Cost-Effectiveness Analysis of MTHFR Polymorphism Screening by Polymerase Chain Reaction in Korean Patients with Rheumatoid Arthritis Receiving Methotrexate

SEONG-KYU KIM, JAE-BUM JUN, AHMED EL-SOHEMY, and SANG-CHEOL BAE

ABSTRACT. ***Objective.*** To determine whether a strategy based on methylenetetrahydrofolate reductase (MTHFR) genotype screening is more cost-effective than the conventional strategy in reducing the risk of methotrexate (MTX)-related toxicity in patients with rheumatoid arthritis (RA).

Methods. We consecutively enrolled 385 patients with RA (355 female, 30 male) who had received MTX and identified toxicity associated with MTHFR C677T genotypes. We designed a hypothetical decision model to compare the genotype-based strategy with the conventional strategy. The time horizon was set as 1 year, and direct medical costs were used. The measured outcomes were the total expected cost, the effectiveness, and the incremental cost-effectiveness ratio.

Results. MTHFR genotype distribution revealed 133 patients (34.6%) with 677CC, 193 (50.1%) with 677CT, and 59 (15.3%) with 677TT. A total of 154 patients (40.0%) exhibited MTX-related toxicity. Compared to RA patients with the CC genotype, the odds ratio (95% confidence interval) for risk of toxicity was 3.8 (2.29–6.33) for the CT genotype, and 4.7 (2.40–9.04) for the TT genotype. In the base-case model, the total expected cost and the probability of continuing MTX medication for the conventional and genotype-based strategies were 851,415 Korean won (US$ 710) and 788,664 Korean won (US$ 658), and 94.03% and 95.58%, respectively.

Conclusion. The MTHFR C677T polymorphism may be an important predictor of MTX-related toxicity in patients with RA. The cost-effectiveness analysis suggests that the genotype-based strategy is both less costly and more effective than the conventional strategy for MTX therapy. (First Release June 1 2006; J Rheumatol 2006;33:1266–74)

Key Indexing Terms:
METHYLENETETRAHYDROFOLATE REDUCTASE RHEUMATOID ARTHRITIS
METHOTREXATE POLYMORPHISM COST-EFFECTIVENESS ANALYSIS

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Methotrexate (MTX) is the most commonly used and the first-line antirheumatic agent in the treatment of rheumatoid arthritis (RA). Since the mid-1980s, several investigators have demonstrated that the potential antirheumatic and antiinflammatory properties of low-dose MTX make it one of the most useful therapeutic agents for RA[1,2]. Although MTX has faster action and higher efficacy than other antirheumatic drugs, patients treated with MTX can experience various MTX-related adverse effects such as liver dysfunction, gastrointestinal (GI) dysfunction, hair loss, oral ulcers, pneumonitis, and hematological abnormalities[3,4]. In nearly 30% of patients with RA, the various side effects result in discontinuation of MTX therapy.

The precise molecular basis for the toxicity of MTX remains unclear. MTX is also associated with other metabolic pathways such as purine, pyrimidine, and homocysteine-methionine metabolism[5]. Among these metabolic pathways, MTX-related toxicity may depend on the level of 5-methyl tetrahydrofolate (5-CH3-THF)[5]. However, the full spectrum of toxicities is unlikely to be caused solely by its action as a

folate antagonist. The methylenetetrahydrofolate reductase (MTHFR) enzyme may be an important regulator of 5-CH3-THF levels. The MTHFR enzyme catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-CH3-THF, which is essential for the methylation of homocysteine to methionine[5]. The C677T MTHFR polymorphism is a C→T mutation at nucleotide position 677, resulting in an alanine-to-valine substitution and producing a thermolabile enzyme with deficient activity[6]. The prevalence of the C677T MTHFR polymorphism varies among racial and ethnic groups, with European-Caucasian and Asian populations exhibiting higher frequencies (24–40%) of the T allele than African-Americans (< 11%)[7].

It has been shown that a single nucleotide polymorphism in the MTHFR gene strongly influences the MTX-related metabolic pathways[8-11]. Some clinical studies have shown that the C677T polymorphism is significantly associated with MTX-induced toxicity in RA[8,9]. The use of pharmacogenomics for individual drug therapy provides the potential to reduce drug-induced toxicity and increase drug efficacy. If a polymerase chain reaction (PCR) analysis having high sensitivity and specificity is used for MTHFR genotyping, it may be possible to predict MTX-related toxicity in RA patients treated with MTX.

We investigated the association between MTX-related toxicities and MTHFR C677T genotypes. Using an analytic decision-tree model, we also assessed whether a genotype-based strategy as a screening test for MTX-related toxicity provides a cost-effectiveness benefit over the conventional strategy.

MATERIALS AND METHODS

Subjects. A total of 385 patients with RA taking low-dose MTX were consecutively enrolled from outpatient clinics at the Hospital for Rheumatic Diseases, Hanyang University Medical Center, Seoul, Republic of Korea, from October 2001 to December 2003. This study was approved by the Institutional Review Board of Hanyang University Medical Center. The patients provided written informed consent. All enrolled patients were diagnosed as having RA on the basis of fulfilling the 1987 American College of Rheumatology (ACR) criteria[12]. The patients were 24 to 80 years of age (50.5 ± 11.0 yrs, mean ± SD), and included 355 women (92.2%). The patients were taking low-dose MTX (5–20 mg/wk), and the current and total accumulated dosages of MTX were calculated at the time of participation in this study based on retrospective reviews of medical records (Table 1). The patients had histories of variable medications during the disease course, comprising antimalarial drugs (200–400 mg), cyclosporine (25–100 mg), sulfasalazine (500–2000 mg), bucillamine (100–200 mg), and/or systemic glucocorticoid (prednisone 2.5–7.5 mg) as combined treatments. NSAID (nonsteroidal anti-inflammatory drugs) and folate (1 mg) were administered to all RA patients. In addition, we investigated the duration of RA, age at disease onset, serum levels of rheumatoid factor (RF) and C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and degree of progression of RA based on functional status classification[13], radiographic classification[14], and the Korean Health Assessment Questionnaire[15].

