Metaanalysis of Methylenetetrahydrofolate Reductase (MTHFR) Polymorphisms Affecting Methotrexate Toxicity

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ABSTRACT. ***Objective.*** Methotrexate (MTX) is an effective therapy for rheumatoid arthritis (RA) but it is also associated with toxicity. Pharmacogenetics is the systematic evaluation of the role of genetic differences in the efficacy and toxicity of therapeutic interventions. Because the results of small pharmacogenetic studies are often misleading, we undertook a metaanalysis of published studies to determine the role of polymorphisms in the therapeutic efficacy and toxicity of MTX.

Methods. A search of PubMed produced 55 publications, which were then reviewed for relevance to MTX toxicity and efficacy in patients with RA. To ensure that no study was missed, each polymorphism found was then entered as an independent search string and all results were reviewed again.

Results. Only 2 polymorphisms [C677T and A1298C in methylenetetrahydrofolate reductase (MTHFR); total 8 studies] relevant to MTX metabolism and efficacy had sufficient data to allow a metaanalysis of their association with toxicity; there was no polymorphism with sufficient data to perform a metaanalysis of efficacy. In a fixed-effects model, the C677T polymorphism was associated with increased toxicity (OR 1.71, 95% CI 1.32–2.21, $p < 0.001$). The A1298C polymorphism was not associated with increased toxicity (OR 1.12, 95% CI 0.79–1.6, $p = 0.626$).

Conclusion. As pharmacogenetics evolves, more data are needed to assess the role of various polymorphisms for drug efficacy and toxicity. These results illustrate the paucity of reliable pharmacogenetic data on a commonly used antirheumatic drug and the potential role of pharmacogenetics in tailoring drug therapy for an individual patient. (First Release Feb 1 2009; J Rheumatol 2009; 36:539–45; doi:10.3899/ jrheum.080576)

Key Indexing Terms:
METHOTREXATE POLYMORPHISM TOXICITY PHARMACOGENETICS
METAANALYSIS RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is among the best studied chronic inflammatory diseases. One of the most effective therapies for RA, and the anchor of many therapeutic regimens, is methotrexate (MTX). Reports of the use of aminopterin (MTX) to treat RA date from 1951, and the initial clinical trials showing the efficacy of MTX date from the mid-1980s[1-5]. While highly effective, it is also associated with toxicity, including worsening of nodulosis, pneumonitis, neurologic toxicity, gastrointestinal complications including nausea, vomiting and diarrhea, transaminitis, hematologic abnormalities, rash, stomatitis, and alopecia[6-14].

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MTX is a structural analog of folic acid[15]. It enters cells via solute carrier family 19 member 1 (SLC19A1), the reduced folate carrier, and then needs to be activated by a gamma-glutamyl hydrolase (GGH) to a polyglutamated form. This blocks the enzyme dihydrofolate reductase (DHFR), inhibiting purine metabolism and impairing protein synthesis by blocking the conversion of other amino acids. In addition, the polyglutamated MTX can also interfere with thymidylate synthetase (TYMS) and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase (also called ATIC). Lastly, the polyglutamated MTX can interfere with methylenetetrahydrofolate reductase (MTHFR), causing elevated homocysteine levels and toxicity[15].

The sequencing of the human genome and the understanding of the potential function of single-nucleotide polymorphisms (SNP) of appropriate genes have provided a vast amount of data to study associations between toxicity or efficacy of different medications, a burgeoning field known as pharmacogenetics. As the technology to study SNP has advanced, reducing costs, more studies of SNP related to specific medications have been published. However, many of these studies are small and of limited utility.

The results of pharmacogenetic studies are often both

conflicting and difficult to understand. A 2002 study examining genetic associations found that of 166 putative associations that had been studied 3 or more times between a SNP and disease susceptibility, only 6 could be reproduced consistently[16].

Because the results of small pharmacogenetic studies are often misleading we undertook a metaanalysis of published studies to determine the role of polymorphisms in MTHFR, an enzyme affected by MTX and its metabolites, in the therapeutic efficacy and toxicity of MTX.

