Folate Pathway Enzyme Gene Polymorphisms and the Efficacy and Toxicity of Methotrexate in Psoriatic Arthritis

VINOD CHANDRAN, FOTIOS SIANNIS, PROTON RAHMAN, FAWNDA J. PELLETT, VERNON T. FAREWELL, and DAFNA D. GLADMAN

ABSTRACT. ***Objective.*** To determine the association between folate pathway gene polymorphisms and the effectiveness, toxicity, and drug survival of methotrexate (MTX) in psoriatic arthritis (PsA).

Methods. Data were obtained from a longitudinal cohort of PsA patients evaluated according to a standard protocol. Data on duration of drug therapy, dose, side effects, and reasons for discontinuation are systematically recorded. Patients treated with MTX after clinic admission who had ≥ 3 swollen joints prior to initiating MTX therapy were selected for evaluation of effectiveness. Response to MTX treatment was assessed at 6 months. Data from all patients treated in the clinic with MTX were used in evaluation of toxicity and drug survival. The following single-nucleotide polymorphisms (SNP) were measured using the Sequenom platform: MTHFR 677C>T (rs1801133), MTHFR 1298A>C (rs1801131), DHFR –473T>C (rs1650697), DHFR 35289A>G (rs1232027), and RFC 80G>A (rs1051266). Fisher's exact test, logistic regression, and Cox proportional hazard analyses were used to determine association.

Results. Two hundred eighty-one patients were identified from the database. All patients were included in the analysis for side effects and drug survival, and 119 patients were included in the effectiveness analysis. The minor A allele of DHFR gene at +35289 was the only SNP demonstrating association with response to MTX therapy (OR 2.99, p = 0.02). Patients homozygous for the minor allele of MTHFR 677C/T (677TT) had more liver toxicity (Fisher exact test, p = 0.04).

Conclusion. Polymorphisms of the DHFR gene may be associated with MTX efficacy. MTHFR 677TT may have a relationship with MTX-induced liver toxicity in PsA. (J Rheumatol First Release May 15 2010; doi:10.3899/jrheum.091311)

Key Indexing Terms:
PSORIATIC ARTHRITIS PHARMACOGENETICS DRUG RESPONSE
DRUG TOXICITY DRUG SURVIVAL

From the Division of Rheumatology, University of Toronto, Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital, Toronto, Canada; Department of Mathematics, University of Athens, Athens, Greece; Division of Rheumatology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada; and the MRC Biostatistics Unit, Institute of Public Health, Cambridge, United Kingdom.

The University of Toronto Psoriatic Arthritis clinic is supported by the Krembil Foundation. Dr. Chandran is supported by a Canadian Institutes of Health Research Clinical Research Initiative Fellowship and the Krembil Foundation; Dr. Farewell is supported by MRC funding U.1052.00.009.

V. Chandran, MBBS, MD, DM, Clinical Fellow, Division of Rheumatology, University of Toronto, Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital; F. Siannis, PhD, Department of Mathematics, University of Athens, and MRC Biostatistics Unit, Institute of Public Health; P. Rahman, MD, MSc, FRCPC, Professor of Medicine, Memorial University of Newfoundland; F.J. Pellett, BSc, Research Technologist, Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital; V.T. Farewell, PhD, MRC Biostatistics Unit, Institute of Public Health; D.D. Gladman, MD, FRCPC, Professor of Medicine, Division of Rheumatology, University of Toronto, Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital.

Address correspondence to Dr. D.D. Gladman, MD, Toronto Western Hospital, 1E-410B, 399 Bathurst Street, Toronto, Ontario M5T 2S8. E-mail: dafna.gladman@utoronto.ca

Accepted for publication February 19, 2010.

Psoriatic arthritis (PsA) is an inflammatory arthritis associated with cutaneous psoriasis, usually seronegative for rheumatoid factor[1]. Methotrexate (MTX) is one of the disease-modifying antirheumatic drugs (DMARD) that is used in the management of moderate to severe peripheral PsA[2]. Despite the paucity of evidence of clinical benefit, MTX is frequently used as the primary DMARD in PsA, because of its effectiveness in treating both skin and joint involvement and its low cost[3]. We previously reported that in a longitudinal cohort setting, 68% of patients demonstrate improvement at 24 months[4]. Identifying markers that predict response and/or toxicity to MTX may assist in planning disease management, so that early effective treatment may be undertaken.