MTX-related toxicities. The assessment of toxicities associated with MTX was based on medical records and patient interviews. The physicians in charge reviewed the medical records and interviewed the patients while blinded to the genotyping results. The physicians first reviewed the medical records and identified the toxicities, and then interviewed the patients to confirm these results. Uncertain causes were considered as having no adverse drug reactions.

Abnormal liver function, leukopenia, and megaloblastic anemia were measured using objective, quantitative methods such as biochemistry and complete blood cell count. Abnormal liver function was defined as 2 successive abnormal values (greater than the upper limit of normal) in liver function tests, and no evidence of abnormality in the serologic test for hepatitis B or hepatitis C viral marker and in imaging evaluations of liver and spleen by abdominal ultrasound graph[8,10]. Leukopenia was defined as a total white blood cell count below 3500/mm^3 after MTX medication or a reduction of more than 75% compared with that prior to MTX treatment[10], and confirmed by 2 successive analyses of the complete blood cell count. Hair loss, dizziness, and GI dysfunction were estimated through medical records and patient interview. Hair loss was assessed as objectively as possible on the basis of an abnormal increase in diffuse loss compared to before the use of MTX. MTX pneumonitis was defined as no infiltration in lung parenchyma before MTX treatment that changed into newly developed infiltration as confirmed by high resolution computerized tomography without evidence of viral, bacterial, or fungal infection.

MTX-related toxicities were classified into 2 subgroups (temporary and permanent withdrawal) using real data as a substitute for grading toxicities.

Analysis of MTHFR genotype. MTHFR genotypes were determined by PCR–restriction fragment length polymorphism (PCR-RFLP) analysis. DNA was extracted from the peripheral blood of all participants using phenol-chloroform. About 25 ng of DNA was amplified using the GeneAmp® PCR System 2700 thermal cycler (Applied Biosystems, Foster, CA, USA). The forward (5'-TGA AGG AGA AGG TGT CTG CGG GA-3') and reverse (5'-AGG ACG GTG CGG TGA GAG TG-3') primers were used to amplify a 198-bp band, and the variant was digested into 175 and 23-bp bands using 4 U of *Hinf*I. The C677T polymorphism in MTHFR was detected as described by Frosst, *et al*[16]. PCR conditions were an initial denaturing at 95°C for 15 min followed by 35 cycles of denaturing at 95°C for 1 min, annealing at 62°C for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min. The final 10 μl reaction mixture contained 0.5 U HotStar Taq polymerase (Qiagen, Mississauga, Canada), 0.8 μM of each primer, and 0.2 mM of each dNTP. PCR products were digested overnight and resolved by agarose gel electrophoresis. Sensitivity and specificity of genetic analysis was assumed to be 100%.

Statistical analysis. Differences in the demographic variables and clinical parameters and additional disease-modifying antirheumatic drugs (DMARD) were analyzed by Spearman's rank correlation test or independent t test according to each MTHFR genotype. The descriptive analysis was performed using nominal scales according to each genotype. Toxicity frequencies and odds ratios (OR) for toxicity according to genotypes with 95% confidence intervals (95% CI) were analyzed by chi-square test. A probability value of $p < 0.05$ was considered statistically significant. All statistical analyses were performed using standard software (SPSS version 11.0 for Windows, SPSS, Chicago, IL, USA).

Cost-effectiveness analysis of MTHFR genotyping as a predictor for MTX-related toxicity

Goals of the study. We investigated whether a genotype-based therapy of MTX could result in better outcomes, primarily from the viewpoint of cost-effectiveness rather than efficacy-based outcomes. Therefore, the study compared the cost-effectiveness of a genotype-based strategy of MTX relative to the conventional strategy in patients with RA.

Study design. As recently recommended[17], a decision-tree model was designed to evaluate 2 treatment strategies: (1) conventional dosage and (2) genotype-based dosage management (Figure 1). This analysis was based on a hypothetical cohort of RA patients who began low-dose MTX as the initial therapy for controlling the activity and progression of RA. We then projected the progression of the cohort according to the basic scenario, with the time horizon set at 1 year.

In developing a decision tree, our retrospective clinical values were used to determine the decision probabilities of each branch leading from each chance node. Expected costs were calculated by combining the probabilities and costs of each branch. Decision-tree analysis was conducted from a socie-

Table 1. Characteristics of patients with rheumatoid arthritis (RA) according to MTHFR genotype. Values are mean ± SD unless otherwise indicated.

