ABCB1 C3435T polymorphism influences methotrexate sensitivity in rheumatoid arthritis patients

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Abstract

Objective

Methotrexate (MTX) is most widely used for the treatment of rheumatoid arthritis (RA). However, it has certain drawbacks with regard to individual differences in its therapeutic effects as well as the differences in the patients' response to MTX therapy. We investigated whether multi-drug resistance-1 (ABCB1) C3435T, reduced folate carrier-1 (RFC1) G80A, 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (ATIC) C347G and a 6bp-deletion polymorphism in the 3'-untranslated region of the thymidylase synthase (TYMS) gene are predictive of MTX sensitivity and its adverse effects.

Methods

Patients whose last maintenance dosage of MTX was ≤ 6 mg/week were regarded as responders, while patients whose last maintenance dosage of MTX was > 6 mg/week or those in whom MTX therapy was changed due to poor response to MTX were regarded as non-responders. The data of 124 RA patients who had received MTX treatment were retrospectively analyzed for polymorphisms in the ABCB1, RFC1, ATIC and TYMS genes, MTX sensitivity and MTX toxicity.

Results

There were no significant differences in MTX sensitivity among the genotypes of RFC1, ATIC and TYMS genes. ABCB1 3435TT cases included statistically significantly more non-responders than 3435CC cases according to univariate analysis (crude odds ratio (OR) = 8.91, p = 0.001) and multivariate analysis (adjusted OR = 8.78, p = 0.038). There were no significant differences in MTX toxicity among the genotypes of all the genes.

Conclusion

These results suggested that the genetic diagnosis of ABCB1 C3435T can be applied to determine MTX sensitivity for the treatment of RA patients. However, further pharmacokinetics studies are required in this regard.

Key words

Rheumatoid arthritis, methotrexate, ABCB1, polymorphism, drug sensitivity.

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Introduction

Currently, methotrexate (MTX) is the most frequently prescribed disease-modifying anti-rheumatic drug (DMARD) (1). In routine clinical practice, different clinical responses have sometimes been observed in patients treated with MTX. This indicates the presence of individual differences in MTX sensitivity; that is, the existence of responders non-responders. MTX has unpredictable adverse effects such as gastrointestinal disturbances, hypersensitivity pneumonitis and bone marrow suppression (2). It is therefore necessary to avoid long-term administration of MTX to non-responders in consideration of the risk of these adverse effects.

Previous studies suggest that the therapeutic effects of MTX can be confirmed when it is administered continuously for 6 or more months (1, 2), while a recent report suggests that a longer period of MTX administration is required to confirm its effects (3). This is due to the lack of an efficient method for monitoring the therapeutic effects of MTX as well as the difficulty in predicting the optimum dosage of MTX required on an individual basis. On the other hand, genetically engineered biological agents have been recommended for use in non-responders to MTX (4, 5). In spite of their potent efficacy in the treatment of rheumatoid arthritis (RA), these agents are expensive and frequently induce complications that involve serious adverse effects. Therefore, these agents should preferably be used only after the effects of MTX have been accurately determined. Recent reports indicate that the bone destruction associated with RA progresses immediately after the onset of the disease (6, 7). Therefore, it would be extremely useful if we could predict the response of a patient to MTX before initiating low-dose MTX administration and determine the individual drug-treatment plan with higher doses of MTX or with genetically engineered biological agents, instead of evaluating individual MTX sensitivity after a long period of MTX treatment.

In order to establish a new indicator of MTX sensitivity, it is necessary to understand the pharmacokinetics and pharmacodynamics of MTX. After entering the body, MTX enters the cells by the action of reduced folate carrier (RFC), which is a member of the solute carrier (SLC) family of uptake-type transporters. On the other hand, MTX is transported outside the cells by the action of ABCC1, ABCC2, ABCC3, ABCC4 and ABCB1, which are members of the ATP-binding cassette (ABC) family of discharge-type transporters (8). Most of the MTX present inside the cells is polyglutamated to form MTX polyglutamate (MTX-PG), which inhibits thymidine synthase (TYMS) in pyrimidine synthesis. Since methylenetetrahydrofolate reductase (MTHFR) acts to pool folic acid in the cells, it could also have an influence on MTX activity. However, the inhibitory activity of MTX-PG on 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase (ATIC), an important enzyme in purine synthesis, is believed to be the most important mechanism in the anti-rheumatic effects of MTX (9). Due to this inhibition of ATIC, AICAR accumulates within the cells and inhibits adenosine deaminase (ADA). Hence, adenosine, which has potent anti-inflammatory action, is released outside the cells.

