Potential role of pharmacogenetics for optimalization of drug therapy in rheumatoid arthritis

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Chapter 3:

Relationship between genetic variants in the adenosine pathway and outcome of methotrexate treatment in patients with recent-onset rheumatoid arthritis

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Abstract

Objective.
Among patients with rheumatoid arthritis (RA), there is a high degree of interindividual variability in the degree of response to methotrexate (MTX) treatment. This study was undertaken to explore polymorphisms in genes contributing to anti-inflammatory adenosine release as novel predictors of MTX treatment outcome.

Methods.
In 205 patients with newly diagnosed RA, 5 polymorphisms in 5 genes coding for enzymes related to the release of adenosine were analyzed. All patients received standardized MTX treatment (up to 25 mg per week orally), combined with folic acid. MTX efficacy was evaluated by the Disease Activity Score (DAS) and compared among genotypes. The association between MTX-related adverse events and genotype was also assessed. The following polymorphisms were determined: *AMPD1* 34C>T, *ATIC* 347C>G, *ITPA* 94C>A, *MTR* 2756A>G, and *MTRR* 66A>G. When significant differences were found by chi-square analysis, odds ratios (ORs) and 95% confidence intervals were calculated.

Results.
Patients carrying the *AMPD1* 34T allele, *ATIC* 347CC, or *ITPA* 94CC were more likely to have a good clinical response, as defined by a DAS of <2,4 (OR [95% confidence interval] 2,1 [1,0–4,5], 2,5 [1,3–4,7], and 2,7 [1,1–8.1], respectively). The likelihood of a good clinical response was increased if patients possessed all 3 favorable genotypes (OR 27.8 [95% confidence interval 3,2–250]). Regarding toxicity, only *ATIC* G allele carriers experienced a greater frequency of adverse events (OR 2,0 [95% confidence interval 1,1–3,7]).

Conclusion.
Polymorphisms in the *AMPD1, ATIC*, and *ITPA* genes are associated with good clinical response to MTX treatment. These findings indicate that genotyping may help in the identification of patients who will benefit most from MTX treatment and may assist clinicians in making treatment decisions regarding patients with recent-onset RA.

Introduction

Patients with rheumatoid arthritis (RA) show considerable variation in their clinical course and response to treatment (1,2). Despite the fact that most clinical study findings support the use of combination therapy to optimally suppress disease activity, most patients with newly diagnosed RA begin with monotherapy; methotrexate (MTX) is the preferred first-line disease-modifying anti-rheumatic drug (DMARD) (3–6).

Although results of randomized controlled clinical trials indicate that MTX alters the clinical course of RA, only ~40% of the patients exhibit a good clinical response (7–9). While achieving good response early in the disease process is key to minimizing the joint damage and functional decline characteristic of RA (6,10,11), it is not yet possible to predict which patients will respond to MTX. In most studies to date that have demonstrated MTX efficacy, predictors for response have not been specifically investigated. Clear predictors of response to MTX would be useful in directing treatment choices in the early phase of the disease.

In candidate gene–driven pharmacogenetic studies, polymorphisms in genes coding for proteins involved in pharmacokinetic or pharmacodynamic pathways related to the drug under study are selected, and possible associations with treatment outcome are investigated (12–14). Specific to MTX, several studies have shown that single-nucleotide polymorphisms (SNPs) in genes coding for the folate pathway enzymes are associated with treatment response (15–17). Although MTX may act in RA through inhibition of folate pathway enzymes, more recent reports indicate that its efficacy may be related to the release of endogenous anti-inflammatory adenosine (18–20) (Figure 1).

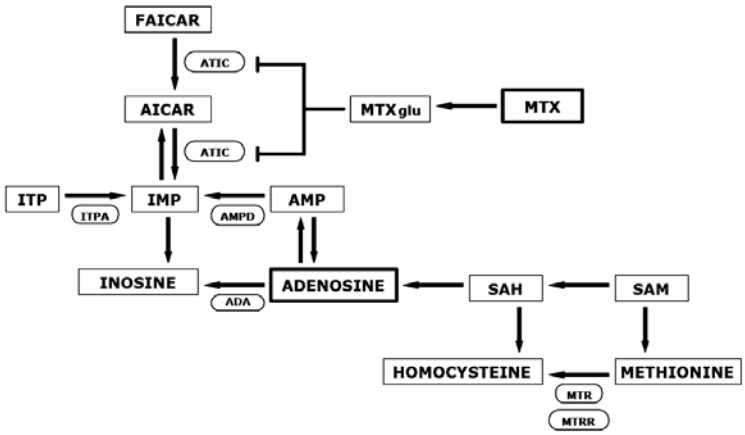

Figure 1. Simplified representation of the adenosine metabolism pathway[a,b,c]

a. Shown are enzymes and metabolites involved in the stepwise release of adenosine.
b. Abbreviation(s): FAICAR= formyl–5-aminoimidazole-4-carboxamide ribonucleotide, ITPA= inosine triphosphate pyrophosphatase, IMP= inosine monophosphate, ATIC= aminoimidazole carboxamide ribonucleotide transformylase, MTXglu= methotrexate polyglutamate, AMPD= adenosine monophosphate deaminase, ADA= adenosine deaminase, SAH= S-adenosylhomocysteine, SAM= S-adenosylmethionine, MTRR= methionine synthase reductase.
c. See ref. 18 for detailed information on the mechanism of action of MTX.

Studies on clinical outcome in patients with other complex conditions such as cardiovascular diseases have already alluded to the relevance of polymorphisms in genes coding for enzymes related to adenosine release (15,21–25). We hypothesized that genetic variants in these genes are associated with MTX treatment outcome. To investigate this, we assessed the relationship between SNPs in genes related to adenosine release and MTX treatment outcome in patients with recent-onset RA.

Patients and Methods

Role of the funding source

The rheumatologists participating in the Foundation for Applied Rheumatology Research were responsible for the study design and data collection in the BeSt study. The authors are responsible for the current subcohort data analysis, including genotyping, interpretation of data, preparing this manuscript, and the decision to publish. Centocor and Schering-Plough did not participate in any of these activities.