		MTHFR C677T Genotype		
	Total, n = 385 Mean ± SD	CC, n = 133 Mean ± SD	CT, n = 193 Mean ± SD	TT, n = 59 Mean ± SD
Age, yrs	50.4 ± 11.0	50.3 ± 10.9	50.5 ± 11.2	50.3 ± 11.2
Sex, female (%)	355 (92.2)	123 (92.5)	177 (91.7)	55 (93.2)
Onset age, yrs	37.5 ± 11.7	37.1 ± 11.9	37.6 ± 11.3	38.1 ± 12.8
Disease duration, yrs	13.9 ± 7.5	14.2 ± 7.8	14.0 ± 7.6	13.2 ± 6.6
RF, positive (%)	308 (80.0)	105 (78.9)	156 (80.8)	47 (79.7)
ESR, mm/h	42.1 ± 31.2	42.0 ± 30.3	40.5 ± 30.2	47.9 ± 35.9
CRP, mg/dl	1.2 ± 1.6	1.2 ± 1.5	1.2 ± 1.6	1.4 ± 1.6
Functional class (%)				
I	87 (22.6)	32 (24.1)	43 (22.3)	12 (20.4)
II	114 (29.6)	41 (30.8)	60 (31.1)	13 (22.0)
III	102 (26.5)	32 (24.1)	52 (26.9)	18 (30.5)
IV	82 (21.3)	28 (21.0)	38 (19.7)	16 (27.1)
Progression stage (%)				
I	42 (10.9)	18 (13.5)	19 (9.8)	5 (8.5)
II	83 (21.6)	22 (16.5)	50 (25.9)	11 (18.6)
III	175 (45.5)	56 (42.1)	91 (47.2)	28 (47.5)
IV	85 (22.0)	37 (27.8)	33 (17.1)	15 (25.4)
KHAQ	1.0 ± 0.8	1.0 ± 0.8	1.0 ± 0.7	1.0 ± 0.8
MTX				
Current dose, mg	10.8 ± 4.5	10.8 ± 5.0	10.8 ± 4.3	11.1 ± 4.1
Total dose, mg	2488.7 ± 1434.5	2524.2 ± 1497.0	2468.6 ± 1425.7	2474.2 ± 1337.9
Duration of use, week	214.1 ± 121.7	215.0 ± 123.3	215.1 ± 124.6	208.5 ± 109.3
Mean dose, mg	11.6 ± 2.4	11.6 ± 2.4	11.6 ± 2.5	11.8 ± 2.1
DMARD*, n (%)				
Hydroxychloroquine	163 (42.3)	59 (44.4)	77 (39.9)	27 (45.8)
Sulfasalazine	147 (38.2)	53 (39.8)	73 (37.4)	21 (35.6)
Bucillamine	105 (27.3)	31 (23.3)	56 (29.0)	18 (30.5)
Steroid	266 (69.1)	90 (67.7)	135 (69.9)	41 (69.5)
Cyclosporine	42 (10.9)	14 (10.5)	18 (9.3)	10 (16.9)

* The number and percentage of patients taking other concurrent DMARD or steroids at any time during the disease course, but not the medication at the time of assessing the toxicity. MTHFR: methylenetetrahydrofolate reductase. RF: rheumatoid factor. ESR: erythrocyte sediment rate. CRP: C-reactive protein. KHAQ: Korean Health Assessment Questionnaire. DMARD: disease modifying antirheumatic drugs. MTX: methotrexate. Steroids include prednisolone, triamcinolone, and deflazacort.

tal perspective, and initially performed on the base case with our real values and followed by sensitivity analysis with the range of values from the literature.

Outcome measures. Outcomes were assessed based on total expected cost incorporating the treatment of adverse events, effectiveness, and incremental cost-effectiveness. The total expected cost was derived from direct health-care-related consumption during 1-year followup. Effectiveness was defined as the probability of continuing medication without permanent withdrawal of MTX, irrespective of any type of adverse event of any severity. The incremental cost-effectiveness ratio was calculated as the ratio of the incremental total cost expected during the 1-year followup divided by the probability of continuing MTX medication.

Basic scenario. The following assumptions were made in formulating the analytic decision-tree model:

1. The dosage of MTX and the laboratory monitoring scheme for its toxicity were according to the 2002 American College of Rheumatology Subcommittee on RA guidelines[18].

2. In the conventional strategy, the initial MTX dosage was 7.5 mg/week, increased by 2.5 mg/week at 4-week intervals. Laboratory monitoring was performed at 4-week intervals until the development of toxicity. If MTX-related toxicity did not appear during the study period, the MTX dose was increased to the maximum dose of 20 mg irrespective of genotype. However, if MTX-related toxicity developed, MTX was discontinued for 4 weeks irrespective of hospital admission status due to toxicity. In patients who tolerated toxicity, laboratory monitoring was performed every 2 weeks for 8 weeks after restarting the reduced dosage of MTX. If the patient subsequently exhibited tolerability, followup laboratory testing was performed every 4 weeks.

3. In the genotype-based strategy, the starting dose of MTX in patients with wild-type and mutant (carriers of the T allele) genotypes was 7.5 and 5 mg/week, respectively. A longer followup period with laboratory monitoring intervals of 8 and 4 weeks was applied in the wild-type and mutant groups, respectively. The maximum doses for patients without toxicity in the wild-type and mutant groups were 20 and 15 mg/week, respectively. Once toxicity had developed, the MTX rest period and the period of laboratory monitoring were the same as in the conventional strategy.

4. We assumed that the decision conditions of the wild-type and mutant genotype-based strategies, including the incidence of MTX-related toxicity, hospital admission rate, and temporary and permanent withdrawal rates of MTX, were the same as those of the wild-type in the conventional strategy since we had only real data for this strategy.