In this study, we focused on the ABCB1, RFC1, ATIC and TYMS genes and investigated whether ABCB1 C3435T, RFC G80A, ATIC C347G and a 6bp-deletion polymorphism in the 3'-untranslated region (UTR) of the TYMS gene are predictive of MTX sensitivity and toxicity during the treatment of RA patients.

Methods

Patients

This study included 124 RA patients (110 Japanese females and 14 Japanese males) who had received MTX therapy. These patients had received MTX for ≥ 3 months and a maintenance dosage of MTX for ≥ 2 months or stopped taking MTX due to the onset of severe adverse effects. All subjects consented to peripheral blood collection and gene analysis. The subjects were treated at the rheumatic outpatient clinic of the

Department of Orthopaedics at Kyoto Prefectural University of Medicine between December 2003 and March 2004. All patients had been diagnosed according to the 1987 American College of Rheumatology (ACR) criteria.
This study was approved by the Ethics Committee and Ethical Review Board on Human Genome/Gene Analysis Research of Kyoto Prefectural University of Medicine, and written informed consent was obtained from all patients.

Clinical information
We collected patient data regarding sex, age, the number of tender or swollen joints (a maximum of 46 joints: the metacarpophalangeal joints, proximal interphalangeal joints, wrists, elbows, shoulders, metatarsophalangeal joints, ankles, knees, hips, sternoclavicular joints and the temporomandibular joints), serum levels of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), matrix metalloproteinase-3 (MMP-3), anti-agalactosyl IgG antibody (CARF), physician's and patient's global assessments of disease activity using the 100-mm visual analogue scale (VAS), modified Health Assessment Questionnaire (mHAQ) (10), maintenance dosage of MTX, history of MTX administration, MTX toxicity and the last concomitant drugs used, which included oral steroids and other DMARDs. The maintenance dosage of MTX was defined as a stable dosage that had not been changed for ≥ 2 months.

Evaluation of MTX toxicity
We assessed MTX toxicity based on the presence or absence of adverse effects during MTX therapy. Blood and urine tests were performed periodically, and the test results were used to ascertain the presence or absence of adverse reactions such as renal and hepatic dysfunctions and pancytopenia. Physicians specializing in rheumatology, respiratory diseases, gastroenterology and dermatology assessed the MTX administration, physical findings and test findings.

Evaluation of MTX sensitivity
After the initiation of MTX therapy, we evaluated MTX sensitivity based on the subjective symptoms that were scored by using the VAS, the number of tender or swollen joints, activities of daily living (ADL) that were scored by using mHAQ, and blood and biochemical findings such as white blood cell count, CRP, ESR and MMP-3. Subsequently, we controlled the dosages of MTX and other concomitant drugs (11). Among the patients who were administered continuous MTX therapy for at least 3 months, excluding those with MTX toxicity, the patients whose last maintenance dosage of MTX was not > 6 mg/week were regarded as responders. The patients whose last maintenance dosage of MTX was > 6 mg/week or those in whom MTX was substituted with other DMARDs because of poor response to MTX were regarded as non-responders. Patients who discontinued MTX therapy due to severe adverse effects were excluded from the sensitivity and maintenance dosage analyses. We investigated the differences in the number of non-responders and responders.

