	24	Elevated liver enzyme levels	4 (12.5)	Non-folate supplementation	(42)
Quintin et al.	1571	10.2–11.6	105–157.6	Transaminases three-times ULN	41 (2.61)	MTX exposure (weeks) and MTX weekly dose (mg)	(31)
Curtis et al.	1953	10–17.5	> 20	Transaminases level >1–2 ULN	429 (22)	PsA, leflunomide used concomitantly	(41)
Kent et al.	481	12.6	DNS	ULN >1	304 (63)	Non-folate supplementation, elevated BMI, untreated hyperlipidemia	(45)
Visser et al.	2199	12.5	168	ULN >1	31	Alcohol, diabetes and obesity	(16)

DNS: Data not shown; MTX: Methotrexate; PsA: Psoriasis; RA: Rheumatoid arthritis; ULN: Upper limit of the normal; BMI: body mass index.

Folate status may also be of importance since the use of at least 5 mg folic acid/week with MTX therapy reduces gastrointestinal and liver toxicity (42-46). A meta-analysis of nine studies including a total of 788 RA patients also indicated that folic acid supplementation reduces gastrointestinal and liver toxicity of MTX without reducing drug efficacy (47). Van Ede et al. (44) showed that 53% of RA patients treated with MTX and who did not take folic acid had elevated liver enzymes levels versus 13% of patients who took folic acid only.

The toxicity profile of MTX may also be dependent on the route of administration. Comparing MTX 15 mg/week subcutaneously versus orally in RA patients showed more frequent discontinuations due to toxicity after subcutaneous MTX administration without a clear difference in type of adverse event except in increase of ALT, which was lower in patients who received subcutaneous MTX (48).

Pharmacogenetic risk factors

Genetic susceptibility plays an important role in the occurrence of adverse drug effects including hepatotoxicity (49). In recent years, several genetic markers have been associated with an increased risk of developing DILI (50, 51). These genetic markers are usually not generic but highly drug specific and potentially lead to a better understanding of the hepatotoxic mechanism and preventative strategies (52). Genetic polymorphisms in several genes have been related to MTX-induced toxicity in RA patients. Particularly variants in *MTHFR* have been related to low-dose MTX-induced hepatotoxicity (Table 2.2).

MTHFR

The *MTHFR* gene is the best studied gene with respect to MTX metabolism. At least 82 polymorphisms have been described (53, 54), although functional data on these variants are available for only a few. The SNPs found to be related to MTX hepatotoxicity are C677T and A1298C (55). The C677T polymorphism leads to an alanine to valine amino acid change at codon 222 and has known functional effects, such as leading to the formation of an enzyme with reduced activity (55). There is a wide range of clinical effects associated with these polymorphisms, for example increased gastrointestinal side effects and increased liver toxicity (56), and various adverse effects (57). Approximately, 50% of the Caucasian population carries at least one copy of the *MTHFR* C677T variant allele (58). Heterozygotes (CT) retain 60% of the MTHFR enzyme activity and represent approximately 40% of the Caucasian population. The homozygous variant TT genotype represents 10% of Caucasians and confers only 30% of normal MTHFR activity (55, 59).

Table 2.2: Pharmacogenetic association studies of methotrexate-induced liver injury in *MTHR* gene

SNP	Authors	n	Mean dose MTX (mg/ week)	Duration (weeks)	Definition of hepatotoxity	Hepatotoxity, n (%)	Genotypes	OR	95% CI	p-value	Ref.
677C>T (rs1801133)	van Ede et al.	236	7.5–25	48	ALT ≥ 3 ULN	30 (12.7)	CT or TT>CC	2.38*	1.06–5.34	0.035	(46)
	Caliz et al.	468	10–25	DNS	DNS	DNS	TT>CT + CC	1.42	1.01–1.98	0.043	(64)
	Aggarwal et al.	150	10.9–11.2	104 ± 82	DNS	10 (6.7)	CC>CT + TT	8 (9.2%) vs 3 (4.8%)			(66)
	Kim et al.	385	6.3–15.3	92–335.8		48 (12.46)	CC>CT or TT	6 (4.5%) vs 42 (16.7%)			(67)
A1298C (rs1801131)	Dervieux et al.	48	7.5–25	24	AST > ULN	4 (8.3)	AC or CC>AA	DNS	DNS	DNS	(63)
	Mena et al.	70	7.5	DNS	ALT or AST > 2 ULN	13 (19)	AC + CC>AA	2.75	1.11–6.75	0.023	(65)
Total		889				105 (11.81)					

The patients included in the study by Caliz et al. were not included in the total because they do not specify the exact number and percentage of patients with MTX-induced hepatotoxity. MTHFR catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. ALT: Alanine transaminase; AST: Aspartate transaminase; DNS: Data not shown; MTX: Methotrexate; OR: Odds ratio; ULN: Upper limit of the normal. * Relative risk (RR).