5. In patients who were still intolerant to MTX after its temporary withdrawal, MTX was completely withdrawn and replaced with new agents

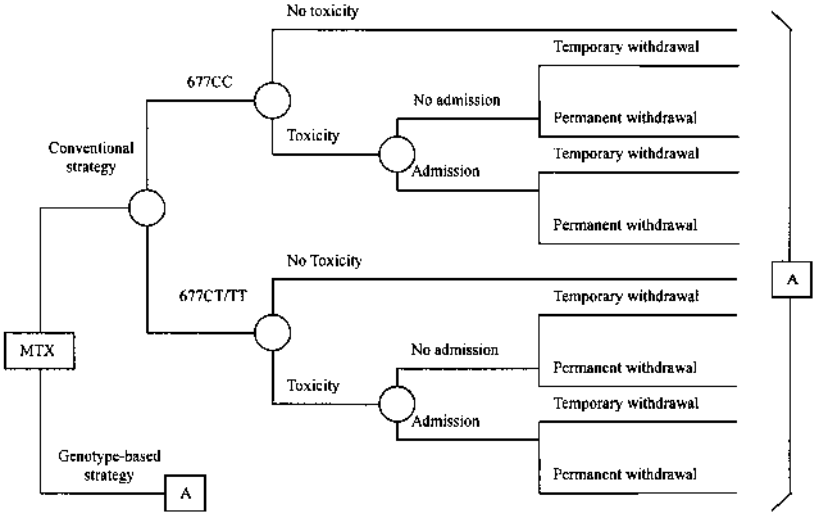

Figure 1. Decision-tree model framework. The base-case patient with RA was treated on the basis of a conventional or genotype-based dosage strategy for MTX. The other arm (A) was shared with the conventional strategy. 677CC: wild-type of the MTHFR C677T gene; 677CT/TT: mutant type of the MTHFR C677T gene.

(cyclosporine alone, combination of hydroxychloroquine and sulfasalazine, or leflunomide alone) until end of study.

6. The sensitivity and specificity of MTHFR PCR genotyping in forming the analytic decision tree was not considered in the assessment of the prevalence of MTHFR polymorphism, because other studies have indicated that this genotyping is nearly 100% accurate[19].

Probabilities and cost estimates. The data for the prevalence of the MTHFR C677T polymorphism, the incidence of MTX-related toxicity, hospital admission rate, and the rates of permanent and temporary withdrawal in wild-type and mutant groups were determined from our retrospective clinical data. Estimates used in the evaluation of base-case analysis are given in Table 2.

This analysis considered only direct medical costs, which included costs to patients themselves and to the healthcare scheme according to the 2004 Korean health insurance system. The cost of the MTX drug was based on the mean price of products sold in Korean pharmacies during the study period of 1 year. Monitoring laboratory tests for MTX toxicity included complete blood count, creatinine, and liver function, in accordance with the 2002 recommendation of the ACR[18]. Hospital admission cost was estimated on the basis of our 3 real RA patients admitted at Hanyang University Hospital, Seoul, Republic of Korea, due to MTX-related toxicity, and the mean cost (2,951,900 Korean won; US$ 2,460) for admission was applied in the base-case analysis (Table 2). The PCR cost of MTHFR C677T genotyping was determined from Hanyang University Hospital as 60,000 Korean won (US$ 50). All costs were calculated in Korean won, and the quoted equivalent US dollar values were calculated using an exchange rate of US$ 1 = 1,200 Korean won.

Sensitivity analysis. After performing the base-case analysis, sensitivity analyses were performed with the range of prevalence of MTFHR polymorphism[7-11], incidence of MTX-induced toxicities among mutants[8-10], rate of permanent discontinuation of MTX, PCR cost of MTHFR genotyping, and hospital admission cost. The most likely estimates for the genotype-based strategy from the literature were applied. The lowest value of admission cost among our 3 real-patient results was also applied. Since the PCR test for MTHFR genotyping is not commercially established in Korea, we performed a sensitivity analysis with 2 estimates of the PCR cost. In addition, analyses were performed for the threshold that maintained the superiority of the genotype-based strategy.

Table 2. Estimates in base-case analysis. All variables except PCR cost and hospitalization cost were derived from our retrospective clinical data.

Variable	Estimate in Base-Case Analysis
Prevalence of MTHFR mutant genotype, %	65.45
Incidence of toxicity in wild genotype, %	20.30
Incidence of toxicity in mutant genotype, %	50.40
Admission rate in wild genotype, %	0.75
Admission rate in mutant genotype, %	1.19
Temporary withdrawal of MTX in wild genotype, %	15.79
Temporary withdrawal of MTX in mutant genotype, %	43.65
Permanent withdrawal of MTX in wild genotype, %	4.51
Permanent withdrawal of MTX in mutant genotype, %	6.75
PCR cost, Korean won (US dollars)	60,000 (50)
Hospitalization cost due to adverse effects, Korean won (US dollars)	2,951,900 (2,460)

PCR: polymerase chain reaction.

RESULTS

Demographic characteristics, clinical manifestations, and MTHFR polymorphism in RA. The demographic characteristics of sex, age, disease-onset age, and duration of disease did not differ significantly between each MTHFR genotype, classified into normal (677CC) and mutant (677CT, 677TT; Table 1). There were no significant differences between the genotypes in clinical variables of RF, ESR, CRP, functional status classification, radiographic classification, and MTX or other antirheumatic drugs.

Association between MTHFR genotypes and MTX-related toxicities.

MTX-related toxicity was identified in 154 (40%) of the 385 patients with RA. The frequency of toxicity was 20.3% in patients with the CC genotype, 49.2% in patients with the CT genotype, and 54.2% in patients with the TT genotype (Table 3). Compared to patients with the CC genotype, the OR (95% CI) for risk of MTX-related toxicity was 3.8 (2.29–6.33) and 4.7 (2.40–9.04) for patients with the CT and TT genotypes, respectively. The MTX-related toxicity was greater among carriers of the T allele than among those with the CC genotype ($p < 0.001$, OR 4.0, 95% CI 2.45–6.51).