MATERIALS AND METHODS

We searched PubMed using keywords "methotrexate," "arthritis," and either "SNP" (single-nucleotide polymorphism) or "polymorphism." Fifty-five articles were identified, and each was individually reviewed for relevance to efficacy of treatment of RA or toxicity. Over 20 different polymorphisms were identified that affected either efficacy or toxicity (Table 1). Because several of the efficacy trials had widely disparate definitions of efficacy, it was the opinion of the authors that an adequate metaanalysis could not be done on that literature. Only 2 SNP were identified with 3 or more articles published with sufficient data on toxicity, MTHFR C677T and MTHFR A1298C (Table 2).

Metaanalysis was performed on those studies examining toxicity, using both random-effects model and fixed-effects models. There were insufficient data on populations to know if appropriate haplotype stratification had been done in each study to know which model was appropriate.

All analyses were done using Comprehensive Meta Analysis Version 2.2.046.

RESULTS

Of the 55 studies identified in the literature, 8 discussed the C677T polymorphism[17-24]. Of those 8, 5 also discussed the A1298C polymorphism[18,20-22,24]. Table 1 shows a list of all polymorphisms identified with studies documenting effects on efficacy, toxicity, or both[17-33]. Table 2 shows the details of the studies in this analysis. Figures 1, 2, and 3 show funnel plots of the effects of the studies.

Of the 8 studies that assessed the C677T polymorphism, either homozygous or heterozygous, only 3 showed a significant increase in toxicity with the use of MTX[19,21,23]. Two others also showed an increase in toxicity, although it was not significant[18,22]. The other 3 studies showed a possible decrease in toxicity, although again not approaching significance[17,20,24]. When assessed together, and weighting for the relative sizes of the different studies, assuming a fixed-effects model, there was a significant, although small, increase in toxicity (odds ratio 1.71, 95% confidence interval 1.32–2.21, $p < 0.001$). Assuming a random-effects model, however, the confidence interval crosses the null hypothesis (OR 1.60, 95% CI 0.90–2.86, $p = 0.11$).

Of the 5 studies that assessed the A1298C polymorphism, again either homozygous or heterozygous, only one showed a significant increase in toxicity[24]. Three of the remaining studies showed almost no influence at all[20-22], and the fourth showed a possible decrease in toxicity[18], approaching but not reaching significance in a fixed-effects model (OR 1.12, 95% CI 0.79–1.6, $p = 0.53$). A random-effects model

Table 1. All methotrexate single-nucleotide polymorphisms studied in RA efficacy and toxicity.

Polymorphism	Articles	Efficacy, Toxicity, or Both
MTHFR C677T	van Ede, 2001[23]	Toxicity
	Urano, 2002[22]	Both
	Kumagai, 2003[20]	Both
	Berkun, 2004[18]	Toxicity
	Wessels, 2006[24]	Both
	Kim, 2006[19]	Toxicity
	Dervieux, 2006[26]*	Both
	Aggarwal, 2006[17]	Both
	Taniguchi, 2007[21]	Both
	Kurzawski, 2007[29]*	Efficacy
MTHFR A1298C	Kumagai, 2003[20]	Both
	Berkun, 2004[18]	Toxicity
	Wessels, 2006[24]	Both
	Dervieux, 2006[26]*	Both
	Taniguchi, 2007[21]	Both
	Kurzawski, 2007[29]*	Efficacy
TYMS 3'UTR	Kumagai, 2003[20]	Both
	Takatori, 2006[31]	Both
TSER*2*3	Dervieux, 2004[27]*	Efficacy
	Dervieux, 2006[26]*	Both
RFC1 G80A	Dervieux, 2004[27]*	Efficacy
	Wessels, 2006[24]*	Both
	Dervieux, 2006[26]*	Both
	Takatori, 2006[31]	Both
	Drozdzik, 2006[28]	Efficacy
ATIC C347G	Dervieux, 2004[27]*	Efficacy
	Wessels, 2006[24]*	Both
	Dervieux, 2006[26]*	Both
	Takatori, 2006[31]	Both
ITPA C94A	Wessels, 2006[24]	Both
MTXPGs	Dervieux, 2004[27]	Efficacy
DHFR–G473A	Wessels, 2006[24]	Both
DHFR G35289A	Wessels, 2006[24]	Both
HLA-G 14b	Rizzo, 2006[30]	Efficacy
HLA DRB1	Ali, 2006[25]	Efficacy
HLADQB1	Ali, 2006[25]	Efficacy
MDR1 C3435T	Drozdzik, 2006[28]	Efficacy
AMPD1 C34T	Wessels, 2006[24]	Both
MTR A2756G	Wessels, 2006[24]	Both
MS A2756G	Dervieux, 2006[26]*	Both
MTRR A66G	Wessels, 2006[24]	Both
	Dervieux, 2006[26]*	Both
GGH C401T	Dervieux, 2006[26]*	Both
GGH C452T	van der Straaten, 2007[32]	Both
GGH T16C	van der Straaten, 2007[32]	Both
SHMT1 C1420T	Dervieux, 2006[26]	Both
ABCB1 C3435T	Takatori, 2006[31]	Both
FPGS A1994G	van der Straaten, 2007[32]	Both
FPGS G114A	van der Straaten, 2007[32]	Both

