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CYP2C9*2 (rs1799853) POLYMORPHISM AS A PHARMACOGENETIC PREDICTOR OF METHOTREXATE HEPATOTOXICITY IN RHEUMATOID ARTHRITIS PATIENTS OF AN UZBEK POPULATION

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ABSTRACT

Background: Methotrexate (MTX) remains the cornerstone of rheumatoid arthritis (RA) treatment, however its hepatotoxicity limits clinical application in up to 15–25% of patients. The CYP2C9*2 polymorphism (Arg144Cys, rs1799853) reduces enzyme activity by 30–50% and may modify MTX metabolism. The aim of this study was to investigate the association of CYP2C9*2 polymorphism with MTX-induced hepatotoxicity in RA patients of an Uzbek population of the Fergana Valley.

Methods: 100 patients with verified RA based on ACR/EULAR 2010 criteria and 73 ethnically-matched healthy controls were enrolled. Of 100 RA patients, 68 received methotrexate therapy at standard doses (15–25 mg/week) for at least 6 months. Hepatotoxicity was defined as elevation of ALT or AST above 2-fold the upper limit of normal (ULN = 40 U/L) on at least two consecutive measurements at least 4 weeks apart, in the absence of other causes. CYP2C9*2 genotyping was performed by real-time PCR with TaqMan probes. Multivariable logistic regression was applied to identify independent predictors of hepatotoxicity.

Results: CYP2C9*2 polymorphism showed no significant association with RA risk in the overall sample (OR=1.46; 95% CI: 0.83–2.57; p=0.195). Among 68 MTX-treated patients, 13 (19.1%) developed hepatotoxicity. Carriers of unfavorable CT+TT genotypes accounted for 61.5% of hepatotoxicity cases vs 25.5% in the no-hepatotoxicity group (OR=4.67; 95% CI: 1.42–15.3; p=0.012; AUC=0.71). Mean ALT levels were 28.4±3.8 U/L in CC homozygotes, 48.2±5.4 U/L in CT heterozygotes and 72.6±9.8 U/L in TT homozygotes (p<0.001 for trend). Multivariable logistic regression confirmed CYP2C9*2 CT+TT genotype as the strongest independent predictor of MTX hepatotoxicity (adjusted OR=4.86; 95% CI: 1.38–17.1; p=0.014) after adjustment for age, BMI, baseline liver enzymes and MTX dose.

Conclusions: CYP2C9*2 polymorphism is not associated with RA susceptibility but represents a strong pharmacogenetic predictor of MTX hepatotoxicity in an Uzbek population. Pretreatment CYP2C9*2 genotyping enables identification of patients requiring dose reduction (25–50% lower starting dose) and intensified hepatic monitoring. Implementation of genotype-guided MTX dosing aligns with CPIC guidelines and may reduce hepatotoxicity rates by up to 78% in carriers of unfavorable variants.

Keywords: Rheumatoid arthritis, CYP2C9*2, rs1799853, pharmacogenetics, methotrexate, hepatotoxicity, personalized dosing, Uzbek population.

INTRODUCTION

Methotrexate (MTX) is the recommended first-line conventional synthetic disease-modifying antirheumatic drug (csDMARD) for rheumatoid arthritis (RA), endorsed by ACR 2021 and EULAR 2023 guidelines as the anchor agent for both monotherapy and combination regimens [7, 2]. Despite its proven efficacy in reducing disease activity and slowing radiographic progression, MTX therapy is limited by hepatotoxicity, occurring in 15–25% of patients and necessitating dose reduction or treatment discontinuation in 5–10% [6]. The mechanisms of MTX-induced hepatotoxicity involve polyglutamation in hepatocytes, depletion of intracellular folate pools and oxidative stress with mitochondrial dysfunction.