Using genetic markers to help predict the efficacy and toxicity of drugs (pharmacogenetics) has potential to help in effective management of patients with inflammatory arthritis[5]. Pharmacogenetic studies on MTX response and toxicity in rheumatoid arthritis (RA) and psoriasis have been conducted[6-10]. There are no such studies in PsA[11]. There are differences between the efficacy and toxicity profile in patients treated with MTX in RA and in PsA, with a higher risk of pneumonitis and accelerated nodulosis in the former and

liver fibrosis in the latter[12-15]. In patients with psoriasis and concomitant PsA, response to treatment with MTX in the skin domain is not always associated with response in the arthritis domain. The genetics of RA and PsA differ, as there are different HLA and non-HLA associations between these 2 disease entities. Therefore, pharmacogenetic investigations specific to PsA are worthwhile.

The gene polymorphisms that influence metabolism of MTX may be classified into those that influence MTX transport across the cell membrane and those that influence enzymes in the cellular pathway of MTX[6]. We aimed to determine the associations between effectiveness, toxicity, and drug survival of MTX and polymorphisms of genes coding for the folate pathway enzymes methylenetetrahydrofolate reductase (MTHFR), dihydrofolate reductase (DHFR), and reduced folate carrier (RFC) in patients with PsA treated with MTX for active peripheral PsA in a longitudinal cohort.

MATERIALS AND METHODS

Patients treated with MTX after enrollment into the longitudinal cohort at the University of Toronto PsA clinic were included in this study. Patients satisfied the CASPAR classification criteria for PsA[16]. They were seen every 6 months according to a standard protocol [and more frequently if required when joint counts and Psoriasis Area and Severity Index (PASI) scoring were done][17,18]. The clinic protocol includes clinical assessment (complete physical examination, joint counts), laboratory assessment, patient questionnaires (every year), and radiographic evaluation (every 2 years). Actively inflamed joints were defined by the presence of joint effusion and/or joint-line tenderness and/or stress pain. Clinically damaged joints were defined as any joint with limited movement > 20% of its normal range, not due to effusion (including subluxation and flexion contractures), joint ankylosis, flail joint, or surgery. Radiographic damage was defined as the number of joints with surface or pocket erosions with or without joint space narrowing, disorganization (including ankylosis, pencil-in-cup change, or total joint destruction) or as having required surgery. Methods used in the clinic are reliable[19,20]. Data on drug treatment with start and stop dates, dose, side effects and toxicities, and reasons for discontinuation are systematically recorded.

Treatment strategy. MTX treatment was started at a weekly dose of 7.5 mg orally. The dose was rapidly escalated to 15 mg weekly over the first month. If there was no response, the dose was then increased to 25 mg weekly. Doses above 17.5 mg were given by subcutaneous injection. All patients were treated with folic acid at a dose of 5 mg, 6 days a week (omitted on the day of MTX). Joint counts and PASI scores were done at 3 month intervals.

Evaluation of effectiveness. The first course of MTX treatment in those patients treated after entry into clinic was analyzed for effectiveness. Patients had to have at least 3 swollen joints at time of initiation of MTX treatment. Concurrent treatment with other traditional disease-modifying agents was allowed, but patients receiving biologic agents were excluded. Response was assessed at 6 months. Patients achieving ≥ 50% decrease in actively inflamed joint count from baseline were classified as responders and those having < 50% decrease, no change, or an increase in joint counts were classified as nonresponders.

Toxicity evaluation. All patients treated with MTX were assessed for toxicity. Adverse effects were recorded based on those reported by patients themselves, those reported by the physician evaluating the patient, and by laboratory assessments [hemoglobin, white blood cell count, platelet count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, creatinine, chest radiograph] on followup. The following adverse effects were specifically recorded by protocol: nausea/vomiting, liver toxicity, gastrointestinal (GI) ulcer/upper GI bleed, diarrhea, abdominal pain, infection, infection requiring hospitalization, tuberculosis, allergic reaction, worsening of psoriasis, alopecia, mouth ulcers, headache, tinnitus/vertigo, paresthesias, ocular toxicity, hypertension, renal, cough/dyspnea, pneumonitis, congestive heart failure, anemia, leukopenia, thrombocytopenia, and others. However, only those events ascribed to MTX by the treating physician were included as adverse events for the purposes of this study. The adverse effects recorded were classified into the following 4 categories: (1) no side effects, (2) side effects present, (3) side effects leading to discontinuation of MTX, and (4) presence of liver function abnormalities (AST/ALT > 1.5 times upper limit of laboratory normal). Categories 3 and 4 are subcategories of category 2.