A total of 167 toxicity events appeared in the 154 patients exhibiting toxicity in our study (Table 4). These events comprised GI dysfunction, abnormal liver function, hair loss, nodulosis, dizziness, leukopenia, megaloblastic anemia, interstitial pneumonitis, and oral ulcer. The major toxicities in our study were GI dysfunction (34.7%), hair loss (29.3%), and abnormal transaminase level (28.8%). The frequencies of these 3 major toxicities were much higher in the mutant genotype group than in patients with the CC genotype. Toxicity events that interrupted MTX administration occurred at least once in 30% of the 167 toxicity events. Abnormal transaminase was more common than GI dysfunction or hair loss among patients with a history of temporary withdrawal of MTX. Twenty-three of the 167 events resulted in permanent withdrawal of MTX, the most common cause of which was GI dysfunction.

Cost-effectiveness analysis of MTHFR genotyping as a predictor for MTX-related toxicity

Base-case analysis. The healthcare resources used by individual patients comprised the MTX drug cost, laboratory cost of monitoring the toxicity, prescribing fee, and hospitalization cost (Table 5), of which toxicity monitoring comprised the largest portion (60% for the conventional dosage strategy and 55% for the genotype-based dosage strategy). The total expected costs and the probability of continuing MTX use dif-

Table 3. Analysis of odds ratios (OR) between genotypes of MTHFR and adverse effects of MTX in patients with RA.

	MTHFR C677T Genotype				
	677CC, n = 133	677CT, n = 193	677TT, n = 59	677CT/677TT, n = 252	All Genotypes, n = 385
Toxicity, n (%)	27 (20.3)	95 (49.2)	32 (54.2)	127 (50.4)	154 (40.0)
OR (95% CI)	—	3.8* (2.29–6.33)	4.7* (2.40–9.04)	4.0* (2.45–6.51)	—
p	—	< 0.001	< 0.001	< 0.001	—

* The OR for development of MTX-related toxicity between individual genotypes (677CT or 677TT) or pairs of genotypes (677CT and 677TT) and the wild genotype.

Table 4. MTX-related toxicity profiles and toxicity frequencies according to genotypes.

	No. of Toxicity Events* (%)			History of Withdrawal of MTX† (%)			Permanent Withdrawal of MTX (%)		
		Genotype			Genotype			Genotype	
	Total, n = 385	677CC, n = 133	677CT/TT, n = 252	Total, n = 385	677CC, n = 133	677CT/TT, n = 252	Total, n = 385	677CC, n = 133	677CT/TT, n = 252
MTX-related toxicities									
GI dysfunction	58 (15.1)	13 (9.8)	45 (17.8)	17 (4.4)	7 (5.3)	10 (4.0)	10 (2.6)	5 (3.8)	5 (2.0)
Abnormal transaminase	48 (12.5)	6 (4.5)	42 (16.7)	22 (5.7)	3 (2.3)	19 (7.5)	8 (2.1)	1 (0.8)	7 (2.8)
Hair loss	49 (12.7)	7 (5.3)	42 (16.7)	7 (1.8)	1 (0.8)	6 (2.4)	4 (1.0)	0 (0.0)	4 (1.6)
Nodulosis	4 (1.0)	1 (0.8)	3 (1.2)	1 (0.3)	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)
Oral ulcer	3 (0.8)	1 (0.8)	2 (0.8)	1 (0.3)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Dizziness	2 (0.5)	1 (0.8)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Leukopenia	1 (0.3)	1 (0.8)	0 (0.0)	1 (0.3)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Megaloblastic anemia	1 (0.3)	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Interstitial pneumonitis	1 (0.3)	0 (0.0)	1 (0.4)	1 (0.3)	0 (0.0)	1 (0.4)	1 (0.3)	0 (0.0)	1 (0.4)

* Some of the 154 patients experiencing toxicity had more than one adverse event. † Number of events and frequencies for temporary or permanent withdrawal of MTX due to toxicity.

Table 5. Expected cost and effectiveness in the base case. Expected costs were calculated on the basis of an individual patient receiving treatment for 1 year.

Strategy for MTX	Individual Components of Treatment Cost, Korean won (US dollars)					Expected Cost, Korean Won (US dollars)	Effectiveness, %
	Drug Cost	Toxicity Monitoring Cost	Prescription Fee	Hospital Admission Cost	PCR Cost		
Conventional dosage strategy	105,258 (88)	494,175 (412)	221,313 (184)	30,669 (26)	0 (0)	851,415 (710)	94.03
Genotype-based dosage strategy	78,848 (66)	416,186 (347)	210,628 (175)	23,002 (20)	60,000 (50)	788,664 (658)	95.58

fered considerably between the 2 strategies. The total expected costs of the conventional and genotype-based strategies were 851,415 Korean won (US$ 710) and 788,664 Korean won (US$ 658), respectively. The probabilities of continuing MTX medication without interruption due to MTX-related toxicity in the conventional and genotype-based strategies were 94.03% and 95.58%, respectively (Table 5). The genotype-based strategy was found to be less costly and more effective than the conventional strategy (Figure 2).