Gene analysis
Genomic DNA was obtained from peripheral blood by using the DNeasy™ Tissue Kit (QIAGEN GmbH, Germany). The sequences of purified polymerase chain reaction (PCR) fragments were determined on an automated DNA sequencer (ABI PRISM377) by using BigDye Terminator cycle sequencing reactions (Applied Biosystems, Perkin-Elmer, Inc., Massachusetts, USA).
Specific oligonucleotide primers used for the PCR amplification of ABCB1 C3435T, RFC1 G80A and a 6bp-deletion polymorphism in the 3'-UTR of the TYMS gene from genomic DNA were derived from known sequences. The sequences of the primers were as follows: forward, 5'-TTCAGCTGCTTGATGGCAAA-3' and reverse, 5'-AGGCAGTGACTCGATGAAGG-3' for ABCB1; forward, 5'-AGTGTCACCTTCGTCCCCTC-3' and reverse, 5'-CTCCCGCGTGAAGTTCTT-3' for RFC1; and forward, 5'-CAAATCTGAGGGAGCTGAGT-3' and reverse, 5'-CAGATAAGTGGCAGTACAGA-3' for TYMS. The PCR amplification program consisted of an initial denaturation step at 94 ºC for 5 min, followed by 35 cycles of denaturation at 94 ºC for 1 min, annealing at 58 ºC for 1 min, extension at 72 ºC for 1 min and a final extension at 72 ºC for 5 min.
ATIC C347G was obtained by employing a real-time TaqMan allelic discrimination method using the Applied Biosystems 7300 Real-Time PCR System and the ABI PRISM 7000 Sequence Detection System. The sequences of the primers were as follows: forward, 5'-AAGACAGTGGCTTCTCCAGGTGTAA-3' and reverse, 5'-AATTTGCTCCACAGCCTCCTCAACA-3'. Allelic discrimination was performed using 5'-VIC-TGGCTTCTCCAGGTGTAACTGTT-MGB-3' and 5'-FAM-TGGCTTCTCCAGGTGTAAGTGTT-MGB-3' probes. The PCR amplification program consisted of an initial denaturation step at 95 ºC for 10 min, followed by 40 cycles of denaturation at 92 ºC for 15 sec and annealing and extension at 60 ºC for 1 min.
Negative and positive controls were performed for each sequencing plate. The Hardy-Weinberg equilibrium was assessed for the genotypes of all the genes by using the χ^2 tests. The allele and genotype frequencies of all the genes were determined in all 124 patients.

Statistical analysis
Statistical significant differences between the responders and non-responders, and patients with or without adverse drug events and their genotypes were assessed using Fisher's exact test, the Wilcoxon rank-sum test and the Kruskal-Wallis test. The crude and adjusted odds ratios (OR) and their 95% confidence interval (CI) were calculated by using the logistic regression model. The logistic regression model in multivariate analysis included the variables whose p value in univariate analysis was < 0.2. With regard to the VAS score, the VAS scores of only the patients were included in the multivariate model because a strong correlation was observed between the VAS scores of the physicians and the patients. The cut-off value for significant differences

Table I. Overview of adverse effects of MTX.

	n	Withdrawal
Adverse effect	48	19
Hepatic toxicity	31	4
Gastrointestinal complaint	8	6
Bone marrow toxicity	2	2
Pulmonary toxicity	2	2
Renal toxicity	2	2
Rash	2	2
Epilation	2	2

One patient had both hepatic and renal toxicity.

(p value) was defined as $p < 0.05$. The patients who discontinued MTX therapy because of severe adverse effects were defined as missing values in MTX sensitivity. All these analyses were conducted using the Statistical Analysis System software package (version 8.02; SAS Institute Inc., Cary, North Carolina, USA).

Results

Relationship between MTX toxicity and the general characteristics of the subjects

Adverse effects during MTX therapy were observed in 48 of the 124 patients (38.7%) (Table I). In 29 of these 48 patients, MTX therapy was continued along with folic acid supplementation. MTX therapy was discontinued in the remaining 19 patients because of severe adverse effects and they were administered other drugs. According to the results of the Wilcoxon rank-sum test, these patients had received lower MTX dosages and a shorter duration of treatment than the non-responders or responders (median MTX dosage: 4.0 versus 6.0 mg/week, $p = 0.004$; median treatment duration: 6 versus 37.5 months, $p = 0.002$).

Table II shows the subjects' general characteristics that were evaluated for studying MTX toxicity. Univariate analysis did not reveal any significant differences in sex, the number of tender or swollen joints, ESR, CARF, mHAQ score, physician's or patient's VAS score, the use of oral steroid or other DMARDs, MTX dosage or treatment duration between patients with and without adverse effects. According to the findings of the Wilcoxon rank-sum test, patients with adverse effects were significantly younger than those without adverse effects (median age: 56.5 versus 63.0 years, $p = 0.010$). Furthermore, patients with adverse effects had significantly higher CRP levels than those in patients without adverse effects (median value: 1.45 versus 0.70 mg/dL, $p = 0.035$). Multivariate analysis showed a statistically significant correlation between MTX toxicity and age, CRP and MTX dosage (age: adjusted OR = 0.95, 95%CI 0.91–0.99, $p = 0.013$; CRP: adjusted OR = 1.28, 95%CI 1.03–1.59, $p = 0.028$; MTX dosage: adjusted OR = 0.71, 95%CI 0.54–0.95, $p = 0.019$).