* Insufficient data in article to permit inclusion in metaanalysis. MTHFR: methylenetetrahydrofolate reductase, TYMS: thymidylate synthase, TSER: thymidylate synthase enhancer region, RFC1: reduced folate carrier1, ATIC 5: aminoimidazole-4-carboxamide ribonucleotide transformylase, ITPA: inosine triphosphate phosphatase, MTXPGs: methotrexate polyglutamates, DHFR: dihydrofolate reductase, HLA: human leukocyte antigen, MDR1: multidrug resistance 1, AMPD1: adenosine monophosphate deaminase 1, MTR: methionine synthase, MTRR: methionine synthase reductase, GGH: gamma-glutamyl hydrolase, MS: methionine synthase, SHMT1: serine hydroxymethyl transferase 1, ABCB1: ATP binding cassette transporter B1, FPGS: folylpoly-gamma-glutamase synthetase.

Table 2. Methotrexate studies included in analysis.

Article	CT or TT, n	CC, n	OR	95% CI	p
C677T					
van Ede, 2001[23]	114	122	2.383	1.063–5.341	0.035
Urano, 2002[22]	71	35	3.623	0.989–13.274	0.052
Kumagai, 2003[20]	69	46	0.626	0.295–1.328	0.222
Berkun, 2004[18]	48	45	1.200	0.512–2.813	0.675
Kim, 2006[19]	252	133	3.989	2.445–6.507	0.000
Aggarwal, 2006[17]	63	87	0.757	0.332–1.729	0.509
Wessels, 2006[24]	111	89	0.802	0.437–1.471	0.475
Taniguchi, 2007[21]	90	66	3.242	1.460–7.200	0.004
Fixed			1.708	1.321–2.207	0.000
Random			1.603	0.897–2.864	0.111
A1298C					
Urano, 2002[22]	32	74	0.908	0.317–2.602	0.857
Kumagai, 2003[20]	35	80	1.029	0.464–2.285	0.944
Berkun, 2004[18]	43	50	0.438	0.181–1.059	0.067
Wessels, 2006[24]	115	83	2.319	1.206–4.456	0.012
Taniguchi, 2007[21]	32	74	1.016	0.486–2.125	0.965
Fixed			0.826	0.0541–1.260	0.375
Random			0.826	0.0541–1.260	0.375