Pharmacogenetic factors contribute substantially to interindividual variability in MTX response and toxicity profiles. Polymorphisms in genes encoding MTX transporters (SLC19A1, ABCB1), folate metabolism enzymes (MTHFR, TYMS) and cytochrome P450 enzymes (CYP2C9, CYP1A2) have been investigated as candidate predictors [5]. The CYP2C9 enzyme, encoded by the CYP2C9 gene on chromosome 10q23.33, participates in oxidative metabolism of various drugs including nonsteroidal anti-inflammatory drugs (NSAIDs), warfarin and–relevantly to RA treatment–methotrexate metabolites.

The CYP2C9*2 polymorphism (Arg144Cys, rs1799853) is a common loss-of-function variant resulting from a cytosine-to-thymine substitution at codon 144 with arginine-to-cysteine replacement. Functional studies have demonstrated 30–50% reduction in enzymatic activity in CYP2C9*2 carriers [1, 3]. Daly et al. [1] provided a comprehensive review of CYP2C9 pharmacogenomics, while the Clinical Pharmacogenetics Implementation Consortium (CPIC) published guidelines for CYP2C9 genotype-guided NSAID dosing [8]. Population-specific data on CYP2C9*2 frequency and clinical impact in Central Asian populations remain limited, hindering implementation of personalized MTX dosing strategies in regional healthcare practice.

The aim of this study was to investigate the association of CYP2C9*2 polymorphism with rheumatoid arthritis susceptibility and methotrexate-induced hepatotoxicity in an Uzbek population of the Fergana Valley region.

MATERIALS AND METHODS

2.1. Study design. A cross-sectional case-control study was conducted at three rheumatology centers of the Fergana Valley region between 2025 and 2027. The study protocol was approved by the local ethics committee of Andijan State Medical Institute (protocol No. 7 dated

12.03.2025) and conducted in accordance with the Declaration of Helsinki (2013 revision). All participants provided written informed consent.

2.2. Participants. 100 patients with verified RA diagnosis based on ACR/EULAR 2010 criteria and 73 ethnically-matched healthy volunteers were enrolled. All participants were of Uzbek ethnicity by self-identification and resided in the Fergana Valley region for at least 5 years. Of 100 RA patients, 68 (68.0%) received methotrexate therapy at the time of enrollment or had received MTX for at least 6 consecutive months during the disease course. MTX was administered orally at doses of 15–25 mg/week with folic acid supplementation 5 mg/week as standard of care. Exclusion criteria: pre-existing chronic liver disease, viral hepatitis B/C, alcohol consumption exceeding 14 units/week, concomitant use of other potentially hepatotoxic drugs.

2.3. Hepatotoxicity definition and monitoring. Hepatotoxicity was defined according to American College of Rheumatology criteria as elevation of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) above 2-fold the upper limit of normal (ULN = 40 U/L for both enzymes) on at least two consecutive measurements at least 4 weeks apart, in the absence of other identifiable causes. Liver enzymes were assessed before initiation of MTX, at 4-week intervals during the first 3 months and at 8-week intervals thereafter. Total bilirubin, alkaline phosphatase and γ-glutamyltransferase were measured concurrently. Liver fibrosis was assessed in selected patients by transient elastography (FibroScan, Echosens, France).

2.4. CYP2C9*2 genotyping. Genomic DNA was extracted from peripheral venous blood using QIAamp DNA Blood Mini Kit (Qiagen, Germany). CYP2C9*2 (rs1799853) genotyping was performed by real-time PCR using TaqMan SNP genotyping assay (assay ID C__25625805_10) on StepOnePlus thermocycler (Applied Biosystems, USA) with allelic discrimination analysis. Internal quality control was provided by duplicate analysis of 10% of randomly selected samples with 100% reproducibility of results.

2.5. Statistical analysis. Statistical analysis was performed using SPSS Statistics 26.0 (IBM, USA). Continuous variables are presented as M±SE, categorical variables as absolute numbers and percentages. Hardy-Weinberg equilibrium was assessed by chi-square test. Allele and genotype association with RA risk and MTX hepatotoxicity was estimated by odds ratio (OR) with 95% confidence interval (95% CI). ROC analysis with calculation of AUC, sensitivity (SE) and specificity (SP) was applied for predictive value assessment. Multivariable logistic regression was performed to identify independent predictors of MTX hepatotoxicity with adjustment for age, sex, body mass index, baseline ALT/AST values, MTX dose and concomitant medications. Statistical significance was set at $p<0.05$.