Sensitivity analysis. To improve the robustness of the analytical model, we calculated the threshold values at which the genotype-based strategy became superior (Table 6). Even when the lowest prevalence of MTHFR polymorphism reported worldwide was applied to our decision-tree model framework, the genotype-based strategy was still superior to the conventional strategy (Figure 2). Indeed, if the prevalence of carriers of the T allele was greater than 7% in this model, genotype-based management of MTX was always superior to the conventional strategy. For the toxicity incidence of 26.86% in patients with a mutant genotype, the incremental

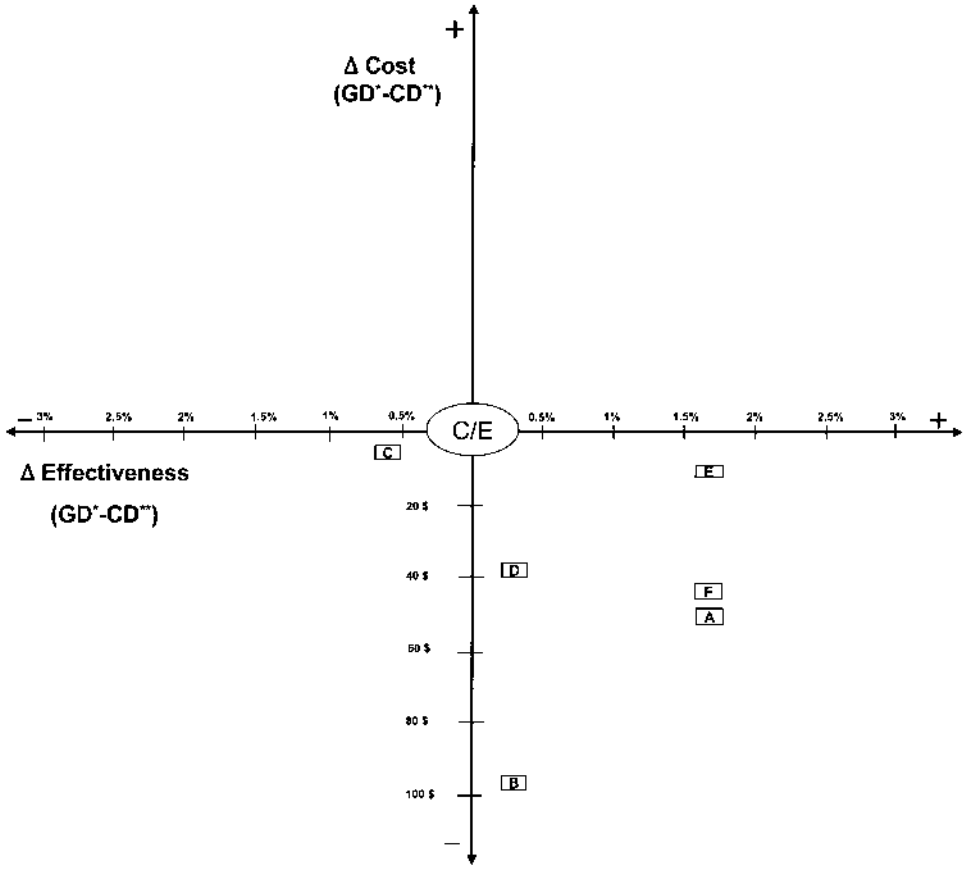

Figure 2. The cost-effectiveness diagram in base-case (A) and sensitivity (B to F) analyses. A. Base-case analysis. B. Prevalence of MTHFR polymorphism of 13.25%. C. Incidence of toxicity in mutant genotype of 26.86%. D. Permanent withdrawal of MTX in mutant genotype of 4.76%. E. Cost for PCR of 120,000 Korean won (US$ 100). F. Cost for hospital admission of 2,555,308 Korean won (US$ 2,129). GD: genotype-based dosage strategy; CD: conventional dosage strategy.

Table 6. Probability estimates and one-way sensitivity analysis.

Variable	Estimate Against Genotype-based Strategy[reference] and Threshold		Conventional Strategy Expected Cost, Korean Won (US dollars)	Conventional Strategy Effectiveness, %	Genotype-Based Strategy Expected Cost, Korean Won (US dollars)	Genotype-Based Strategy Effectiveness, %	Incremental Cost Effectiveness, Korean Won (US dollars)
Prevalence of MTHFR polymorphism, %	Estimate	13.25[7]	812,057 (677)	95.32	694,815 (579)	95.58	Genotype-based strategy is superior
	Threshold	7.00	802,124 (668)	95.32	681,765 (568)	95.32	If greater than threshold, genotype-based strategy is superior
Incidence of toxicity in mutant genotype, %	Estimate	26.86[9]	800,917 (667)	96.10	788,664 (657)	95.58	23,563 (20)
	Threshold	33.00	811,687 (680)	95.58	788,664 (657)	95.58	If greater than threshold, genotype-based strategy is superior
Permanent withdrawal of MTX in mutant genotype, %	Estimate	4.76*	832,900 (694)	95.32	788,664 (657)	95.58	Genotype-based strategy is superior
	Threshold	3.97	825,494 (688)	95.84	788,664 (657)	95.58	If greater than threshold, genotype-based strategy is superior
PCR cost, Korean Won (US dollars)	Estimate	120,000 (100)†	851,415 (710)	94.03	848,664 (707)	95.58	Genotype-based strategy is superior
	Threshold	123,238 (103)	851,415 (710)	94.03	851,902 (710)	95.58	If less than threshold, genotype-based strategy is superior
Hospitalization cost, Korean Won (US dollars)	Estimate	2,555,308 (2,129)**	847,294 (706)	95.03	785,574 (655)	95.58	Genotype-based strategy is superior
	Threshold	0 (0)	820,746 (684)	95.03	765, 662 (638)	95.58	Genotype-based strategy is superior

* Values derived from our clinical data. † Authors' own estimates. ** Lowest admission cost among 3 real patients.

cost was 23,563 Korean won (US$ 20) per effectiveness gain. However, if the incidence of toxicity was greater than 33%, the genotype-based therapy was superior.

When we doubled the cost of PCR testing [120,000 Korean won (US$ 100)], the genotype-based strategy was still superior to the conventional strategy. In our model the PCR cost at which the strategies were equal was 123,238 Korean won (US$ 103), but this appears unreasonably high for a genotyping test in the current Korean health insurance system.