Patients

The 247 patients enrolled in this study comprised a subcohort of the 508 patients participating in the BeSt (Behandelstrategieën voor Reumatoide Artritis [Treatment Strategies for Rheumatoid Arthritis]) study (26). Inclusion criteria for the study included fulfillment of the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 revised criteria for RA (27), age of ≥18 years, and disease duration of <2 years. Patients also had to have active disease, defined as at least 6 swollen joints (of 66) and at least 6 tender joints (of 68), and either an erythrocyte sedimentation rate (ESR) of ≥28 mm/hour or a score of >20 mm on a 100-mm visual analog scale (VAS) for patient assessment of global health (0 mm = best; 100 mm = worst). Individuals were ineligible for the BeSt study if they had previously been treated with DMARDs other than antimalarial agents or were receiving concomitant treatment with an experimental drug. The local ethics committee at each participating hospital approved the study protocol, and all patients provided written informed consent before enrollment into the study.

Study design

The BeSt study was a randomized, multicenter, single-blind, clinical study comparing the clinical efficacy of 4 different treatment strategies in early RA: sequential monotherapy starting with MTX (n = 126), step-up from MTX to combination therapy with MTX and sulfasalazine (SSZ) (n = 121), initial combination therapy with MTX, SSZ, and high-dose (with tapering) prednisolone (n = 133), or initial biologic therapy with infliximab plus MTX (n = 128). Only patients who had been allocated to single use of MTX (n = 247) were included in the current analysis.

The primary goal of therapy in the BeSt study was clinical response as defined by a European League Against Rheumatism (EULAR) Disease Activity Score (DAS) of ≤2.4 (28,29). The DAS is a validated composite outcome measure consisting of the Ritchie Articular Index (RAI) (30), the number of swollen joints (of 44), general well-being as indicated by the patient on a VAS, and the ESR. A research nurse who was blinded with regard to the allocated treatment group assessed the DAS every 3 months.

All patients included in this analysis started on a regimen of oral MTX 7.5 mg weekly, increasing to 15 mg weekly after 4 weeks, in combination with folic acid (1 mg per day). In the event of insufficient clinical response (DAS ≤2.4) at the 3-month followup visit, the MTX dosage was increased stepwise to 25 mg weekly, given either orally or parenterally according to the rheumatologist's judgment. If the clinical response remained insufficient at the 6-month followup visit, patients were treated ac-

cording to the next step of the BeSt protocol, i.e., patients assigned to MTX sequential monotherapy were switched to SSZ 1,000 mg twice daily, and SSZ 1,000 mg twice daily was added to the MTX regimen for patients assigned to initial step-up combination therapy. Concomitant treatment with nonsteroidal antiinflammatory drugs and intraarticular injections of corticosteroids were allowed for all treatment groups. For the current analysis, clinical data from the first 6 months of followup were used to represent MTX treatment only. Responders were defined as patients with a DAS of ≤2.4 (good clinical response) based on the EULAR response criteria (28,29), and nonresponders as patients with a DAS of ≤2.4 at the 6-month followup visit.

Toxicity was evaluated by tabulating reported adverse drug events. Adverse drug events were spontaneously reported by the patients, were ascertained from nonspecific questioning by the investigator about the patient's well-being, or were found upon physical examination or determination of clinical laboratory parameters during the study. In cases of adverse drug events, MTX treatment was continued at the lowest tolerated dose or, if MTX was not tolerated at all, the DMARD therapy was changed. The following noninfectious adverse drug events were specifically evaluated: gastrointestinal adverse drug events (defined as general well-being, nausea, vomiting, diarrhea, or constipation); liver adverse drug events (defined as elevated liver enzyme levels resulting in MTX dosage adjustment or discontinuation), pneumonitis, and skin and mucosal disorders. Patients were also monitored for leukopenia (white blood cell count $<4 \times 10^9$/liter) and for elevations in levels of alanine aminotransferase and alkaline phosphatase to >3 times the upper limit of normal (i.e., >135 units/liter and >360 units/liter, respectively).

Five SNPs in genes related to adenosine release (31) (Figure 1) were selected, taking into consideration the following criteria: validated SNP, SNP causes nonsynonymous amino acid change, indications for clinical relevance from previous publications (15,21–25,32,33), and a preferred minimal genotype frequency of ~10%. The 5 selected genes were those coding for adenosine monophosphate deaminase (*AMPD1*), aminoimidazole carboxamide ribonucleotide transformylase (*ATIC*), inosine triphosphate pyrophosphatase (*ITPA*), methionine synthase (*MTR*), and methionine synthase reductase (*MTRR*). The following SNPs were analyzed: *MTRR* 66A>G (rs1801394), *MTR* 2756A>G (rs1805087), *AMPD1* 34C>T (rs17602729), *ITPA* 94C>A (rs1127354), and *ATIC* 347C>G (rs2372536).

DNA was isolated from peripheral white blood cells by a standard manual salting-out method. As a quality control, positive controls (Control DNA CEPH 347-02; Applied Biosystems, Foster City, CA) and negative controls (water) were used. In addition, 5–10% of samples were genotyped in duplicate, and no inconsistencies were observed.

Genotyping was performed using real-time polymerase chain reaction with TaqMan, according to the protocol provided by the manufacturer (Applied Biosystems). Genotype frequencies were in Hardy-Weinberg equilibrium, and the success rate was 99.5% for *MTRR* 66A>G, 100% for *MTR* 2756A>G, 99.5% for *AMPD1* 34C>T, 99.5% for *ITPA* 94C>A, and 100% for *ATIC* 347C>G. Genotype distributions were as follows: for *AMPD1* 34C>T, 74% CC, 25% CT, 1% TT; for *MTRR* 66A>G, 20% AA, 53% AG, 28% GG; for *MTR* 2756A>G, 70% AA, 27% AG, 2% GG; for *ITPA* 94C>A, 85% CC, 15% CA, 0% AA; and for *ATIC* 347C>G, 47% CC, 45% CG, 8%GG.

Statistical analysis.