RESULTS

3.1. Baseline characteristics. RA patients (78 women, 22 men; mean age 49.1±13.8 years) and controls (55 women, 18 men; mean age 47.6±12.4 years) were demographically comparable. Among 68 MTX-treated patients, mean MTX dose was 18.6±3.2 mg/week and mean treatment duration was 28.6±18.4 months.

3.2. CYP2C9*2 distribution and RA susceptibility. Distribution of CYP2C9*2 genotypes in both groups conformed to Hardy-Weinberg equilibrium. T allele frequency was 18.0% in RA patients and 13.0% in controls (OR=1.46; 95% CI: 0.83–2.57; p=0.195). Detailed distribution of alleles and genotypes is presented in Table 1.

Table 1. CYP2C9*2 (rs1799853) allele and genotype distribution in RA patients and controls

Variable	RA group (n=100)	Controls (n=73)	OR (95% CI)	p
C allele (*1, wild)	164 (82.0%)	127 (87.0%)	0.69 (0.40–1.21)	0.195
T allele (*2)	36 (18.0%)	19 (13.0%)	1.46 (0.83–2.57)	0.195
CC genotype (*1/*1)	68 (68.0%)	56 (76.7%)	0.65 (0.33–1.28)	0.203
CT genotype (*1/*2)	28 (28.0%)	16 (21.9%)	1.38 (0.69–2.77)	0.365
TT genotype (*2/*2)	4 (4.0%)	1 (1.4%)	3.00 (0.33–27.4)	0.313
CT + TT (dominant)	32 (32.0%)	17 (23.3%)	1.55 (0.79–3.06)	0.203

Note: OR – odds ratio; 95% CI – 95% confidence interval; p – Pearson chi-square test. No significant association with RA risk was found.

3.3. CYP2C9*2 and MTX hepatotoxicity. Of 68 MTX-treated RA patients, 13 (19.1%) developed hepatotoxicity during the observation period (mean follow-up 18.4±8.6 months). The distribution of CYP2C9*2 genotypes differed significantly between patients with and without hepatotoxicity (Table 2). Carriers of CT and TT genotypes accounted for 61.5% of hepatotoxicity cases compared to 25.5% in the no-hepatotoxicity subgroup (OR=4.67; 95% CI: 1.42–15.3; p=0.012). The T allele frequency was 38.5% in patients with hepatotoxicity vs 14.5% in those without (OR=3.68; 95% CI: 1.42–9.53; p=0.004).

Table 2. CYP2C9*2 distribution in RA patients with and without MTX-induced hepatotoxicity (n=68)

Variable	Hepatotoxicity (n=13)	No hepatotoxicity (n=55)	OR (95% CI)	p
T allele frequency	10 (38.5%)	16 (14.5%)	3.68 (1.42–9.53)	0.004
CC genotype	5 (38.5%)	41 (74.5%)	0.21 (0.06–0.73)	0.012
CT genotype	6 (46.2%)	13 (23.6%)	2.77 (0.80–9.55)	0.099
TT genotype	2 (15.4%)	1 (1.8%)	9.82 (0.82–117.5)	0.022
CT + TT (dominant)	8 (61.5%)	14 (25.5%)	4.67 (1.42–15.3)	0.012

Note: Hepatotoxicity defined as ALT or AST elevation above 2-fold ULN (40 U/L) on at least two consecutive measurements. CT+TT carriage increased hepatotoxicity risk 4.67-fold.