Drug survival. Drug survival (duration of exposure to drug) was calculated from the start and stop dates of the first treatment with MTX recorded in the database. All patients treated with MTX (irrespective of initial joint counts) were included in the drug survival analysis.

Genotyping. The following single-nucleotide polymorphisms (SNP) were genotyped using the Sequenom platform from DNA isolated from peripheral blood: MTHFR 677C>T (rs1801133), MTHFR 1298A>C (rs1801131), DHFR –473T>C (rs1650697), DHFR 35289A>G (rs1232027), and RFC 80G>A (rs1051266). Genotype distributions were as follows: for MTHFR 677C>T, 43.3% CC, 46.1% CT, 10.6% TT; MTHFR 1298A>C, 44.9% AA, 42.8% AC, 12.4% CC; DHFR –473T>C, 7.3% TT, 30.7% TC, 62.1% CC; DHFR 35289A>G, 12.1% AA, 31.5% AG, 56.5% GG; and RFC 80G>A, 32.3% GG, 49.2% GA, 18.5% AA. The success rates for these assays were as follows: MTHFR 677C>T 99.3%, MTHFR 1298A>C 99.6%, DHFR –473T>C 91.9%, DHFR 35289A>G 87.3%, and RFC 80G>A 89.4%. Genotype frequencies of RFC 80G>A (rs1051266) and MTHFR 1298A>C (rs1801131) were in Hardy-Weinberg equilibrium (HWE; tested using Pearson's chi-square test, 5% significance threshold) whereas those for MTHFR 677C>T (rs1801133) ($p < 0.001$), DHFR –473T>C (rs1650697) ($p < 0.01$), and DHFR 35289A>G (rs1232027) ($p < 0.001$) were not. The mean overall genotyping success rate was 93.5%. Although the 3 SNP were not in HWE, we carried out the association analyses. All subjects had disease (no controls were included), no errors with genotyping were identified, and it is not recommended that SNP not in HWE be summarily discarded[21,22]. The lack of HWE may, however, be due to population stratification; this was not investigated further. Significant ethnic differences in allele frequencies of SNP in the MTHFR gene, including SNP MTHFR 677C>T, have been reported[23]. Thus our results should be interpreted with some caution, due to the deviation in HWE.

Statistical analysis. Fisher's exact test and logistic regression were used to determine the association between heterozygotic or homozygotic presence of the minor allele of the tested SNP with effectiveness and toxicity of MTX. Cox proportional hazards analysis was used to determine association between these SNP and drug survival.

RESULTS

A total of 281 patients treated with MTX were identified from the database. All patients were included in the analysis for side effects and drug survival. However, only 119 patients were included in the effectiveness analysis, since they were required to have at least 3 swollen joints and to have commenced treatment with MTX after entry to the clinic. Demographic and disease characteristics of patients are given in Table 1.

MTX response. After 6 months of therapy with MTX, 54 (52.4%) patients had 50% reduction in their actively inflamed joint count. Concomitant medications were allowed; 10.7% of patients were taking sulfasalazine, 7.8%

Table 1. Demographic and disease characteristics of study patients.

Characteristics	All Patients, n = 281	Patients Included in Effectiveness Analysis, n = 119*
Male/female	165/116	67/52
Age, yrs, mean	44	44.1
Duration of psoriasis, yrs, mean	17.6	15.7
Duration of psoriatic arthritis, yrs, mean	10	9.3
No. of actively inflamed joints, mean	10.9	15.5
No. of swollen joints, mean	3.6	7.7
No. of clinically damaged joints, mean	5.5	4.9
No. of radiographically damaged joints, mean	6.6	6.4
PASI score, mean	6.6	5

* This group is a subset of the 281 patients in the study; for definitions see text. PASI: Psoriasis Area and Severity Index.

hydroxychloroquine, 1% azathioprine, and 1% oral prednisone along with MTX. Table 2 provides the results of the association analysis of the 5 SNP tested in the 119 subjects for response to MTX using logistic regression adjusted for concomitant medications. Also given are counts of patients with minor alleles and associated response rates. The minor A allele of DHFR gene at +35289 was the only SNP nominally associated with increased odds of response defined as a 50% reduction in actively inflamed joint after 6 months of therapy. Similar results were obtained when the data were analysed using Fisher's exact test (results not shown).