Differences in baseline characteristics were analyzed by Student's *t*-test for continuous variables or chi-square test for dichotomous variables. For response and toxicity, differences in genotype distribution were tested by 3 x 2 cross-tabulations for each genotype, and by 2 x 2 cross-tabulations for carriers versus noncarriers, with analysis by 2-sided chi-square test. When genotype distributions differed, we used binary logistic analysis to calculate odds ratios

(ORs) for achieving good response or experiencing adverse drug events. Age and sex were identified as possible confounders and were used as covariates in all regression analyses. The primary efficacy end point was good clinical response (DAS ≤2.4) at 6 months. For classification as having good clinical response based on the DAS, patients had to be available for evaluation at a given time point; no values were carried forward. Secondary end points were good clinical improvement, defined as a change of >1.2 in the DAS, and moderate clinical improvement, defined as a change of >0.6 in the DAS. Additionally, for efficacy analyses, the following possible confounding factors were identified: DAS at baseline, duration of joint symptoms before enrollment, duration of RA before enrollment, rheumatoid factor (RF) positivity, modified Sharp/van der Heijde radiographic score (34) at baseline, ESR, RAI, and C-reactive protein level.
For safety analyses, all patients whose MTX regimen was altered prior to the 6-month followup visit were assessed for adverse drug events after the change in therapy and were included in the safety analyses. Analyses of laboratory measurements were performed for completers only. In the toxicity regression analysis, the following potential confounding factors were tested: body weight, creatinine clearance rate, MTX dosage group (15 mg/week or 25 mg/week), and alcohol use.
All statistical analyses were performed using SPSS 11.5 software (SPSS, Chicago, IL). Since 5 hypotheses were tested, Bonferroni adjustment was performed for multiple comparisons. Both adjusted and unadjusted *P* values were calculated. *P* values less than 0.05 were considered significant.

Results

Patient disposition and baseline characteristics.
DNA samples could be obtained from 205 of the 247 patients randomized to receive MTX monotherapy in the BeSt study. There were no statistically significant differences in baseline characteristics between patients with and those without available DNA samples (data not shown). Baseline demographic and disease characteristics of the 205 RA patients who were genotyped are presented in Table 1. The reported ethnicity distribution in the study population was 93% Caucasian (n = 191), 2.4% Asian (n = 5), 1.0% African (n = 2), and 3.4% other (n = 3 Hindustani, 3 Surinamese, 1 Israeli). All results remained similar when performed with and without inclusion of non-Caucasian patients.

Characteristics	Baseline value
Demographic	
Gender [female / male %]	68.8 / 31.2
Age [years] (sd)	54.6 (±13.3)
RF positivity [%]	67.3
Disease duration in weeks [median] (range)	2.0 (0-104.7)
Measures of disease activity	
Duration of joint complaints in weeks [median] (range)	25.0 (1.1-584.3)
DAS (sd)	4.5 (± 0.8)
ESR [median mm/hr] (range)	38 (2 - 143)
CRP [median mg/L] (range)	23 (0 - 238)
RAI [median] (range)	13 (2 - 47)

Swollen joints [median] (range)	13 (3 - 36)
Sharp van der Heijde score [median] (range)	4 (4 - 49.5)

Table 1. Baseline demographic and disease characteristics among the 205 patients with genotyping data

Abbreviation(s): DAS= Disease Activity Score in 44 joints, ESR= Erythrocyte Sedimentation Rate, RF= Rheumatoid factor, CRP= C-reactive protein, RAI=Ritchie Articular Index.

Association of *AMPD1* 34C>T, *ATIC* 347C>G, and *ITPA* 94C>A polymorphisms with good clinical response to MTX therapy.

At 6 months, 186 patients remained in the study, of whom 47% had a good clinical response (DAS ≤2.4) (n = 87) (Figure 2). Among these responders, 43% were receiving MTX 15 mg weekly and 57% were receiving MTX 25 mg weekly.

Three of the 5 selected genetic polymorphisms were associated with good clinical response at 6-month followup (Figure 3). Patients carrying the *AMPD1* T allele were 2)1 times more likely to achieve good clinical response when compared with patients possessing the *AMPD1* CC variant. For *ATIC* and *ITPA,* associations between the CC genotype and good clinical response were found (Figure 3). The numbers and percentages of responders by genotype are presented in Table 2).

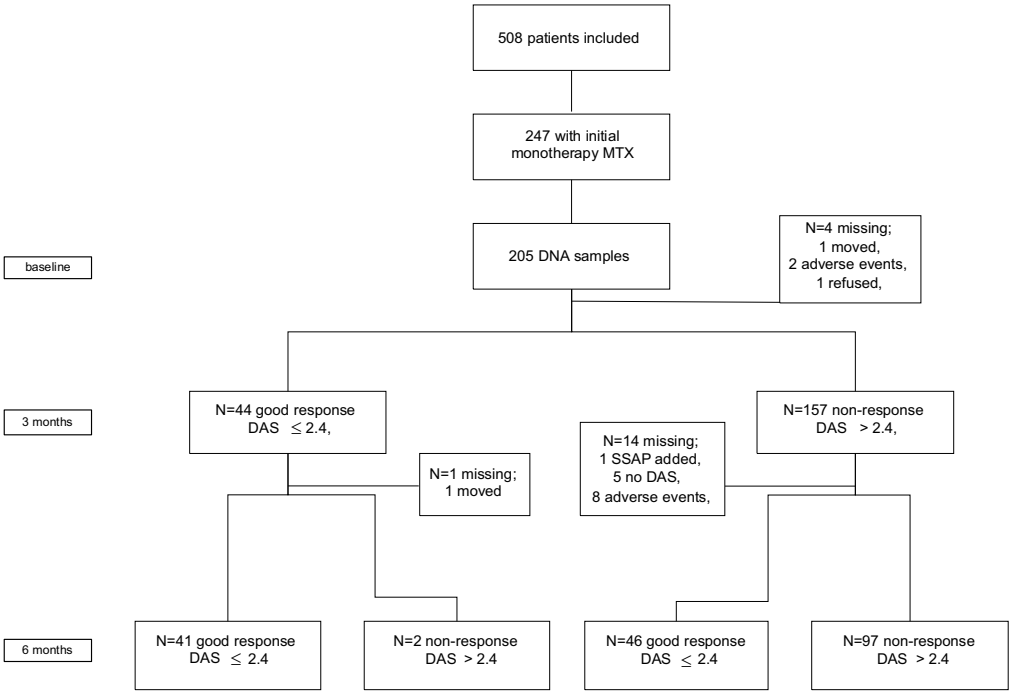

Figure 2. Disposition of the patients

Abbreviation(s): MTX= methotrexate; DAS= Disease Activity Score; SSAP= sulfasalazine.