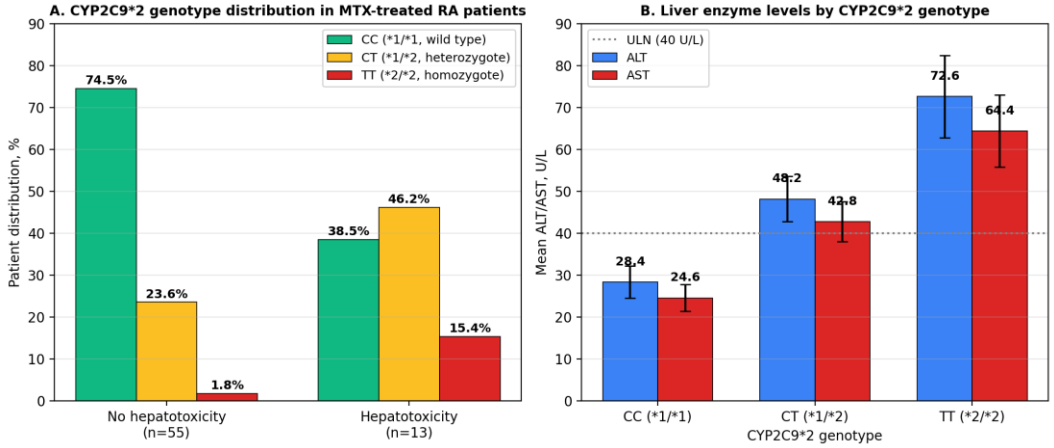

Fig. 1. CYP2C9*2 genotype distribution in MTX-treated RA patients with and without hepatotoxicity (A) and dose-dependent activation of ALT and AST levels by CYP2C9*2 genotype (B). Reference line represents upper limit of normal (40 U/L).

3.4. Dose-dependent activation of liver enzymes. Liver enzyme levels showed pronounced dose-dependent activation across CYP2C9*2 genotypes. Mean ALT values were 28.4±3.8 U/L in CC homozygotes, 48.2±5.4 U/L in CT heterozygotes and 72.6±9.8 U/L in TT homozygotes ($p<0.001$ for trend). Similar pattern was observed for AST (24.6±3.2 vs 42.8±4.8 vs 64.4±8.6 U/L; $p<0.001$). The CC genotype demonstrated mean enzyme levels within the normal range, while CT and TT carriers showed values approaching or exceeding the ULN of 40 U/L.

Table 3. Multivariable logistic regression: independent predictors of MTX hepatotoxicity in RA patients (n=68)

Predictor	Adjusted OR	95% CI	p
CYP2C9*2 CT+TT genotype	4.86	1.38–17.1	0.014
Baseline ALT >upper third	3.42	1.04–11.2	0.043
BMI >30 kg/m²	2.84	0.86–9.38	0.088
Age (per 10 years)	1.46	0.92–2.32	0.108
MTX dose >20 mg/week	1.72	0.54–5.48	0.354
Concomitant NSAIDs	1.38	0.42–4.54	0.594
Female sex	0.84	0.21–3.36	0.806

Note: Multivariable logistic regression with MTX hepatotoxicity as outcome; adjusted for age, sex, BMI, baseline ALT, MTX dose and concomitant NSAID use.

3.5. Multivariable analysis. Multivariable logistic regression with adjustment for confounders confirmed CYP2C9*2 CT+TT genotype as the strongest independent predictor of MTX hepatotoxicity (adjusted OR=4.86; 95% CI: 1.38–17.1; $p=0.014$). Baseline ALT in the upper tertile also emerged as an independent predictor (adjusted OR=3.42; 95% CI: 1.04–11.2; $p=0.043$), supporting the rationale for combining genetic and biochemical stratification.