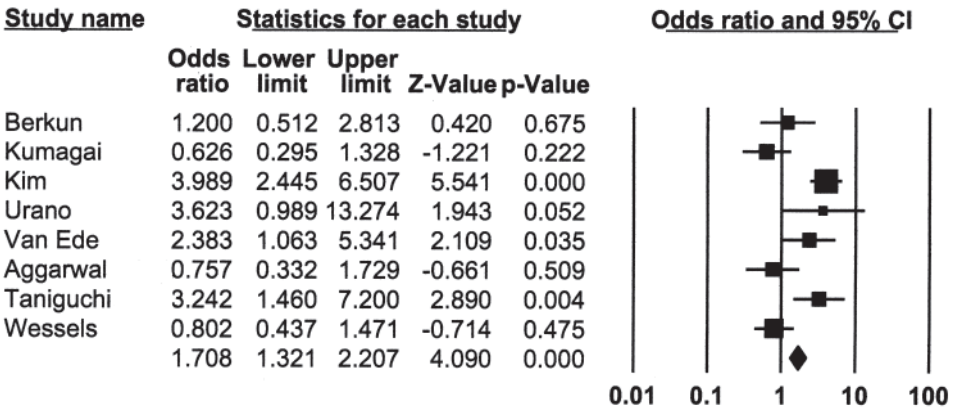

Figure 1. Funnel plot for SNP C677T fixed-effects model.

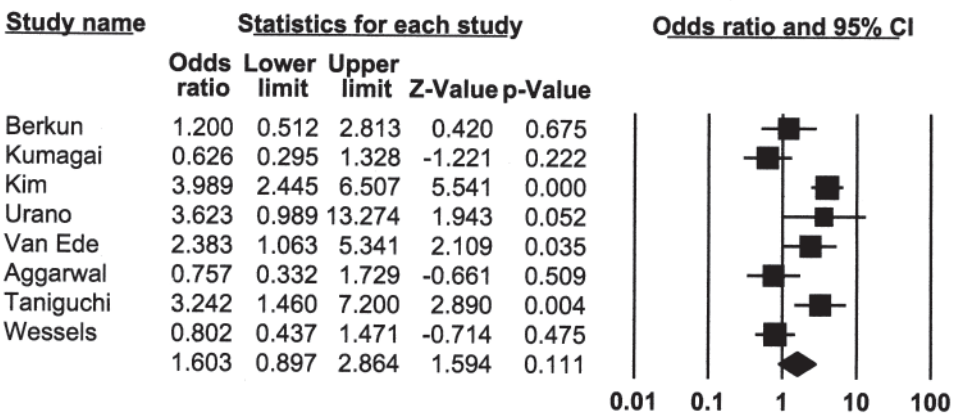

Figure 2. Funnel plot for SNP C677T random-effects model.

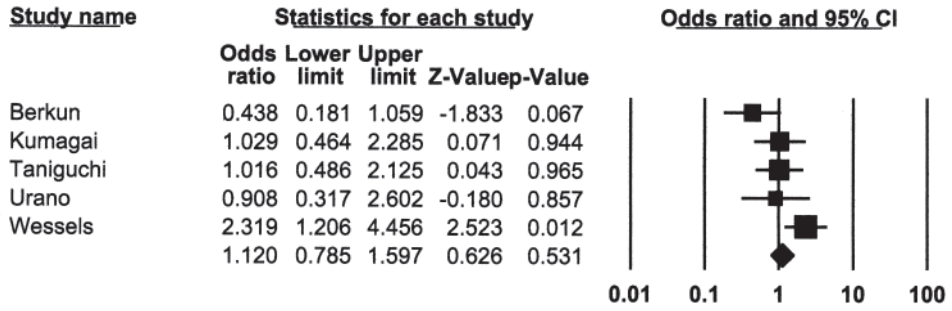

Figure 3. Funnel plot for SNP A1298C fixed-effects model.

showed similar results (OR 1.04, 95% CI 0.6–1.81, p = 0.88).

All studies used "any toxicity" as an endpoint. As such, a mild elevation in liver function test (LFT) results or stomatitis was treated the same as nausea or as LFT elevations greater than 3 times the upper limit of normal. In addition, almost all studies did not discriminate between whether patients had only one copy of the polymorphism or 2 copies of the polymorphism.