To assess whether these 3 favorable polymorphisms showed an additive effect with regard to response to MTX therapy, additional analyses were performed for each combination of the *AMPD1*, *ATIC*, and *ITPA* genotypes. Among patients carrying the combinations *AMPD1* T allele and *ATIC* CC (n = 22), *AMPD1* T allele and *ITPA* CC (n = 41), and *ATIC* CC and *ITPA* CC (n = 82), the percentages with good clinical response at 6 months increased to 68%, 63%, and 56%, respectively. Among the 16 patients who carried all 3 favorable genotypes, 88% achieved a good clinical response. Logistic regression analyses revealed that the OR for achievement of good clinical response in this group was 27.8) The explained variance (R^2) of these combined favorable genotypes for MTX treatment response was 24.2% (Figure 3). In contrast, if patients carried all 3 unfavorable genotypes, i.e., the *AMPD1* CC and *ITPA* CA genotypes and the *ATIC* G allele (n = 10), the response rate at 6 months was only 10%.

	AMPD1			*ATIC*			*ITPA*	
	CC	**CT**	**TT**	**CC**	**CG**	**GG**	**CC**	**CA**
Population	151 (73%)	50 (24%)	3 (2%)	97 (47%)	92 (45%)	16 (8%)	174 (85%)	30 (15%)
Good clinical response at six months	57 (38%)	28 (56%)	1 (33%)	51 (53%)	30 (33%)	6 (38%)	79 (45%)	7 (23%)
Methotrexate 15 mg weekly	25/36 (69%)	15/15 (100%)	1/1 (100%)	22/25 (88%)	15/22 (68%)	4/5 (80%)	38/47 (81%)	3/5 (60%)
Methotrexate 25 mg weekly	30/98 (31%)	13/29 (45%)	0/2 (0%)	28/61 (46%)	14/60 (23%)	2/9 (22%)	39/107 (36%)	4/22 (18%)
Adverse drug events at six months	42/146 (29%)	16/50 (32%)	1/3 (33%)	21/94 (22%)	33/91 (36%)	6/15 (40%)	51/169 (30%)	8/30 (27%)

Table 2. Methotrexate response and adverse drug events at 6 months by AMPD1, ATIC and ITPA genotypes[a,b,c,d]

a. *MTR* and *MTRR* were not associated with methotrexate (MTX) efficacy or toxicity. Values are the number [%].
b. Genotype data missing on 1 of the 205 patients.
c. Data on MTX dosage missing on 2 of the 87 responders at 6 months.
d. Abbreviation(s): *AMPD1* = adenosine monophosphate deaminase, *ATIC* = aminoimidazole carboxamide ribonucleotide transformylase, *ITPA* = inosine triphosphate pyrophosphatase.

After adjustment for multiple comparisons, the association of the *ATIC* CC genotype with MTX response remained significant *(P* = 0.035), and the combination of favorable *AMPD1*, *ATIC*, and *ITPA* genotypes remained significantly associated with good clinical response (Figure 3). The regression analysis using the parameter good clinical improvement as opposed to good clinical response also revealed an association with the *ATIC* CC genotype in comparison with G allele carriers (OR 2.5 [95% confidence interval 1.3– 4.8], *P* = 0.007). No associations between the *MTRR* and *MTR* polymorphisms and good clinical response were found (data not shown).
In the regression analysis to predict good clinical response, only DAS at baseline and RF positivity appeared to be significant predictive factors (Figure 3). Patients who had a lower DAS at baseline and/or were RF negative were more likely to show good clinical response at 6 months. We also investigated whether the possible confounding factors were affected by genotype; no significant associations between the possible confounding factors examined and genotype variants were observed.

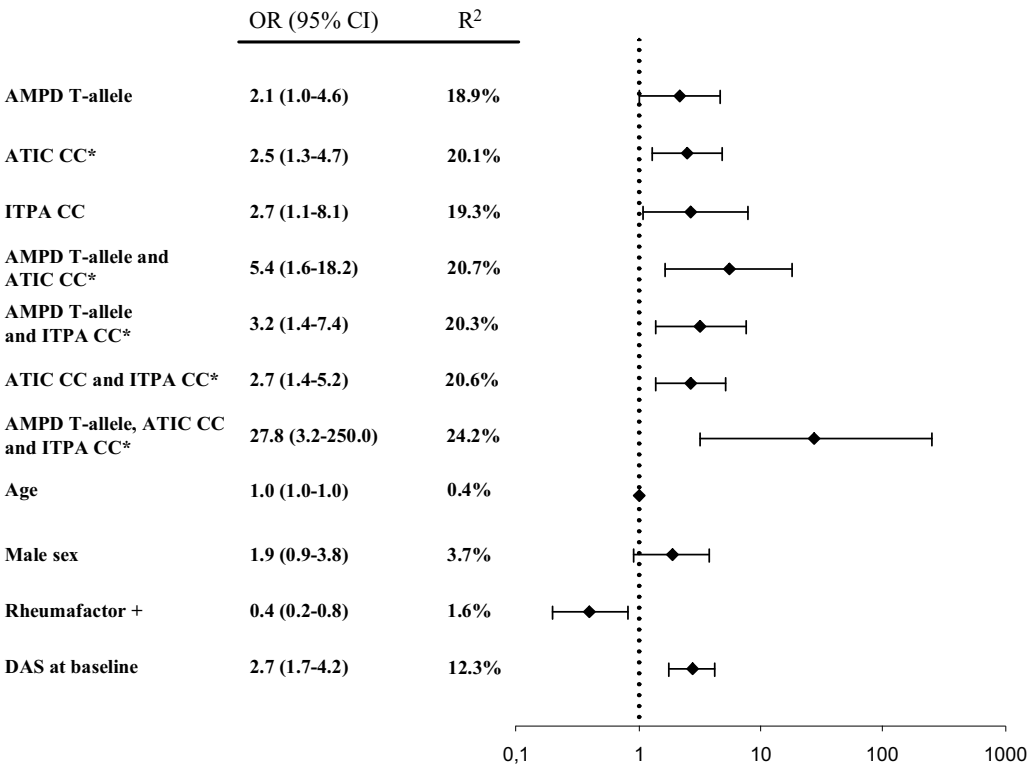

Figure 3. Associations between *AMPD1* 34C>T, *ATIC* 347C>G, and *ITPA* 94C>A polymorphism and good clinical response to methotrexate[a,b,c]