Relationship between MTX sensitivity and the general characteristics of the subjects

Table III shows the subjects' general characteristics that were evaluated for studying MTX sensitivity. Fisher's exact test did not reveal any significant differences in sex, the use of oral steroid or other DMARDs or the presence or absence of adverse effects between the responders and non-responders. The Wilcoxon rank-sum test did not reveal any significant differences in the number of swollen joints, ESR, CARF, mHAQ score, physician's VAS score or treatment duration between the responders and non-responders. According to the results of this test, the non-responders were significantly younger than the responders (median age: 57.0 versus 63.0 years, $p = 0.043$), and the number of tender joints in the non-responders was significantly higher than that in the responders (median number of tender joints: 6.0 versus 3.0, $p = 0.033$). The patient's VAS scores were significantly higher in the non-responders than in the responders (median VAS scores: 46.0 versus 31.0 mm, $p = 0.006$). The CRP levels were also significantly higher in the non-responders than in the responders (median CRP level: 1.60 versus 0.60 mg/dL, $p = 0.001$). Furthermore, the MMP-3 levels were significantly higher in the non-responders than in the responders (median MMP-3 level: 191.0 vs 117.0 ng/mL, $p = 0.004$). Multivariate analysis revealed a statistically significant correlation between CRP and MTX sensitivity (adjusted OR = 2.20, 95%CI 1.30–3.71, $p = 0.003$).

Relationship between the genotypes and MTX toxicity or sensitivity

Each sequencing reaction was considered successful after the negative and positive controls were evaluated. None of the genotypes showed any significant deviation from Hardy-Weinberg equilibrium. The allele frequencies were C:0.601 T:0.399 for ABCB1, G:0.492 A:0.508 for RFC1, C:0.778 G:0.222 for ATIC and 6bp:0.363 and del:0.637 for TYMS. There were no significant differences in MTX toxicity among the genotypes of all the genes (Table II).

There were no significant differences in MTX sensitivity among the genotypes of the RFC1, ATIC and TYMS genes (Table III). ABCB1 3435TT cases included statistically significantly more non-responders than 3435CC cases according to univariate analysis ($p = 0.002$) and multivariate analysis (adjusted OR = 8.78, 95%CI: 1.13-68.5, $p = 0.038$). No statistically significant difference in the number of non-responders was observed between the 3435CC and 3435CT cases (Table III). It was suggested that C3435T had a large influence on the responses to MTX as a factor independent of other factors. The probability of non-responders among the cases with 3435TT was nine times as high as that among the 3435CC cases.

ABCB1 C3435T polymorphism

Table IV shows the ABCB1 genotypes for each group; no significant differences were observed between the patients who discontinued MTX therapy and the non-responders or responders in this regard. The Kruskal-Wallis test revealed differences in the MTX maintenance dosage among the ABCB1 3435CC, 3435CT and 3435TT cases ($p = 0.002$). In other words, the MTX maintenance dosage increased from CC to CT to TT. No significant differences were observed in the treatment duration among the ABCB1 genotypes.

Table II. Characteristics for assessment of MTX toxicity among 124 patients.