DISCUSSION

The primary findings of our investigation are the increased odds ratio of MTX toxicity used to treat RA associated with the C677T polymorphism in a fixed-effects model. There was no association between the A1298C polymorphism and toxicity.

This metaanalysis illustrates the paucity of data about the pharmacogenetics of one of the most commonly used disease-modifying antirheumatic drugs. The C677T and A1298C polymorphisms are just 2 of over a dozen polymorphisms reported in the MTHFR gene; of those 12, only 7 have been associated with efficacy or toxicity in RA[34]. The C677T polymorphism was first described in the mid-1990s; this SNP leads to decreased activity of the MTHFR enzyme; the homozygous variant has about 30% of the function of the wild type[35,36]. The heterozygous variant has about 60% of the function of the wild type. The A1298C polymorphism was first discovered in 1998; the homozygous variant has about 60% of the function of the wild type[37,38].

In attempting to draw a collective conclusion from the individual trials, it is important to comment on the strengths and weaknesses of each. The first article assessing the connection between the C677T polymorphism and toxicity, van Ede, *et al* 2001[23], focused on discontinuation due to toxicity or elevation of LFT. In addition, patients filled out a "standard toxicity questionnaire" to assess other side effects. The primary purpose of the study was actually to assess in a prospective manner the effects of folic acid and folinic acid supplementation on MTX efficacy and toxicity in patients with RA, and this analysis used only a random subset of patients from the original study. This study is confounded somewhat by the variable use of folic acid supplementation among the RA patients — one-third of patients received placebo, one-third received daily folic acid, and one-third received folinic acid weekly. While this study's[2,3] strengths include a thorough statistical analysis, including definition of patient numbers needed for adequate power, toxicity in the study was defined as discontinuation. Many patients suffer from side effects insufficient to warrant discontinuation, and most of the other studies did not discriminate between minor and more significant toxicities in their analyses.

Urano and colleagues assessed the role of both the C677T and A1298C polymorphisms[22]. There is no discussion of numbers needed for adequate power of this cross-sectional analysis, and there is no description of how these patients were chosen from the outpatient clinic population in Tokyo. In addition, patients in the study did not receive doses of MTX higher than 12.5 mg, markedly different from conventional therapy elsewhere. The authors also do not discriminate between transaminitis and less severe side effects, such as stomatitis or alopecia. The authors do note that no patient in their study had both the 677T and 1298C haplotype. Urano's group published a second report on MTX polymorphisms several years later, this time with Taniguchi as the lead author[21]. The purpose of the study was to validate their previous work. The design was retrospective, with patients chosen randomly from their outpatient clinic population at the Institute of Rheumatology, Tokyo Women's Medical University. The study also examined both polymorphisms. Again, there is no discussion of power. In addition, less than one-third of the patients in the study received folic acid supplementation, and more than half of patients received 6 mg or less MTX. Toxicities and adverse events are not clearly defined in the study beyond a definition of transaminitis.

Kumagai, *et al*[20], another group based in Japan, studied both polymorphisms. This was a prospective analysis, with the primary purpose of assessing the effects of several polymorphisms. The authors do not state where patients were recruited from. They also do not discuss how many patients they needed for adequate power[20]. The authors also employed a maximum dose of 12 mg MTX in the study. While toxicities are broken down by frequency, the authors

use the aggregate of all adverse events, not discriminating between minor and more significant side effects. Unlike most of the other studies, this one does discriminate between heterozygous and homozygous genotypes and rate of adverse events.

Berkun and colleagues also studied both polymorphisms[18]. This was a prospective study, with 93 consecutive RA patients recruited from 3 different rheumatology outpatient clinics in Israel. In contrast to the other studies, the definition of toxicity is more clearly described. However, the authors use a composite "side effects" result, and do not discuss severe versus mild effects. MTX doses are a little higher in this population, with an average dose just under 12 mg weekly. In addition, patients received an average dose of over 5 mg folate supplementation daily.