a. Data presented are odds ratios (ORs) (diamonds), 95% confidence intervals (95% CIs) (bars), and R^2 values with correction for the potential confounding factors of age, sex, rheumatoid factor (Rheumafactor) positivity, and Disease Activity Score (DAS) at baseline.
b. Odds ratios presented for age, sex, rheumatoid factor positivity, and DAS at baseline are results found without inclusion of genotypes as independent variables.
c. * P< 0.05 after Bonferroni adjustment.

Safety findings.

Safety data were available on 200 patients at 6 months; 4 patients did not return for the 6-month followup visit and 1 patient had moved away. Thirty percent of these patients (n = 60) experienced at least 1 adverse drug event during 6 months of treatment (Table 3). The percentage of patients experiencing an adverse drug event was similar in both dosage groups, although more patients receiving MTX 25 mg per week discontinued therapy due to adverse drug events.

During 6 months of treatment, patients carrying the *ATIC* G allele were twice as likely to experience any adverse drug event compared with patients without the allele (Figure 4). However, after adjustment for multiple comparisons, the association between the *ATIC* G allele and adverse drug events did not remain significant. No other associations with MTX-induced adverse events were identified. In the logistic regression analysis, none of the identified potential confounding factors was predictive of adverse drug events.

Adverse Drug Event	Frequency at 6 months
Skin and mucosa disorders	17 (8.5%)
Pneumonitis	0 (0%)
Hepatic elevated liverenzymes	16 (8%)
Gastrointestinal (general wellbeing, nausea, vomiting,diarrhoea, constipation)	26 (13.0%)
Overall adverse drug events in total population	60 (30%)

Table 3. Number of patients (percentage) with adverse drug events during six months of treatment

Values for overall adverse drug events are the number (%) of patients experiencing 1 event; values for the individual types of event are the number of events (% of patients).

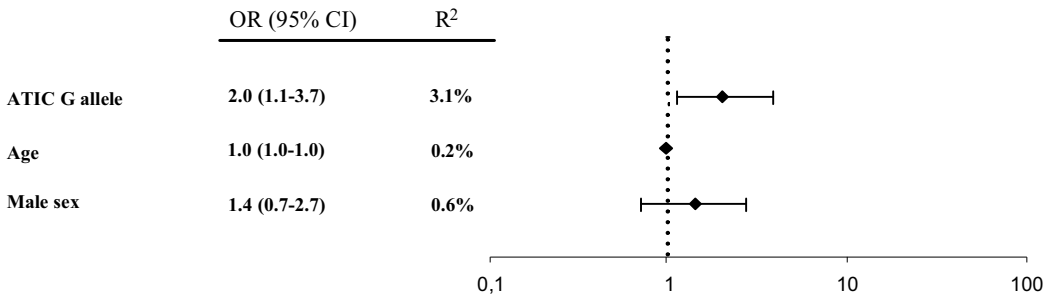

Figure 4. Association between *ATIC* 347C>G polymorphism and the occurrence of adverse drug events during 6 months of methotrexate therapy

Data presented are odds ratios (ORs) (diamonds), 95% confidence intervals (95% CIs) (bars), and R^2 values with correction for the potential confounding factors of age and sex.

We also examined the interaction between achievement of good clinical response (DAS ≤2.4) at 6 months, the *AMPD1*, *ATIC*, and *ITPA* genotypes, and the occurrence of adverse drug events. Responders at 6 months (n = 87) were selected, and regression analyses were performed. In general, patients with good clinical response at 6 months experienced fewer adverse drug events compared with nonresponders (OR 0.45, 95% confidence interval 0.22–0.91). This finding was also observed in nonresponders carrying the *ATIC* G allele; the OR of adverse drug events was increased from 2.0 to 2.8 (95% confidence interval 1.1–7.5) in this group.

For responders carrying the *AMPD1* T allele, the single *ATIC* CC or the single *ITPA* CC genotype, or combinations of these genotypes, no associations with the occurrence of adverse drug events were

found. The numbers and percentages of patients experiencing adverse drug events by genotype for *AMPD1*, *ATIC*, and *ITPA* are presented in Table 2)

Discussion

Results of this analysis show an association between allelic variants in the adenosine monophosphate deaminase (AMPD), 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase, and inosine triphosphate pyrophosphatase (ITPA) genes and clinical response to MTX therapy in patients with recent-onset RA. Patients carrying the *AMPD1* T allele, the *ATIC* CC genotype, or the *ITPA* CC genotype are 2–3 times more likely to have a good clinical response, defined by a DAS of ≤2.4, following 6 months of MTX therapy. Additionally, the rate of good clinical response is increased substantially in patients carrying the 3 favorable genotypes.

With regard to the occurrence of adverse drug events, the only association found was with the *ATIC* G allele. This association was not significant after adjustment for multiple testing. No associations between methionine synthase or methionine synthase reductase and MTX efficacy or toxicity were found.

Previously, only the contribution of the *ATIC* 347C>G polymorphism has been studied in relation to the efficacy and safety ofMTX. In 2 articles, Dervieux et al report that RA patients with a higher mutation index respond better to MTX therapy (15,35). This composite mutation index was calculated for each patient by summing the scores for 3 SNPs in different genes, including *ATIC*. The patients with a higher mutation index showed a linear decline in the number of tender and swollen joints, the physician's global assessment of disease activity on a VAS, and the Health Assessment Questionnaire (36). Furthermore, it was suggested that patients with the *ATIC* 347GG genotype had an increased likelihood of response to MTX treatment. In addition, similar to our findings, Weisman et al showed that patients with the *ATIC* 347GG genotype more frequently experienced side effects overall, and specifically, gastrointestinal adverse drug events (37).