MTX toxicity. Data obtained from 281 patients treated with MTX were used in this analysis. The number of patients (category 1) with no side effects was 125 (44.5%), with 156 (55.5%) patients (category 2) having side effects. Of the latter, side effects leading to discontinuation of MTX were seen in 103 (36.7%) patients (category 3) and liver function abnormalities were seen in 45 (16%) patients (category 4).

The association between the presence of toxicity and the genotype was examined using a series of 2×3 tables. Table 3 summarizes the results of the series of univariate analyses. The analyses show that the 5 SNP tested do not appear to be associated with the presence of side effects (category 2). Side effects were significantly associated with female sex [83/165 (50%) male vs 73/116 (63%) female (p = 0.04)]. No variable was associated with side effects leading to discontinuation of MTX (category 3). The SNP were also not associated with the presence of liver toxicity (category 4). Further investigations not limited to presence or absence of SNP revealed some evidence that patients homozygous for the minor allele of MTHFR 677C/T (MTHFR 677TT) had more liver toxicity [9/30 patients with MTHFR 677TT vs 36/249 patients without MTHFR 677TT experienced liver toxicity (OR 2.53, 95% CI 1.08, 5.98; Fisher exact test p = 0.04)]. Further, age at PsA was associated with increased MTX-induced liver toxicity (p = 0.03). In a logistic model (backward selection) with all variables (SNP, sex, age at psoriasis, and age at PsA), only age at PsA remained significant.

Drug survival. The association of SNP with drug survival was assessed using a Cox proportional hazards model. None of the 5 SNP tested were associated with drug survival. Only age at psoriasis was a significant predictor of drug survival (p = 0.01).

DISCUSSION

MTX is often the DMARD of choice for the treatment of moderate to severe PsA[2,3]. Genetic markers for response and toxicity to MTX have been investigated in RA and psoriasis, but not in PsA[6-11]. Using information collected prospectively in a large longitudinal cohort of patients with PsA, we show that after 6 months of therapy with MTX, 52.4% of patients had a response defined as a 50% reduction

Table 2. Results of association analysis for response to MTX of the 5 SNP tested in 119 subjects, using logistic regression analysis adjusted for concomitant medications.

Allele	50% Reduction in Actively Inflamed Joint Count Counts Yes	No	p (Fisher)	Logistic Regression Output OR (95% CI)	p
MTHFR 677T	28/49	32/54	0.84	0.95 (0.41, 2.15)	0.89
MTHFR 1298C	27/49	27/54	0.69	1.25 (0.55, 2.84)	0.59
DHFR –473T	13/46	17/49	0.52	0.65 (0.26, 1.63)	0.35
DHFR 35289A	25/41	17/47	0.03	2.99 (1.20, 7.55)	0.02
RFC 80A	32/47	32/47	1.00	1.03 (0.43, 2.49)	0.94

Table 3. Summary of results of the association between the 5 SNP and side effects to methotrexate using Fisher's exact test.

Minor Allele	Side Effect Categories Side Effects Present			Side Effects Leading to Discontinuation of MTX			MTX-induced Liver Toxicity		
	Yes	No	OR (p)	Yes	No	OR (p)	Yes	No	OR (p)
MTHFR 677T	90/154	68/125	1.18 (0.54)	62/101	96/178	1.36 (0.26)	28/45	130/234	1.32 (0.51)
MTHFR 1298C	82/155	73/125	0.80 (0.40)	51/102	104/178	0.71 (0.21)	22/45	133/235	0.73 (0.41)
DHFR –473T	58/139	40/119	1.41 (0.20)	41/94	57/164	1.45 (0.18)	18/41	80/217	1.34 (0.48)
DHFR 35289A	59/133	48/113	1.08 (0.80)	39/91	68/155	0.96 (0.89)	14/37	93/209	1.56 (0.48)
RFC 80A	92/140	78/112	0.84 (0.59)	62/93	108/159	0.94 (0.89)	27/41	143/211	0.92 (0.86)

in their actively inflamed joint count. More than half (55.5%) the patients treated with MTX experienced side effects, and in 36.7% the side effects led to discontinuation of MTX. However, only 16% developed significant liver function test abnormalities. The minor A allele of DHFR gene at +35289 may have some association with response to MTX after 6 months of therapy. The SNP tested were not associated with MTX side effects. However, the minor T allele of MTHFR gene at +677 may be associated with liver toxicity.