	Adverse effects			Odds Ratio			
	Present (n = 48)	Absent (n = 76)	p value	Crude OR(95% CI)	p value	†Adjusted OR(95% CI)	p value
Sex:							
Female	42 (87.5%)	68 (89.5%)	0.776*	1			
Male	6 (12.5%)	8 (10.5%)		1.21 (0.39-3.75)	0.735		
Age (years):							
Median	56.5	63.0	0.010 #	per 1 year		per 1 year	
Mean	56.8	61.2		0.96 (0.92-0.99)	0.024	0.95 (0.91-0.99)	0.013
No. of swollen joints (maximum 46):							
Median	4.0	5.0	0.975 #	per 1			
Mean	5.5	5.6		1.00 (0.90-1.10)	0.952		
No. of tender joints (maximum 46):							
Median	4.0	3.5	0.880 #	per 1			
Mean	5.9	6.7		0.99 (0.93-1.05)	0.623		
CRP (mg/dL):							
Median	1.45	0.70	0.035 #	per 1		per 1	
Mean	2.13	1.34		1.24 (1.02-1.52)	0.035	1.28 (1.03-1.59)	0.028
ESR (mm/h):							
Median	59.0	51.5	0.361 #	per 1			
Mean	62.9	58.3		1.00 (0.99-1.02)	0.465		
MMP-3 (ng/mL):							
Median	127.0	129.0	0.254 #	per 1			
Mean	193.7	179.8		1.00 (1.00-1.00)	0.712		
CARF (AU/mL):							
Median	53.4	67.2	0.549 #	per 1			
Mean	318.5	217.2		1.00 (1.00-1.00)	0.422		
mHAQ score:							
Median	0.75	1.00	0.500 #	per 1			
Mean	1.12	1.25		0.84 (0.54-1.31)	0.444		
VAS score (physician):							
Median	45.0	40.0	0.487 #	per 1			
Mean	42.6	40.0		1.01 (0.99-1.03)	0.573		
VAS score (patient):							
Median	40.0	32.5	0.438 #	per 1			
Mean	40.4	37.7		1.01 (0.99-1.03)	0.563		
Steroid:							
Absent	21 (43.8%)	24 (31.6%)	0.185*	1			1
Present	27 (56.3%)	52 (68.4%)		0.59 (0.28-1.25)	0.171	0.93 (0.39-2.21)	0.874
Other DMARDs:							
Absent	19 (39.6%)	38 (50.0%)	0.273*	1			
Present	29 (60.4%)	38 (50.0%)		1.53 (0.73-3.18)	0.258		
MTX dose (mg/w):							
Median	6.0	6.0	0.168 #	per 1		per 1	
Mean	5.6	5.9		0.86 (0.68-1.08)	0.199	0.71 (0.54-0.95)	0.019
MTX duration (months):							
Median	37	34	0.362 #	per 1			
Mean	40	46		1.00 (0.99-1.01)	0.419		
ABCB1:							
3435CC	19 (39.6%)	29 (38.2%)	0.277*	1			1
3435CT	17 (35.4%)	36 (47.4%)		0.72 (0.32-1.63)	0.432	0.70 (0.28-1.74)	0.443
3435TT	12 (25.0%)	11 (14.5%)		1.67 (0.61-4.54)	0.319	2.64 (0.86-8.13)	0.092
				(Trend: p = 0.499)			(Trend: p = 0.213)
RFC1:							
80GG	10 (20.8%)	17 (22.4%)	0.582*	1			
80GA	29 (60.4%)	39 (51.3%)		1.26 (0.51-3.16)	0.617		
80AA	9 (18.8%)	20 (26.3%)		0.77 (0.25-2.32)	0.636		
				(Trend: p=0.627)			
ATIC:							
347CC	27 (56.3%)	49 (64.5%)	0.675*	1			
347CG	18 (37.5%)	23 (30.3%)		1.42 (0.65-3.08)	0.375		
347GG	3 (6.3%)	4 (5.3%)		1.36 (0.28-6.54)	0.700		
				(Trend: p=0.406)			
TYMS 3'-UTR:							
6bp/6bp	5 (10.4%)	9 (11.8%)	0.806*	1			
6bp/del	26 (54.2%)	36 (47.4%)		1.30 (0.39-4.33)	0.669		
del/del	17 (35.4%)	31 (40.8%)		0.99 (0.29-3.42)	0.984		
				(Trend: p=0.743)			

Statistical analysis was performed using *Fisher's exact test, #Wilcoxon rank sum test.
†This model included the variables of age, CRP, steroid, MTX dose and ABCB1.

Discussion

Evaluating the responders and non-responders requires caution since it can be influenced by the RA status as well as the use of other concomitant drugs. The average dosage of MTX used in the U.S. is reported to be 7.5–15 mg/week (11). The body size of a Japanese individual is approximately 80% that of a typical American. In addition, a large-scaled clinical study investigating the efficacy and adverse effects of MTX has shown that the optimum dosage of MTX for a Japanese individual is 6 mg/week, and that higher dosages will increase the incidence of adverse effects (12). We used MTX monotherapy at a dosage of 6 mg/week or lower as our first choice of therapy in RA patients. We also implemented concomitant therapy with other DMARDs and small dosages of oral steroids. In addition, we increased the dosage of MTX or switched to genetically engineered biological agents in patients who showed inadequate therapeutic effects of MTX. In this study, responders were defined as patients in whom RA could be effectively controlled by administering MTX up to a maximum optimum dosage (i.e., 6 mg/week), and non-responders were defined as those in whom the dosage of MTX was increased beyond the optimum dosage or those in whom MTX was substituted with a different drug.