Aggarwal, *et al*[17] analyzed only the C677T polymorphism, in a retrospective study selecting patients randomly from an outpatient clinic in Lucknow, India. All patients received folic acid supplementation, and MTX doses were similar to Berkun's study[18]. Toxicity was better defined in this study; the authors broke down rates of toxicity for specific genotypes as any, hematologic, hepatic, gastrointestinal, and pulmonary. Only one other study in this analysis provided similar data on toxicity.

Kim and colleagues[19] also studied only the C677T polymorphism. Of the 8 studies, this prospective study in Seoul, South Korea, was by far the largest. The mean MTX dose was similar to the previous 2 studies, 11.6 mg weekly, and all patients received daily folic acid supplementation. Toxicities were well defined by the authors, and they note which patients required temporary versus permanent withdrawal. The authors also provide data on specific toxicities related to genotype.

Lastly, Wessels, *et al*[24] assessed toxicity related to both C677T and A1298C polymorphisms. The patients were a subcohort of the BeSt trial. All patients received folic acid supplementation, but average MTX dose was not noted. Toxicity was well defined, the authors present data on specific toxicities for each genotype, and they also discriminate between the heterozygous and homozygous genotype.

An additional variable that may have clinical influence is the time from initiation or titration of MTX dose to onset of adverse effects. This clearly would be an important component in assessing the risk of medication and for patient counselling. However, the data presented in the articles in this analysis were insufficient to assess whether the presence or absence of the respective SNP had an effect on time to adverse event.

Another potential issue in studying pharmacogenetics is the influence of multiple SNP on the efficacy or toxicity of a drug. While a single SNP may not have significance, the combination of several SNP for a given protein may lead to significant changes in function that increase or decrease toxicity or efficacy or both. To date, no study has been published assessing the presence and effects of both C677T and A1298C in patients with RA. Other studies have found a correlation between the presence of both SNP and outcome, including increased frequency of neural tube defects, and patients heterozygous for both SNP have significantly decreased MTHFR activity, compared to patients with only one SNP, and the expected increase in homocysteine levels as well[37].

Analyzing the data presented here, it is unclear whether the fixed or random-effects model is the most appropriate analytic tool, as the frequency of the respective SNP in various populations has not been fully explored among all patients with RA. In Caucasians and Asians, 12% to 15% of individuals are homozygous for TT and as many as 50% are heterozygous for the C677T polymorphism[39,40]. The C677T polymorphism has a frequency of about 35% in North America[36,41,42]. For the A1298C polymorphism, the homozygous CC polymorphism among Caucasians was present in 7%–12% of the population, and the allelic frequency was about 33%[37,43,44]. Nonetheless, it is likely that regardless of penetrance of the polymorphism, the clinical impact that it has would be the same no matter where the study was performed or the frequency of the polymorphism within each study population, so the fixed-effects model, which demonstrated a clear and significant association between the C677T polymorphism and MTX toxicity, may be more applicable. It is also notable that none of the studies in our analysis discusses the racial or ethnic background of participants. As the rate of the different SNP may be different in different ethnic groups, these data would be useful to understand the effect of a given SNP and the utility of studying different SNP in different patient populations.

The strengths of our study include the size of the analysis, with over 1400 patients for the C677T analysis and over 660 for the A1298C analysis. In addition, the relative merits of each study are discussed, with a focus on the differences in both the treatments and toxicity analyses of the different studies. This analysis has limitations as well; first, there is an inherent heterogeneity to metaanalysis, and there were differences in definition of toxicity, MTX dose, and use of folic acid supplement among the different studies. Second, not all studies discriminated between the heterozygous and homozygous genotype. Because of this, the metaanalysis was performed combining all patients that deviated from the wild type, allowing all studies to be compared in the metaanalysis.

In conclusion, as pharmacogenetics evolves, more and larger studies are needed to assess the role of various polymorphisms for drug efficacy and toxicity. However, until larger studies are carried out, metaanalysis of pooled data is the best tool to validate genetic associations with efficacy and toxicity. Our results illustrate the paucity of reliable pharmacogenetic data on a very commonly used antirheumatic drug and the potential role that pharmacogenetics can play in tailoring drug therapy for an individual patient.

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