It is difficult to compare these findings with our results since study designs and data analysis differ. We chose to assess the contribution of genetic markers predictive of treatment outcome in the BeSt study because that study had clear and objective outcome measures and standardized treatment regimens in a well-described population of patients with recent-onset RA (26). Multivariate data analysis with Bonferroni adjustment for multiple comparisons was performed after 6 months of treatment, with controlling for identified confounders of response. Moreover, the selected patients were all treated with MTX monotherapy for an identical time period, and had not used any DMARDs prior to enrollment.

In contrast, other investigations have used crosssectional study designs with variable disease durations, MTX dosages, and treatment durations (15,35,37). In one study, combination DMARD therapy was allowed (37). Cross-sectional analyses reflect rheumatology practice, but population stratification may have occurred by selecting patients who are still being treated with MTX. With the design of the present study, the influence of sequential monotherapy and other possible confounders of treatment outcome is excluded.

The association of *ATIC* 347GG with side effects was established without controlling for confounders (37). The associations of clinical efficacy and overall toxicity with higher pharmacogenetic indexes were found in multivariate analysis in which other factors were included (15,35,37), but the composite mutation indexes used were calculated with grouping of different genotypes in 2 of the 3 studies. Moreover, the pharmacogenetic index calculation is based on the assumption that the contribution of every polymorphism is small, but that every polymorphism affects the response in the same direction with an equal, additive value. However, there are no data that support this assumption. In summary, different study designs and statistical methods should be taken into account in comparing results from different pharmacogenetic studies. We believe our results are more applicable to patients with recent-onset, non–DMARD-treated RA.

As with most genetic studies, the current study was not sufficiently powered to derive definitive conclusions. Further, while adjusting our results for multiple testing minimized false-positive associations, it also increased the chance of Type II error due to the conservative nature of the Bonferroni adjustment (38,39). Accordingly, we have presented both adjusted and unadjusted results.

Our primary efficacy parameter was good clinical response at 6 months of MTX treatment; in other reports, remission has been described as the primary goal of therapy (7,40,41). To examine whether the identified genotypes for good clinical response at 6 months were also predictive of remission at 1 year of followup, an additional analysis of patients carrying the *ATIC* CC genotype was performed. Results of this analysis showed that in 35% of the 97 patients carrying the *ATIC* CC genotype, disease was in remission, defined as a DAS <1.6, at 1 year; previous reports have indicated that remission has been achieved at 1 year in 10–25% of patients receiving MTX (8,42). This observation thus indicates that this variant may be associated with a prolonged and increased clinical response.

Our data showed that MTX therapy was less beneficial for *ATIC* G allele carriers, *ITPA* A allele carriers, and patients with the *AMPD1* CC genotype. While 47% of the overall population exhibited good clinical response at 6 months, comparison of good clinical response among allelic variants showed that the response percentages were 58% in patients with the *ATIC* CC genotype and 37% in *ATIC* G allele carriers. Also, good clinical response was achieved with 6 months of MTX therapy in 50% of the patients with the *ITPA* CC genotype compared with 26% of the *ITPA* A allele carriers, and by 60% of the *AMPD1* T allele carriers compared with 42% of the patients with the *AMPD1* CC genotype.

These findings suggest that pharmacogenetic testing before initiation of therapy may help to guide clinical treatment decisions, for example, in identifying patients with all 3 favorable genotypes, in whom MTX treatment is more likely to be efficacious. As another example of such clinical use, we analyzed the patients with all 3 unfavorable genotypes, i.e., the ATIC G allele, the ITPA A allele, and the AMPD1 CC genotype. In patients with these genotypes, other DMARD therapy may be chosen rather than MTX, because their response rate at 6 months was only 10%. Thus, such pharmacogenetic testing could avoid ineffective treatment and, at the same time, indicate high potential for effective therapy in 14% of the RA population.

Ideally, our findings regarding the effect of genetic variants in *AMPD1*, *ITPA*, and *ATIC* genes on MTX treatment outcome should be replicated and prospectively tested in a randomized controlled study comparing clinical response in 2 groups of patients (43,44). In such a study, patients in the first group would receive standard MTX treatment. In the second group, the pharmacogenetic test results would dictate whether patients receive standard MTX treatment (patients with the favorable genotypes) or other DMARDs (patients without the favorable genotypes).

The polymorphisms tested were selected based on the hypothesis that the mechanism of action of MTX is related to adenosine release (Figure 1). The enzymes whose genetic polymorphisms were studied relate to adenosine and were chosen because in vitro studies showed that polymorphisms altered their enzyme function or expression. Moreover, other reports have indicated the clinical relevance of these SNPs in different complex traits (15,21–25). Although the effect of variant alleles in relation to cellular adenosine homeostasis has not yet been explored, several in vitro effects have been shown (32,33,45–48).

Adenosine is thought to mediate the antirheumatic effects of MTX via adenosine receptor signaling (48–50). Binding of this compound to specific receptors enhances the antiinflammatory properties of MTX. The *AMPD1* 34C>T mutation generates an AMPD enzyme with lower activity (32). *AMPD1* catalyzes the conversion of AMP to inosine monophosphate (IMP). Alternatively, AMP is converted to adenosine. Thus, deficiency of *AMPD1* could enhance adenosine release. In addition, both *ITPA* and *ATIC* may lead to formation of adenosine. ITPA polymorphisms have been shown to lead to ITPA deficiency. ITPA catalyzes the conversion of ITP to IMP, whereas ITP is formed by phosphory-

lation of IMP. Deficiency of ITPA interrupts this cycle and possibly nfluences its balance with AMP and adenosine (33). Furthermore, MTX inhibits *ATIC*. This leads to cellular accumulation of AICAR, a nucleoside precursor (18,24). AICAR inhibits adenosine deaminase, which results in reduced conversion of adenosine to inosine.