The *MTHFR* A1298C polymorphism is a glutamic acid to alanine substitution at codon 429 and leads to a reduced enzyme activity (60) and also results in altered clinical effects. The reduced enzyme activity leads to high blood homocysteine levels and this disorder is connected to coronary and peripheral artery diseases (61). The C allele has a frequency of 32% in the Caucasian population. Interestingly, patients who are heterozygous for both the C677T and A1298C SNPs (approximately 15% of the Caucasian population) are clinically similar to patients that are homozygous carriers of the C677T polymorphism. The two SNPs are in very strong linkage meaning that the genotypes are strongly related and not independent (55, 58).

Several groups have investigated the effect of these *MTHFR* polymorphisms on MTX efficacy (55, 62) but this is outside the scope of this article.

van Ede et al. were the first to show an association of the *MTHFR* C677T polymorphism and elevated ALT levels during MTX treatment in patients with RA (46). Thirty patients out of 236 (12.7%) withdrew from MTX therapy because of elevated ALT values. Both the heterozygous variant genotype (677CT versus 677CC) and the homozygous variant genotype (677TT versus 677CC) were associated with an increased relative risk of MTX discontinuation owing to elevation of the ALT values, independent of folate supplementation. The authors postulated that an increase of transaminases during MTX treatment in RA patients is mediated by its effects on homocysteine metabolism (46). The ALT elevations were designated 'mild' when < 3 ULN occurring on at least two of four consecutive (every 3 weeks) evaluations, and 'moderate' when ≥ 3 ULN.

In a study by Dervieux et al. MTX naive patients were enrolled in a pharmacogenetic study where they analyzed both C677T and A1298C *MTHFR* polymorphisms. A total of 8.3% of the patients showed symptoms of hepatotoxicity (AST > ULN) and any side effects were associated, among others, with the *MTHFR* 1298AC/CC genotype, but not with *MTHFR* C677T (63).

In a study by Caliz et al. the two *MTHFR* SNPs and their haplotypes were studied in relation to MTX toxicity, including hepatotoxicity, in a retrospective cohort of 468 Spanish patients with RA (64). Eighty-four of the 468 patients (18%) experienced MTX toxicity, most commonly gastrointestinal and hepatotoxicity. The C677T polymorphism was associated with an increased risk of MTX toxicity with an odds ratio (OR) of 1.42 (95% CI: 1.01–1.98) but the A1298C SNP was not related to MTX toxicity. The haplotype 677T-1298A was nominally associated with toxicity, with an OR of 1.40 (95% CI: 1.00–1.96). Thus, the C677T polymorphism in the *MTHFR* gene was found to be associated with the composite endpoint of MTX toxicity but no specific information is given for hepatotoxicity.

Mena et al. analyzed the association of both *MTHFR* C677T and A1298C polymorphisms and the presence of transaminasemia in 70 Mexican RA patients treated with MTX. A total of 19% (13 out of 70) of the patients had an increase in the serum level of transaminases. The *MTHFR* A1298C polymorphism was associated with elevation of transaminases ($p = 0.024$) (65).

Aggarwal et al. studied the relationship between the C677T gene polymorphism and low-dose MTX toxicity and efficacy in a cohort of 150 RA patients on folate supplementation (66). Ten patients (6.7%) presented hepatotoxicity. However, there was no significant difference in overall occurrence, severity or the organ specific toxicity between patients with or without polymorphism (CC: 8 [9.2%] vs CT and TT: 3 [4.8%]).