Statistically significant differences were observed in the number of tender joints, CRP, MMP-3 and patient's VAS score between the responders and non-responders, whereas there were no differences in the use of concomitant drugs. Multivariate analysis also revealed a statistically significant correlation between CRP and MTX sensitivity. The changes in the CRP levels are faster and CRP is more sensitive than ESR. Thus, CRP is a very good indicator of inflammation. It has been shown that if the CRP level is high, joint destruction is considered to be advancing at a faster rate (13). MMP-3 is another indicator of regional joint inflammation, and is useful for predicting advances in joint destruction (14, 15). The levels of CRP and MMP-3 reportedly show a good correlation (16), and these levels are thus very important for predicting disease activity and joint destruction. The CRP levels were higher in the non-responders than in the responders. This indicated that there was considerable validity in the definitions of responders and non-responders in this study, and that the disease state of RA could be reflected based on the patient's response to MTX.

Both univariate and multivariate analysis showed that the ages of the patients with adverse effects were significantly lower than those of the patients without adverse effects. The effects of MTX are expected to be more potent in elderly patients who have decreased gastrointestinal motility due to aging. Therefore, due to this mechanism, the effects of this drug might be enhanced with age; this might have resulted in an increased number of responders (17, 18).

There have been several reports that have predicted MTX sensitivity during RA treatments and the risks associated with the adverse effects. However, many of them have focused on the MTHFR genotypes associated with folic acid metabolism based on the aforementioned pharmacogenetics of MTX (19-21). Kumagai *et al.* have reported that genotyping for TYMS polymorphisms may become a useful indicator for determining the appropriate dosage of MTX in RA patients (22). Dervieux *et al.* have recently reported that the MTX-PG concentrations in peripheral erythrocytes and the genetic polymorphism of enzymes involved in the folate-purine-pyrimidine pathway, including RFC-1 G80A, ATIC C347G and a 28-bp tandem repeat polymorphism in the TYMS enhancer region, are useful for monitoring MTX sensitivity (23). However, in this study, no significant differences were observed in MTX sensitivity among the genotypes of RFC1, ATIC and TYMS genes (Table III). The discrepancy between our results and those of previous studies might be attributed to the differences in the clinical evaluation of the responders and non-responders as well as the difference in the protocols of MTX therapy. Kumagai *et al.*, in their study, regarded the patients whose last maintenance dosage of MTX was 6 mg/week as non-responders (22).

P-glycoprotein (P-gp) is a transport protein that plays an important role in drug absorption and distribution within the body (24). P-gp functions as a membrane protein that transports substrates from inside the cells to the outside using ATP as its energy source. Substrates include a variety of drugs such as carcinostatics, immunosuppressants and antihyperlipidemic drugs (25). The gene that encodes P-gp is a multi-drug resistance-1 (ABCB1) gene, which is a member of the ATP-binding cassette (ABC) transporter family that performs an important role in intracellular transport of MTX (26, 27).

The association between MTX sensitivity and P-gp has been investigated in some diseases. The sensitivity to MTX that was confirmed in lymphoblasts of acute lymphoblastic leukemia is reported to be related to a transporter protein, DHFR levels and the polyglutamation of MTX (28). In addition, accelerated expression of ABCB1 mRNA and increased expression of P-gp have been observed in leukemic cells that have a low sensitivity to MTX (29,30). The overexpression of ABCB1 in ovarian cancer causes resistance to MTX by reducing the intracellular accumulation and the polyglutamation of MTX (31). Previous studies concerning RA have demonstrated a correlation between MTX sensitivity and the expression level of P-gp in lymphocytes (3233). In addition, the P-gp levels in peripheral monocytes are higher in the non-responders than in the responders (34). These results strongly indicate that the polymorphism of the ABCB1 gene, which gives rise to individual differences in the P-gp expression levels and function, can be associated with MTX sensitivity in RA.

Numerous studies have investigated the relationship of the ABCB1 C3435T genotype with the expression levels and function of P-gp; however, the phenotype of ABCB1 C3435T is still under discussion (35-37). Hashida *et al.* have indicated that P-gp is closely involved in the pharmacokinetics of tacrolimus. Additionally, the function of P-gp can

Table III. Characteristics for assessment of MTX sensitivity among 105 patients.