Since understanding of these enzymes, their substrates, and interactions remains imprecise, no conclusions about the mechanism of action of MTX in relation to adenosine release can be drawn. Nevertheless, our results strongly indicate that MTX therapy works via the adenosine pathway. Moreover, we have confirmed that the genetic profile of RA patients is indeed a determinant of response to MTX treatment (15,16,45).

In summary, results of this analysis identify patients with adenosine genotypes who are most likely to achieve good clinical response with MTX. Findings of our pharmacogenetic analysis identified markers in the *ATIC*, *ITPA*, and *AMPD1* genes that may assist the rheumatologist in making clinical treatment decisions for patients with recent-onset RA.

References

(1) Anderson JJ, Wells G, Verhoeven AC, Felson DT. Factors predicting response to treatment in rheumatoid arthritis: the importance of disease duration. *Arthritis Rheum* 2000;43:22–9.

(2) Pincus T. Long-term outcomes in rheumatoid arthritis. *Br J Rheumatol* 1995;34 Suppl 2:59–73)

(3) Aletaha D, Smolen JS. The rheumatoid arthritis patient in the clinic: comparing more than 1,300 consecutive DMARD courses. *Rheumatology* (Oxford) 2002;41:1367–74)

(4) Smolen JS, Aletaha D, Machold KP. Therapeutic strategies in early rheumatoid arthritis. *Best Pract Res Clin Rheumatol* 2005;19:163–77.

(5) O'Dell JR, Haire CE, Erikson N, Drymalski W, Palmer W, Eckhoff PJ, et al. Treatment of rheumatoid arthritis with methotrexate alone, sulfasalazine and hydroxychloroquine, or a combination of all three medications. *N Engl J Med* 1996;334:1287–91)

(6) Pincus T, Yazici Y, Sokka T, Aletaha D, Smolen JS. Methotrexate as the "anchor drug" for the treatment of early rheumatoid arthritis. *Clin Exp Rheumatol* 2003;21(5 Suppl 31):S179–85.

(7) Klareskog L, van der Heijde D, de Jager JP, Gough A, Kalden J, Malaise M, et al. Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. *Lancet* 2004;363:675–81)

(8) Mottonen T, Hannonen P, Leirisalo-Repo M, Nissila M, Kautiainen H, Korpela M, et al and the FIN-RACo trial group. Comparison of combination therapy with single-drug therapy in early rheumatoid arthritis: a randomised trial. *Lancet* 1999;353:1568–73)

(9) Genovese MC, Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, et al. Etanercept versus methotrexate in patients with early rheumatoid arthritis: two-year radiographic and clinical outcomes. *Arthritis Rheum* 2002;46:1443–50.

(10) Matteson EL, Weyand CM, Fulbright JW, Christianson TJ, Mc-Clelland RL, Goronzy JJ. How aggressive should initial therapy for rheumatoid arthritis be? Factors associated with response to 'non-aggressive' DMARD treatment and perspective from a 2-yr open label trial [published erratum

appears in Rheumatology (Oxford) 2004;43:1065]. *Rheumatology* (Oxford) 2004;43:619–25.

(11) Verstappen SM, Jacobs JW, Bijlsma JW, Heurkens AH, van Booma-Frankfort C, ter Borg EJ, et al. Five-year followup of rheumatoid arthritis patients after early treatment with disease- modifying antirheumatic drugs versus treatment according to the pyramid approach in the first year. *Arthritis Rheum* 2003;48: 1797–807.

(12) Evans WE, McLeod HL. Pharmacogenomics—drug disposition, drug targets, and side effects. *N Engl J Med* 2003;348:538– 49.

(13) Rocha JC, Cheng C, Liu W, Kishi S, Das S, Cook EH, et al. Pharmacogenetics of outcome in children with acute lymphoblastic leukemia. *Blood* 2005;105:4752–8.

(14) Israel E, Chinchilli VM, Ford JG, Boushey HA, Cherniack R, Craig TJ, et al. Use of regularly scheduled albuterol treatment in asthma: genotype-stratified, randomised, placebo-controlled cross-over trial. *Lancet* 2004;364:1505–12)

(15) Dervieux T, Furst D, Lein DO, Capps R, Smith K, Walsh M, et al. Polyglutamation of methotrexate with common polymorphisms in reduced folate carrier, aminoimidazole carboxamide ribonucleotide transformylase, and thymidylate synthase are associated with methotrexate effects in rheumatoid arthritis. *Arthritis Rheum* 2004;50:2766–74)

(16) Urano W, Taniguchi A, Yamanaka H, Tanaka E, Nakajima H, Matsuda Y, 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.

(17) Wessels JA, de Vries-Bouwstra J, Heijmans BT, Slagboom PE, Goekoop-Ruiterman YP, Allaart CF, et al. Efficacy and toxicity of methotrexate in early rheumatoid arthritis are associated with single-nucleotide polymorphisms in genes coding for folate path- way enzymes. *Arthritis Rheum* 2006;54:1807–96.

(18) Cronstein BN. Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis [review]. *Pharmacol Rev* 2005;57:163–72)

(19) Nakamachi Y, Koshiba M, Nakazawa T, Hatachi S, Saura R, Kurosaka M, et al. Specific increase in enzymatic activity of adenosine deaminase 1 in rheumatoid synovial fibroblasts. *Arthritis Rheum* 2003;48:668–74)

(20) Riksen NP, Barrera P, van den Broek PH, van Riel P, Smits P, Rongen G. Methotrexate modulates the kinetics of adenosine in humans in vivo. *Ann Rheum Dis* 2006;65:465–70.

(21) Kalsi KK, Yuen AH, Rybakowska IM, Johnson PH, Slominska E, Birks EJ, et al. Decreased cardiac activity of AMP deaminase in subjects with the AMPD1 mutation: a

potential mechanism of protection in heart failure. *Cardiovasc Res* 2003;59:678–84)

(22) Bosco P, Gueant-Rodriguez RM, Anello G, Barone C, Namour F, Caraci F, et al. Methionine synthase (MTR) 2756 (A>G) polymorphism, double heterozygosity methionine synthase 2756AG / methionine synthase reductase (MTRR) 66AG, and elevated homocysteinemia are three risk factors for having a child with Down syndrome. *Am J Med Genet* 2003;121:219–24

(23) Gaughan DJ, Kluijtmans LA, Barbaux S, McMaster D, Young IS, Yarnell JW, et al. The methionine synthase reductase (MTRR) A66G polymorphism is a novel genetic determinant of plasma homocysteine concentrations. *Atherosclerosis* 2001;157:451–6.