Kim et al. enrolled 385 patients with RA who had received MTX and identified toxicity associated with *MTHFR* C677T genotypes, including hepatotoxicity. Forty-eight patients (12.5%) presented elevated transaminases levels: CT/TT: 42 patients (16.7%) versus CC: six patients (4.5%). They concluded that the *MTHFR* C677T polymorphism may be an important predictor of MTX-related toxicity in patients with RA (67).

According to these articles, MTX-induced hepatotoxicity occurred in approximately 11.81% of patients; a total of 889 patients were examined and 105 presented hepatotoxicity.

Meta-analysis

Fisher et al. conducted a meta-analysis of published studies including 1400 patients for association of the *C677T* polymorphism and over 660 for the *A1298C* variant and demonstrated that the first but not the latter variant was significantly related to toxicity of MTX, including hepatotoxicity (OR: 1.71; CI: 1.32–2.21, $p < 0.001$) (68). This analysis has several limitations. First, there is an inherent heterogeneity to meta-analysis, and there were differences in the definition of toxicity, MTX dose and folic acid supplementation among the different studies examined. Second, not all studies included in the meta-analysis discriminated between the heterozygous and homozygous genotype. Because of this, the meta-analysis was performed combining all patients who deviated from the wild-type, allowing all studies to be compared in the meta-analysis (68).

Recently a second meta-analysis was published (69) looking for possible associations of *MTHFR* polymorphisms with adverse effects, including hepatotoxicity. The findings of this analysis concerning the *C677T* polymorphism and toxicity in RA patients are consistent with Fisher's meta-analysis (68) (TT vs CC [OR: 4.191; 95% CI: 1.642–10.698]; CT vs CC [OR: 1.46; 95% CI: 0.680–3.130]).

Owen et al. examined a retrospective cohort of 309 patients with RA from the UK, for which information on MTX efficacy and toxicity was available (70). Next, 17 studies were selected from the published literature on *MTHFR* C677T and A1298C variants and response in RA, including the cohort study of Owen et al. and a meta-analysis was then performed. Nine SNPs were analyzed including *C677T* and *A1298C*. Preliminary analysis revealed an association between *C677T* and MTX toxicity, which was particularly strong in the non-Caucasian group (OR: 1.93; 95% CI: 1.47–2.55). However, after adjustment for heterogeneity between the toxicity studies by a random-effects model, the association with toxicity did not persist. The authors conclude that none of the SNPs showed association with MTX efficacy or toxicity in this cohort (70).

A fourth meta-analysis, including 1,514 patients with RA, was conducted by Lee et al. (71). They report no significant associations between the toxicity and efficacy of MTX in RA and the *C677T* or *A1298C* polymorphisms of *MTHFR* (OR for adverse effects with 1298 AA versus 1298 AC/CC were 0.942; 95% CI: 0.479–1.851; p = 0.861, and OR for adverse effects with 677CC versus 677CT/TT was 0.633; 95% CI: 0.325–1.234; p =0.180). These results are conflicting with the results Fisher (68) and Spyridopoulou et al. (69) but in line with the results of Owen et al. (70).

DISCUSSION & FUTURE PERSPECTIVE

Our review of the literature shows that there is a limited number of studies that focus specifically on the study of low-dose MTX-induced hepatotoxicity.

Several nongenetic risk factors for MTX-induced liver injury have been identified, such as the use of alcohol, exposure to hepatotoxic drugs and the cumulative dose of MTX. There are more studies for nongenetic risk factors than for genetic risk factors.

Regarding genetic risk factors, the *MTHFR* C677T polymorphism appears to be the most promising predictive genetic marker for low-dose MTX-induced hepatotoxicity. Results for *MTHFR* A1298C are less consistent and require additional studies.

The identification of genetic predictors for MTX-induced hepatotoxicity presents an important potential opportunity to pre-emptively identify individual patients at risk for this debilitating disease. Many studies reported genetic variants, for example *ADORA2a*, associated with enzymes and proteins involved in the mechanism of action of MTX and their relations with efficacy and toxicity, including abnormal liver function test (72-77). However, only a small number of studies have reported variants in genes that are predictive for MTX-induced hepatotoxicity.