				Odds Ratio			
	Non-responders (n = 33)	Responders (n = 72)	p value	Crude OR(95% CI)	p value	†Adjusted OR(95% CI)	p value
Sex:							
Female	28 (84.9%)	65 (90.3%)	0.511*	1			
Male	5 (15.2%)	7 (9.7%)		1.66 (0.49-5.67)	0.420		
Age (years):							
Median	57.0	63.0	0.043 #	per 1 year		per 1 year	
Mean	57.3	61.1		0.96 (0.93-1.00)	0.080	0.97 (0.90-1.04)	0.334
No. of swollen joints (maximum 46):							
Median	7.0	5.0	0.188	per 1		per 1	
Mean	6.6	5.1		1.08 (0.96-1.20)	0.191	0.89 (0.75-1.06)	0.202
No. of tender joints (maximum 46):							
Median	6.0	3.0	0.033 #	per 1		per 1	
Mean	8.6	5.4		1.06 (0.99-1.13)	0.106	1.09 (1.00-1.20)	0.064
CRP (mg/dL):							
Median	1.60	0.60	0.001 #	per 1		per 1	
Mean	2.46	1.02		1.72 (1.26-2.37)	0.001	2.20 (1.30-3.71)	0.003
ESR (mm/h):							
Median	56.0	51.0	0.333 #	per 1			
Mean	61.2	55.6		1.01 (0.99-1.02)	0.427		
MMP-3 (ng/mL):							
Median	191.0	117.0	0.004 #	per 1		per 1	
Mean	218.4	161.9		1.00 (1.00-1.00)	0.237	1.00 (1.00-1.00)	0.823
CARF (AU/mL):							
Median	54.6	78.3	0.603 #	per 1			
Mean	136.9	332.5		1.00 (1.00-1.00)	0.227		
mHAQ score:							
Median	1.1	1.0	0.610 #	per 1			
Mean	1.1	1.2		0.84 (0.51-1.37)	0.473		
VAS score (physician):							
Median	50.0	40.0	0.088 #	per 1			
Mean	47.0	37.7		1.02 (1.00-1.05)	0.087		
VAS score (patient):							
Median	46.0	31.0	0.006 #	per 1		per 1	
Mean	49.4	33.8		1.04 (1.01-1.07)	0.005	1.03 (0.99 -1.07)	0.163
Steroid:							
Absent	11 (33.3%)	25 (34.7%)	1.000 *	1			
Present	22 (66.7%)	47 (65.3%)		1.06 (0.45-2.54)	0.889		
Other DMARDs:							
Absent	12 (36.4%)	39 (54.2%)	0.098 *	1			1
Present	21 (63.6%)	33 (45.8%)		2.07 (0.89-4.83)	0.093	2.65 (0.69-10.2)	0.157
Treatment duration (months):							
Median	40	36	0.456 #	per 1			
Mean	51	46		1.00 (0.99-1.01)	0.541		
Adverse effects:							
Absent	22 (66.7%)	54 (75.0%)	0.481*	1			
Present	11 (33.3%)	18 (25.0%)		1.50 (0.61-3.69)	0.377		
ABCB1:							
3435CC	7 (21.2%)	34 (47.2%)	0.002*	1		1	
3435CT	15 (45.5%)	32 (44.4%)		2.28 (0.82-6.31)	0.114	2.14 (0.47-9.76)	0.325
3435TT	11 (33.3%)	6 (8.3%)		8.91 (2.46-32.2)	0.001	8.78 (1.13-68.5)	0.038
				(Trend: p = 0.001)		(Trend: p = 0.045)	
RFC1:							
80GG	6 (18.2%)	14 (19.4%)	0.958*	1			
80GA	18 (54.6%)	41 (56.9%)		1.02 (0.34-3.09)	0.966		
80AA	9 (27.3%)	17 (23.6%)		1.24 (0.35-4.32)	0.741)		
				(Trend: p = 0.723)			
ATIC:							
347CC	19 (57.6%)	48 (66.7%)	0.490*	1			
347CG	11 (33.3%)	21 (29.2%)		1.32 (0.54-3.26)	0.543		
347GG	3 (9.1%)	3 (4.2%)		2.53 (0.47-13.6)	0.281		
				(Trend: p = 0.268)			
TYMS 3'-UTR:							
6bp/6bp	2 (6.1%)	11 (15.3%)	0.375*	1			
6bp/del	18 (54.6%)	32 (44.4%)		3.09 (0.62-15.5)	0.170		
del/del	13 (39.4%)	29 (40.3%)		2.47 (0.48-12.7)	0.282		
				(Trend: p = 0.554)			

Statistical analysis was performed using *Fisher's exact test, #Wilcoxon rank sum test.
†This model included the variables of age, no. of swollen and tender joints, CRP, MMP-3, patient's VAS score, other DMARDs and ABCB1.