(24) Marie S, Heron B, Bitoun P, Timmerman T, van den Berghe G, Vincent MF. AICA-ribosiduria: a novel, neurologically devastating inborn error of purine biosynthesis caused by mutation of ATIC. *Am J Hum Genet* 2004;74:1276–81)

(25) Marinaki AM, Ansari A, Duley JA, Arenas M, Sumi S, Lewis CM, et al. Adverse drug reactions to azathioprine therapy are associated with polymorphism in the gene encoding inosine triphosphate pyrophosphatase (ITPase). *Pharmacogenetics* 2004;14:181–7.

(26) Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Allaart CF, van Zeben D, Kerstens PJ, Hazes JM, et al.
Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study): a randomized, con- trolled trial. *Arthritis Rheum* 2005;52:3381–90.

(27) Arnett FC, Edworthy SM, Bloch DA. McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24)

(28) Van Gestel AM, Prevoo ML, van 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis: comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. *Arthritis Rheum* 1996;39:34– 40.

(29) Van der Heijde DM, van 't Hof M, van Riel PL, van de Putte LB. Development of a disease activity score based on judgment in clinical practice by rheumatologists. *J Rheumatol* 1993;20:579–81)

30) Ritchie DM, Boyle JA, McInnes JM, Jasani MK, Dalakos TG, Grieveson P, et al. Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. *QJM* 1968;37:393– 406.

(31) National Center for Biotechnology Information. URL: www.ncbi. nlm.nih.gov.

(32) Morisaki T, Gross M, Morisaki H, Pongratz D, Zollner N, Holmes EW. Molecular basis of AMP deaminase deficiency in skeletal muscle. *Proc Natl Acad Sci USA* 1992;89:6457–61

(33) Cao H, Hegele RA. DNA polymorphisms in ITPA including basis of inosine triphosphatase deficiency. *J Hum Genet* 2002;47:620–2

(34) Van der Heijde DM, van Riel PL, Nuver-Zwart IH, Gribnau FW, van de Putte LB. Effects of hydroxychloroquine and sulphasalazine on progression of joint damage in rheumatoid arthritis. *Lancet* 1989;1:1036–8.

(35) Dervieux T, Furst D, Orentas Lein D, Capps R, Smith K, Caldwell J, et al. Pharmacogenetic and metabolite measurements are associated with clinical status in rheumatoid arthritis patients treated with methotrexate: results of a multicentred cross sectional observational study. *Ann Rheum Dis* 2005;64:1180–5.

(36) Fries JF, Spitz P, Kraines RG, Holman HR. Measurement of patient outcome in arthritis. *Arthritis Rheum* 1980;23:137– 45.

(37) Weisman MH, Furst DE, Park GS, Kremer JM, Smith KM, Wallace DJ, et al. Risk genotypes in folate-dependent enzymes and their association with methotrexate-related side effects in rheumatoid arthritis. *Arthritis Rheum* 2006;54:607–12

(38) Huizinga TW, Pisetsky DS, Kimberly RP. Associations, populations, and the truth: recommendations for genetic association studies in Arthritis & Rheumatism. *Arthritis Rheum* 2004;50:2066–71

(39) Newton-Cheh C, Hirschhorn JN. Genetic association studies of complex traits: design and analysis issues. *Mutat Res* 2005;573:54–69.

(40) Puolakka K, Kautiainen H, Mottonen T, Hannonen P, Korpela M, Hakala M, et al. Early suppression of disease activity is essential for maintenance of work capacity in patients with recent-onset rheumatoid arthritis: five-year experience from the FIN-RACo trial. *Arthritis Rheum* 2005;52:36– 41

(41) Verstappen SM, Albada-Kuipers GA, Bijlsma JW, Blaauw AA, Schenk Y, Haanen HC, et al. A good response to early DMARD treatment of patients with rheumatoid arthritis in the first year predicts remission during follow up. *Ann Rheum Dis* 2005;64: 38– 43

(42) Gossec L, Dougados M, Goupille P, Cantagrel A, Sibilia J, Meyer O, et al. Prognostic factors for remission in early rheumatoid arthritis: a multiparameter prospective study. *Ann Rheum Dis* 2004;63:675–80.

(43) Hattersley AT, McCarthy MI. What makes a good genetic association study? *Lancet* 2005;366:1315–23

(44) Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. *Nat Genet* 2001;29:306–9.

(45) Morabito L, Montesinos MC, Schreibman DM, Balter L, Thompson LF, Resta R, et al. Methotrexate and sulfasalazine promote adenosine release by a mechanism that requires ecto-5 -nucleotidase-mediated conversion of adenine nucleotides. *J Clin Invest* 1998;101:295–300.

(46) Cronstein BN, Eberle MA, Gruber HE, Levin RI. Methotrexate inhibits neutrophil function by stimulating adenosine release from connective tissue cells. *Proc Natl Acad Sci USA* 1991;88:2441–5.

(47) Delano DL, Montesinos MC, Desai A, Wilder T, Fernandez P, D'Eustachio P, et al. Genetically based resistance to the antiinflammatory effects of methotrexate in the air-pouch model of acute inflammation. *Arthritis Rheum* 2005;52:2567–75.

(48) Montesinos MC, Desai A, Delano D, Chen JF, Fink JS, Jacobson MA, et al. Adenosine A2A or A3 receptors are required for inhibition of inflammation by methotrexate and its analog MX-68) *Arthritis Rheum* 2003;48:240–7.

(49) Cutolo M, Sulli A, Pizzorni C, Seriolo B, Straub RH. Anti- inflammatory mechanisms of methotrexate in rheumatoid arthritis. *Ann Rheum Dis* 2001;60:729–35.

(50) Chan ES, Cronstein BN. Molecular action of methotrexate in inflammatory diseases [review]. *Arthritis Res* 2002;4:266–73