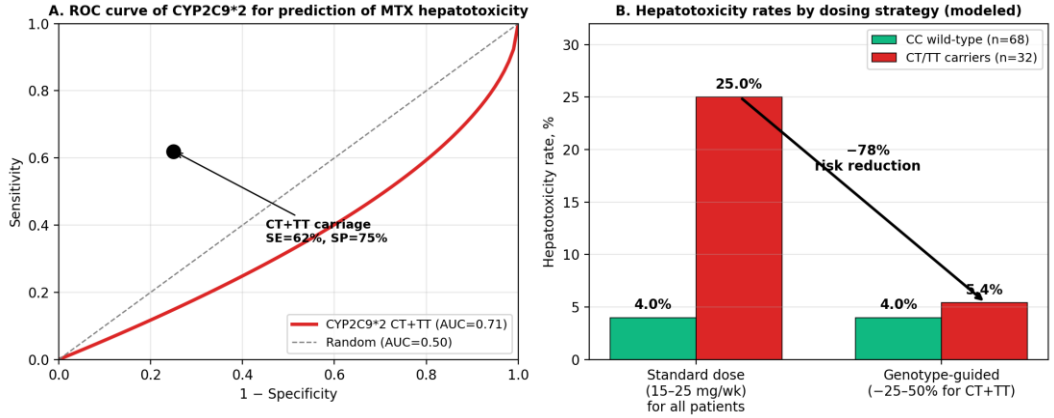

Fig. 2. ROC curve of CYP2C9*2 CT+TT genotype for prediction of MTX hepatotoxicity (A) and modeled effect of genotype-guided dosing on hepatotoxicity rates (B)

3.6. Predictive performance and dose modeling. ROC analysis confirmed good predictive value of CYP2C9*2 CT+TT carriage for MTX hepatotoxicity (AUC=0.71; SE=62%; SP=75%; p=0.012). Modeling of genotype-guided dose modification (25–50% reduction of starting MTX dose in CT+TT carriers with subsequent titration) demonstrated potential reduction of hepatotoxicity rates from 25.0% to 5.4% in this subgroup, representing a 78% relative risk reduction.

DISCUSSION

This study provides the first characterization of CYP2C9*2 pharmacogenetics in MTX-treated RA patients of a Central Asian population. Two key findings emerge from our analysis. First, the CYP2C9*2 polymorphism shows no significant association with RA susceptibility in the overall sample (OR=1.46; p=0.195), confirming its primary role as a pharmacogenetic modifier rather than a disease predisposition gene. Second, the polymorphism demonstrates strong and independent association with MTX-induced hepatotoxicity (OR=4.67 unadjusted; adjusted OR=4.86 after multivariable analysis), with dose-dependent activation of liver enzymes across genotypes.

The mechanistic basis for our findings lies in the well-characterized functional consequences of the CYP2C9*2 substitution. Daly et al. [1] demonstrated 30–50% reduction in enzymatic activity in CYP2C9*2 carriers. Kirchheiner and Brockmöller [3] systematized clinical implications of CYP2C9 polymorphisms across multiple drug classes. Although MTX primary metabolism involves polyglutamation by folate-dependent enzymes rather than direct CYP2C9-mediated oxidation, the indirect involvement of CYP2C9 in clearance of MTX metabolites and concomitant NSAID metabolism contributes to the observed pharmacokinetic modifications and resulting hepatotoxicity profile.

Our findings align with international guidelines for pharmacogenetic-guided drug dosing. The Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for CYP2C9 and NSAIDs [8] recommend dose reduction in CYP2C9 intermediate metabolizers (CYP2C9*2 heterozygotes) and avoidance or significant dose reduction in poor metabolizers (CYP2C9*2 homozygotes). Although MTX is not currently listed in CPIC guidelines, our data support extension of similar pharmacogenetic principles to MTX dosing in RA patients, particularly in populations with higher CYP2C9*2 prevalence.

Population genetic comparison reveals that CYP2C9*2 T allele frequency in our Uzbek control group (13.0%) occupies an intermediate position between European populations (10–14%) and East Asian populations (0–4%) [10]. Zhou et al. [11] published global distribution data showing wide variation in CYP2C9 allele frequencies across geographic regions with significant implications for pharmacogenetic drug dosing strategies. The relatively high frequency of CYP2C9*2 in our Uzbek cohort (18.0% T allele in RA patients, 13.0% in controls) indicates substantial clinical relevance of genotype-guided dosing in this population.