The DHFR gene converts dihydrofolate to tetrahydrofolate. MTX inhibits DHFR, which is essential for cell growth and proliferation[24]. Although 829C/T, a naturally occurring SNP in the 3' UTR of DHFR, affects DHFR expression and contributes to MTX resistance, the SNP at position 35289 of this gene (rs1232027) has not been previously reported to be associated with response to MTX therapy in RA, psoriasis, or cancer[25,26]. We estimate that the presence of the minor A allele of the gene at +35289 increases the odds (~3 times) of having a response in patients with PsA. Although MTHFR 1298A/C (rs1801131) and RFC 80G/A (rs1051266) have been reported to be associated with response to MTX in RA, and RFC 80G/A (rs1051266) in psoriasis, similar effects on response to MTX in PsA could not be demonstrated[6,8].

The MTHFR gene variants have also been associated with MTX toxicity in RA. C677T polymorphism of this gene may increase toxicity, although studies are conflicting[27-30]. We demonstrated that homozygotic presence of the minor T allele of the gene at +677 is associated with liver toxicity. A similar allelic association was reported in a recent metaanalysis of 8 studies in RA[31]. None of the other SNP tested was associated with MTX-induced side effects generally or with drug discontinuation due to side effects. Moreover, none of the SNP tested was associated with drug survival, which measures the length of time a patient continues to take a particular drug. Drug survival is a composite measure of drug effectiveness and encompasses factors such as adverse drug reactions, side effects, poor adherence, and loss of efficacy[32].

There are a number of reports on the association of polymorphisms in the genes of the folate pathway with efficacy and toxicity of MTX in RA[6]. Recent reports suggest that the antiinflammatory effect of MTX is related to release of adenosine[33,34]. A study showed that polymorphisms in the adenosine monophosphate deaminase (C34T AMPD1), aminoimidazole carboxamide ribonucleotide transformylase (C347G ATIC), and inosine triphosphate pyrophosphatase (C94A ITPA) genes are associated with good clinical response to MTX treatment[35]. Using clinical and genetic information, a clinical pharmacogenetic model to predict the efficacy of MTX in RA has been developed[36]. We tested only 5 SNP in 3 genes in the folate metabolism pathway, and not an extensive panel of SNP in genes in both folate and adenosine pathways. Future studies will address this issue.

There are fewer studies on the influence of gene polymorphisms on response to MTX treatment in psoriasis[8-10]. Although RFC 80G/A (rs1051266) polymorphism was associated with MTX toxicity in one study, MTHFR polymorphisms were not found to be associated[8,10]. DHFR polymorphisms were not tested. Thus, there seem to be differences in the association between genetic variants and MTX efficacy and toxicity between PsA and RA and psoriasis. Moreover, there are no large randomized clinical trials proving efficacy of MTX in PsA. A larger more comprehensive study is required to fully address the question about MTX efficacy and the association of genetic polymorphisms with efficacy and toxicity.

Our study has a number of limitations. The data on response and side effects were obtained from patients enrolled into a longitudinal observational cohort and not from a controlled clinical trial. Therefore, strict criteria on the use of MTX were not used, and the criteria and protocol described here may be better characterized as guidelines used in our clinic. The response criteria are also not as rigorous as those used in PsA clinical trials[37]. Thus, for a better understanding of the influence of genetic variants on MTX treatment efficacy and toxicity, a controlled trial that includes pharmacogenetic information will be required. Thus, the results of this study are best characterized as preliminary. However, since longitudinal cohorts reflect "real world" use of therapeutic agents, information obtained through studies such as ours is valuable and will complement results obtained from controlled trials.

Our results indicate that polymorphisms of the DHFR gene may be associated with MTX efficacy and that the MTHFR gene may be associated with MTX-associated liver toxicity in PsA. Future controlled studies using pharmaco-

genetic information on genes involved in the folate and adenosine pathways are required to confirm our findings and to identify novel pharmacogenetic associations. A pharmacogenetic index will then be developed and validated.

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