The currently available evidence for genetic markers for MTX-induced hepatic injury in RA treatment is limited in two ways. First, the number of studies is low with only 19 studies identified and four meta-analyses. Moreover, many of the studies have a small sample size, typically approximately 50–500 patients. With a relative low incidence of MTX-induced hepatotoxicity of approximately 11.81% (a total of 889 patients with 105 presenting with hepatotoxicity) this limits the power to identify genetic markers. The differences in results for the *C677T* polymorphism between the meta-analysis may be primarily caused by the use of different groups of studies or different meta-analysis methods. In addition, with the current data there is a risk for overestimating the effect of genetic markers since smaller studies tend to overestimate the effect of a biomarker and results from small studies are more likely to suffer from publication bias (78). In general, many biomarkers proposed as determinants of disease, risk, prognosis or response to treatment in highly cited studies do not transform to clinical practice. In addition, to be able to correctly assess the potential clinical value of any biomarker it is essential to have the diagnostic test criteria of the related test, for example, the sensitivity, specificity, positive and negative predictive value or percentage explained variance. To date, for many genetic markers, diagnostic test criteria are not commonly reported (79). Unfortunately, this is also the case for the studies identified in Table 2.2. Some data regarding the sensitivity and specificity of abnormal hepatic values are available for predicting liver injury. The American College of Rheumatology guideline for monitoring MTX-induced hepatotoxicity (29, 37) presents diagnostic test criteria, that is, sensitivity and specificity of elevated liver enzymes, to predict hepatotoxicity. The sensitivity of elevated AST levels for predicting fibrosis/cirrhosis was 80% whereas the specificity was 82%. One study suggests that ALT alone might detect 90% of the elevated AST or paired tests (80). To be able to better assess the added value of genetic markers to the classical risk factors for low-dose MTX-induced hepatotoxicity we would, therefore, like to call for the reporting of diagnostic test criteria, such as sensitivity, specificity, positive and negative predictive value or percentage explained variance, in all pharmacogenetic association studies.

The identified studies investigating determinants of MTX-induced hepatotoxicity are very heterogeneous with regard to the methodology. An elevation of liver enzymes was generally taken as a surrogate for hepatotoxicity whereas the gold standard is considered a liver biopsy (23). Although liver biopsy provides the most reliable diagnostic procedure for MTX-induced liver injury (81), it is not without risk and has cost implications. Therefore, controversy exists on the justification of liver biopsies prior to treatment with MTX, especially because of the low absolute risk of MTX-induced liver injury in RA patients (14, 15, 82). Moreover, the use of a standardized severity and causality score for MTX-induced

hepatotoxicity is essential in order to objectify clinical observations (81). Several groups have developed methods to improve the consistency, accuracy and objectiveness in causality assessment of adverse drug reactions in general (83) but mostly they were not applied in the studies examined.

In addition to the use of different definitions of hepatotoxicity, the available evidence is also restricted by differences in MTX dose and folic acid supplementation, small sample size and the lack of replication studies. Owing to the limited sample size of many studies, power to detect the true risk and determinants of MTX hepatotoxicity may be limited. Many of the reported positive associations have either not been replicated or have shown inconsistent findings (55).

In general, from the published studies, *MTHFR* C677T appears to be the most promising genetic marker predicting low-dose MTX-induced hepatotoxicity, although we have to emphasize the limited power of currently available studies to identify genetic biomarkers for hepatotoxicity. So, conflicting results exist limiting its clinical application.

Efforts should be made to further explore genetic and nongenetic risk factors for MTX-induced hepatotoxicity. Adequately powered multicenter studies, stratified by race, are needed to clarify the muddled state that exists in MTX pharmacogenetics today. Future research should focus on a genome-wide association study (GWAS) to explore additional genetic markers. GWAS have revolutionized genetic research as they allow the discovery of multiple gene variants with individually small effects. The advantage of GWAS is that they eliminate the need to choose, *a priori*, candidate genes or variants. GWAS are highly suitable to identify genetic variants contributing to complex phenotypes such as drug-induced toxicity. The GWAS approach enables novel and less obvious genetic markers to be identified, particularly for genetic variation affecting drug pharmacodynamics, which is more complex and often less well understood than drug pharmacokinetics. Recent years have shown numerous examples of the successful application of GWAS to identify genetic markers for drug-induced toxicity, including liver toxicity, hypersensitivity, skin rash and myotoxicity (84, 85). Cooperative efforts should be encouraged to prospectively collect biological samples from well-documented cases with MTX-induced hepatotoxicity and from controls.

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