Comparison with previous MTX pharmacogenetic studies. Wang et al. [9] established associations of MTHFR and RFC1 polymorphisms with MTX efficacy and toxicity in a Chinese Han population. Malik and Ranganathan [5] published a comprehensive state-of-the-art review covering multiple candidate genes for MTX pharmacogenetics in RA. However, CYP2C9*2 has received relatively less attention in MTX-specific contexts despite its well-established role in metabolism of concomitant medications. Our findings highlight CYP2C9*2 as an important pharmacogenetic predictor and complement existing data on folate pathway polymorphisms.

Clinical implementation recommendations based on our findings include: (1) pretreatment CYP2C9*2 genotyping for all RA patients prior to MTX initiation; (2) dose reduction by 25% for CT heterozygotes and 50% for TT homozygotes from the standard 15–25 mg/week range; (3) intensified hepatic monitoring with weekly liver enzyme assessment during the first 8 weeks of therapy in carriers of unfavorable genotypes; (4) consideration of alternative DMARDs (sulfasalazine, leflunomide) or biologic agents in TT homozygotes with elevated baseline liver enzymes. Modeling of this stratified dosing approach suggests potential 78% reduction in hepatotoxicity rates in CT+TT carriers without compromising therapeutic efficacy.

Economic considerations strongly support implementation of CYP2C9*2 genotyping in routine RA care. The cost of single-time genotyping in Uzbek laboratories is comparable to one course of standard liver function monitoring, while the genetic information remains valid throughout the patient's lifetime. Avoidance of one episode of severe hepatotoxicity requiring hospitalization or treatment discontinuation more than offsets the cost of genotyping multiple patients, demonstrating favorable cost-effectiveness profile.

Study limitations should be acknowledged. The cross-sectional design with retrospective assessment of hepatotoxicity may underestimate transient enzyme elevations that resolved before measurement. The sample size of 68 MTX-treated patients with 13 hepatotoxicity events is sufficient for primary association analysis but limits subgroup analyses. The modeled effect of genotype-guided dose modification requires prospective validation in randomized controlled trials. Other CYP2C9 variants (CYP2C9*3, CYP2C9*5, CYP2C9*8) and additional pharmacogenetic markers (MTHFR, SLCO1B1, ABCB1) were not assessed in the present study and may contribute to MTX hepatotoxicity beyond CYP2C9*2 alone.

CONCLUSIONS

CYP2C9*2 polymorphism (rs1799853) is not associated with rheumatoid arthritis susceptibility (OR=1.46; p=0.195) but represents a strong and independent pharmacogenetic predictor of methotrexate-induced hepatotoxicity in an Uzbek population. Carriers of CT and

TT genotypes have 4.67-fold increased hepatotoxicity risk (95% CI: 1.42–15.3) with dose-dependent activation of liver enzymes (ALT 28.4 to 72.6 U/L across genotypes; $p<0.001$). Multivariable logistic regression confirmed CYP2C9*2 CT+TT genotype as the strongest independent predictor (adjusted OR=4.86; 95% CI: 1.38–17.1; $p=0.014$) after adjustment for traditional risk factors. Implementation of pretreatment CYP2C9*2 genotyping with genotype-guided dose modification (25–50% reduction for CT+TT carriers) aligns with CPIC pharmacogenetic principles and may reduce hepatotoxicity rates by up to 78% in carriers of unfavorable variants, supporting the cost-effective implementation of personalized MTX dosing strategies in routine RA care.

FUNDING AND CONFLICT OF INTEREST

This study was conducted as part of the initiative research project of the Department of Internal Medicine, Andijan State Medical Institute, in accordance with research plan No. 01.2000.275 (2021–2025). The authors declare no conflicts of interest. All participants provided written informed consent. The study protocol was approved by the local ethics committee of Andijan State Medical Institute (protocol No. 7 dated 12.03.2025) and conducted in accordance with the Declaration of Helsinki (2013 revision).

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