Table IV. ABCB1 C3435T polymorphism.

	ABCB1			
	3435CC	3435CT	3435TT	p value
Total (n =124)	48 (38.7%)	53 (42.7%)	23 (18.6%)	
Adverse effects:				
Present (n = 48)	19 (39.6%)	17 (35.4%)	12 (35.0%)	0.277*
Absent (n = 76)	29 (38.2%)	36 (47.4%)	11 (14.5%)	
Non-responders or				
Responders (n = 105)	41 (39.1%)	47 (44.8%)	17 (16.2%)	0.295*
Withdrawal (n = 19)	7 (36.8%)	6 (31.6%)	6 (31.6%)	
Non-responders (n = 33)	7 (21.2%)	15 (45.5%)	11 (33.3%)	0.002*
Responders (n = 72)	34 (47.2%)	32 (44.4%)	6 (8.3%)	
MTX maintenance dose (mg/w) (n = 105):				
Median	6.0	6.0	8.0	0.002$
Mean	5.6	5.8	7.2	
Treatment duration (months):				
Median	33	37	32	0.580$
Mean	41	45	49	

Statistical analysis was performed using *Fisher's exact test, or $ Kruskal-Wallis test.

be evaluated by measuring the dose/-concentration (D/C) ratio of tacrolimus; this is determined as the mean dose that can achieve the targeted trough level (38). We had previously investigated the ABCB1 C3435T genotype and the D/C ratio of tacrolimus in Japanese patients with kidney transplants, and found that compared to the 3435CC patients, the 3435TT patients showed an extremely low occurrence of steroid-induced osteonecrosis of the femoral head. Additionally, tacrolimus D/C ratio increased significantly in the 3435TT patients (37). Based on these findings, we believed that the pumping ability of P-gp was more elevated in the 3435TT patients than in the 3435CC patients in Japan. Hence, in the case of the 3435TT genotype, there might be a large number of non-responders due to accelerated release of MTX outside the cells and subsequent decreased MTX sensitivity. Conversely, the ABCB1 C3435T genotype might not be related to RA because this genotype has shown almost identical frequencies (C:0.60 and T:0.40) in our previous study (37).

A correlation between the genetic polymorphism of MTHFR and the frequency of adverse events after administering MTX for RA has been reported as previously mentioned (19-21). A significant relationship between the polymorphisms of ABCB1, RFC1, ATIC and TYMS and adverse effect profiles could not be observed in the present study. There are various types and degrees of severity of adverse effects — some occur immediately after administration of a drug, while others occur only after several months of drug intake; some are dose-dependent, while others are not and some are severe, while others are mild (11). In this study, multivariate analysis showed that the patients with adverse effects had received lower MTX dosages. Patients who discontinued MTX therapy due to severe adverse effects had received lower MTX dosages and had a shorter duration of treatment; however, this was unrelated to the ABCB1 genotype. These observations may suggest that severe adverse effects occur relatively soon after administering MTX at lower dosages. However, since the factors that acted after the drug entered the cells might have a great influence on the occurrence of adverse effects, the individual risks of adverse effects could not be assessed in this study.

The results of this study indicated that patients with ABCB1 3435CC and 3435CT showed higher therapeutic effects of MTX because of a decrease in the P-gp function although it has not yet been demonstrated. In addition, the results suggested that the ABCB1 genotype affected the MTX dosage requirement. It may be possible to diagnose the ABCB1 genetic polymorphism before administering MTX, and treat RA patients by predicting the individual response to MTX. In the case of the 3435TT genotype, increasing the dosage of MTX during the early phase of treatment and substituting it with genetically engineered biological agents such as infliximab that is not affected by P-gp (5) after carefully monitoring the MTX efficacy was considered necessary. Alternatively, the concomitant administration of MTX and drugs such as cyclosporine (39) and tacrolimus (40) that have demonstrated antagonistic and inhibitory effects on P-gp activity should be considered.

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