When the lowest hospitalization cost among our 3 hospitalized cases of 2,555,308 Korean won (US$ 2,129) was used, the genotype-based strategy was still superior to the conventional therapy. Indeed, the genotype-based therapy was superior in the sensitivity analysis even when the hospital admission cost was set to zero.

DISCUSSION

The objective of this study was to determine the cost effectiveness of a pharmacogenomic-based therapy using the MTHFR genotype screening by PCR relative to the conventional approach to reduce MTX-related toxicity in patients with RA. This study was designed and analyzed on the basis of data derived from a retrospective clinical investigation. Our results show that the genotype-based dosage strategy using the PCR method is more cost-effective in the treatment of RA patients than the conventional dosage strategy of MTX. It is able to both reduce the MTX-related toxicity and reduce the economic cost of controlling RA.

MTX is considered an important drug for the treatment of RA, but its use has been limited by the incidence of mild to severe toxicity[5,20]. Only a few determinants for MTX-related toxicity have been identified, such as older age, elevated transaminase, and renal insufficiency[20,21]. Laboratory tests for predicting the development of toxicity due to MTX treatment may not be established, except the possible role of folate deficiency.

The C677T polymorphism is the most common in MTHFR, but its frequency varies between ethnic groups. It has been identified that the frequency of the 677T allele is 24–40% in Europeans, 26–37% in Japanese, and < 11% in black Africans[7]. In our Korean population we found that the frequencies of the T allele and the homozygous mutant are 40% and 15.3%, respectively.

Recent studies have elucidated a significant relationship between the MTHFR polymorphism and the development of MTX-related toxicity[8,9]. Carriers of the T allele were found to be more susceptible to MTX-related toxicities than the wild-type, such as elevation of liver enzymes, hair loss, and GI dysfunction[8,9]. In contrast, other studies suggested that there was no association between the C677T polymorphism and toxicity, although this involved a relatively small number of patients[10,11]. A recent study also suggested that MTX-related

toxicity is associated with the A1298C polymorphism of the MTHFR gene[11]. Individuals with the 1298CC genotype reported fewer MTX-related toxicities. However, the C allele of the A1298C polymorphism has been described as a factor reflecting sensitivity to MTX therapy[9]. Future studies should attempt to determine the precise role of the A1298C polymorphism. Our study shows that carriers of the 677T allele were 4 times more likely to experience toxicity than patients with the CC genotype. Further, the toxicity was 4.7-fold higher in patients with the 677TT genotype than in those with the wild-type 677CC genotype.

We first used a pharmacoeconomic approach for the cost-effectiveness analysis of MTHFR PCR screening and MTX therapy in RA patients based on the data from our clinical study. The base-case analysis revealed that the genotype-based strategy is superior to the conventional strategy in terms of both the total expected cost and the effectiveness. The following factors contribute to the cost-effectiveness of pharmacogenomic strategies: the frequency of the genetic polymorphism, the degree of gene penetrance (association between genotype and phenotype), sensitivity and specificity of the genetic screening, prevalence of the disease of interest, and outcomes and economic effects of the disease and its treatment[17]. In terms of genes, common variant alleles or frequent polymorphisms for the candidate gene and high penetrance influence the cost-effectiveness analysis. The penetrance of the MTHFR gene is relatively low, with a higher prevalence of genotypic polymorphism. PCR-RFLP for MTHFR genotyping is considered a rapid and inexpensive method, and although it is not commercially available in Korea, it exhibits a sensitivity and specificity of nearly 100%[19]. Most patients with RA initially received low-dose MTX, which is relatively inexpensive compared to other DMARD. And although the prevalence of RA is considered to be 0.5–1% in the population, if MTX therapy in RA is interrupted due to its toxicity, management of the disease using expensive DMARD becomes costly. Therefore, a treatment based on this genetic screening is an ideal strategy in terms of both cost and effectiveness.

We applied sensitivity analysis to identify the impact of the validity of the base-case analysis. The reported prevalence of the C677T MTHFR polymorphism varies between ethnic groups from 7% to 67%[7-11]. Although the estimate used in the base-case analysis was 65.45%, which is higher compared with other data, a prevalence of greater than 7% in our analysis results in the superiority of the genotype-based strategy. The prevalence of the MTHFR polymorphism is much higher in the Korean population, and hence the genotype-based strategy may be more beneficial than the conventional strategy for predicting MTX-related toxicity. When the incidence rate of MTX-related toxicity was 26.86% (the lowest reported incidence rate in the literature), we found that the incremental cost-effectiveness ratio was 23,563 Korean won (US$ 20) per 1% of additional effectiveness, which is considered acceptable. The superiority of the genotype-based strategy was maintained if the incidence of toxicity was greater than 33%. The cost of PCR genotyping of the MTHFR gene is also an important factor in the sensitivity analysis. This method is not yet commercially available in Korea, so it is difficult to decide on the appropriate cost of the PCR method. Our analysis assumed that initial analysis of the base-case cost 60,000 Korean won (US$ 50). Even when this cost was doubled, the pharmacogenomic-guided therapy was still superior. It is only when the PCR cost reaches 123,238 Korean won (US$ 103) that the conventional strategy becomes superior. The lowest cost of 3 hospital admission cases was applied in our sensitivity analysis, which again resulted in the superiority of the genotype-based strategy. This was still the case when there was no cost associated with hospital admission.

In our retrospective clinical study involving 385 RA patients treated with MTX, 23 (5.97%) permanently withdrew due to MTX-related toxicity. It has been previously reported that about 30% of RA patients discontinue MTX because of toxic side effects[3,21]. Discontinuation rates of MTX ranging from 2.83% to 24.15% were identified in studies on the association between MTHFR genotypes and MTX-related toxicity, although none of these studies reported discontinuation rates by MTHFR genotype[8-10]. We found that the genotype-based strategy was superior as the discontinuation rate became close to that of the wild-type. However, if the discontinuation rate was less than 3.97%, the conventional strategy became superior.

Several limitations apply to our analyses. First, data on the conventional strategy group with the wild-type genotype were applied to the decision tree of genotype-based strategy, because data for the genotype-based strategy are not yet available. Second, we did not consider the MTHFR A1298C polymorphism that has recently been associated with MTX-related toxicity[11]. However, we consider that there are still insufficient data regarding the sensitivity or toxicity by MTX on the A1298C polymorphism, and further evaluations are therefore needed. Third, only direct cost was considered in the cost evaluation of our analysis. However, inclusion of indirect costs in the analytic decision-tree model will preserve the superiority of the cost-effectiveness analysis in patients of the genotype-based strategy, because more interventions are required in the conventional strategies due to the higher incidence of toxicity. Fourth, our estimate of base-case analysis may not be applicable to other ethnic groups, especially non-Asian populations. However, the robustness of our results as revealed by extensive sensitivity analyses with the data of other ethnic groups suggests that it may be universal. Finally, there is no validation sample for the pharmacogenetics between MTX-related toxicity and MTHFR polymorphism in our study. As the deficiency of the validation sample could be compensated for by others' investigations, we believe that our analysis may have broad application.

Our findings support the use of MTHFR C677T genotyp-

ing as a predictor of MTX-related toxicity in patients with RA. Pharmacogenomic-guided therapy via screening using PCR genotyping for MTHFR may be less costly and more effective than the conventional strategy, and thus is a useful and available option for reducing MTX-related toxicity.

REFERENCES

1. Weinblatt ME, Trentham DE, Fraser PA, et al. Long-term prospective trial of low-dose methotrexate in rheumatoid arthritis. Arthritis Rheum 1988;31:167–75.
2. Kremer JM. Longterm methotrexate therapy in rheumatoid arthritis: a review. J Rheumatol 1985;12 Suppl 12:25–8.
3. Mielants H, Veys EM, Van der Straeten C, Ackerman C, Goemaere S. The efficacy and toxicity of a constant low dose of methotrexate as a treatment for intractable rheumatoid arthritis: an open prospective study. J Rheumatol 1991;18:978–83.
4. Cronstein BN. Molecular therapeutics. Methotrexate and its mechanism of action. Arthritis Rheum 1996;39:1951–60.
5. van Ede AE, Laan RFJM, Blom HJ, De Abreu RA, van de Putte LBA. Methotrexate in rheumatoid arthritis: an update with focus on mechanisms involved in toxicity. Semin Arthritis Rheum 1998;27:277–92.
6. Goyette P, Sumner JS, Milos R, et al. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. Nat Genet 1994;7:195–200.
7. Schneider JA, Rees DC, Liu YT, Clegg JB. Worldwide distribution of a common methylenetetrahydrofolate reductase mutation. Am J Hum Genet 1998;62:1258–60.
8. van Ede AE, Laan RF, Blom HJ, et al. The C677T mutation in the methylenetetrahydrofolate reductase gene: a genetic risk factor for methotrexate-related elevation of liver enzymes in rheumatoid arthritis patients. Arthritis Rheum 2001;44:2525–30.
9. Urano W, Taniguchi A, Yamanaka H, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene were associated with both the efficacy and the toxicity of methotrexate used for the treatment of rheumatoid arthritis, as evidenced by single locus and haplotype analyses. Pharmacogenetics 2002;12:183–90.
10. Kumagai K, Hiyama K, Oyama T, Maeda H, Kohno N. Polymorphisms in the thymidylate synthase and methylenetetrahydrofolate reductase genes and sensitivity to the low-dose methotrexate therapy in patients with rheumatoid arthritis. Int J Mol Med 2003;11:593–600.
11. Berkun Y, Levartovsky D, Rubinow A, et al. Methotrexate related adverse effects in patients with rheumatoid arthritis are associated with the A1298C polymorphism of the MTHFR gene. Ann Rheum Dis 2004;63:1227–31.
12. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
13. Hochberg MC, Chang RW, Dwosh I, Lindsey S, Pincus T, Wolfe F. The American College of Rheumatology 1991 revised criteria for the classification of global functional status in rheumatoid arthritis. Arthritis Rheum 1992;35:498–502.
14. Steinbrocker O, Traeger CH, Batterman RC. Therapeutic criteria in rheumatoid arthritis. JAMA 1949;140:659–62.
15. Bae SC, Cook EF, Kim SY. Psychometric evaluation of a Korean Health Assessment Questionnaire for clinical research. J Rheumatol 1998;25:1975–9.
16. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111–3.
17. Flowers CR, Veenstra D. The role of cost-effectiveness analysis in the era of pharmacogenomics. Pharmacoeconomics 2004;22:481–93.
18. American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines. Guidelines for the management of rheumatoid arthritis: 2002 Update. Arthritis Rheum 2002;46:328–46.
19. Ulrich CM, Yasui Y, Storb R, et al. Pharmacogenetics of methotrexate: toxicity among marrow transplantation patients varies with the methylenetetrahydrofolate reductase C677T polymorphism. Blood 2001;98:231–4.
20. McKendry RJ, Dale P. Adverse effects of low dose methotrexate therapy in rheumatoid arthritis. J Rheumatol 1993;20:1850–6.
21. Buchbinder R, Hall S, Sambrook PN, et al. Methotrexate therapy in rheumatoid arthritis: a life table review of 587 patients treated in community practice. J Rheumatol 1993;20:639–44.