Clin Rheumatol (2016) 35:879–885
DOI 10.1007/s10067-015-2917-y

ORIGINAL ARTICLE

# Reduced folate carrier-1 80G > A gene polymorphism is not associated with methotrexate treatment response in South Indian Tamils with rheumatoid arthritis

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Received: 18 November 2014 / Revised: 24 January 2015 / Accepted: 3 March 2015 / Published online: 15 March 2015


**Abstract** Methotrexate (MTX) is the most commonly used disease-modifying drug to treat rheumatoid arthritis (RA). Although there are no reliable molecular markers to predict the treatment response and adverse effects to MTX therapy, the polymorphisms in genes coding for MTX metabolizing enzymes and transporters may play a crucial role. The reduced folate carrier-1 (*RFC*-1) is a bidirectional anion exchanger which transports MTX and folinic acid. It is reported to influence MTX treatment response and adverse effects in some ethnic populations but not in others. It is also associated with susceptibility to various diseases including systemic lupus erythematosus (SLE). The present study was aimed at investigating the role of *RFC*-1 80G > A gene polymorphism in association with disease susceptibility, MTX treatment response and the MTX-induced adverse events in the South Indian Tamil patients with rheumatoid arthritis. The *RFC*-1 80G > A gene polymorphism was investigated in 327 patients with RA and in 322 healthy controls by PCR-RFLP method. It was found that the heterozygous *RFC*-1 80 GA genotype was associated with protection against RA [$p$=0.02, odds ratio (OR) 0.69, 95 % confidence interval (CI) 0.50–0.95]. However, it was not found to be associated with MTX treatment response. The *RFC-1* G allele frequency was higher in patients with adverse effects, but the difference was not statistically significant ($p$=0.08, OR 1.44, 95 % CI 0.97–2.13). *RFC-1* 80G > A gene polymorphism confers protection for RA. However, it is not associated with MTX treatment response and MTX-induced adverse effects in South Indian Tamil patients with RA.

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**Keywords** Methotrexate · Polyglutamates · RFC-1 polymorphism · Rheumatoid arthritis · South Indian · Treatment response

**Abbreviations**

| | |
|---|---|
| ACPA | Anticitrullinated peptide antibody |
| ACR | American College of Rheumatology |
| AIRE | autoimmune regulator |
| DAS28 | (ESR) score Disease activity score based on 28 joints and erythrocyte sedimentation rate |
| DMARD | Disease-modifying antirheumatic drug |
| HLADR | Human leukocyte antigen |
| MAF | Minor allele frequency |
| MHC2TA | Major histocompatibility complex II transactivator |
| PCR | Polymerase chain reaction |
| RA | rheumatoid arthritis |
| RF | Rheumatoid factor |
| RFC | Reduced folate carrier |
| RFLP | Restriction fragment length polymorphism |

## Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease mainly affecting young and middle-aged females [1]. The primary goal of therapy in RA is to achieve rapid and effective disease control to prevent the long-term damage to joint structure and function [2]. Disease-modifying anti rheumatic drugs (DMARDs) are the mainstay of therapy to control symptoms and modify disease progression in the long term [3]. Methotrexate (MTX) is the most commonly used DMARD and the first-line anchor drug for treating RA. A large interpatient variability is known to

occur in clinical response to MTX [4] as 30 to 40 % of the patients on MTX therapy fail to attain remission [5]. Identifying the genetic predictors of treatment response in RA will help in early institution of alternate or combination DMARD therapy in patients unlikely to respond well to methotrexate monotherapy so as to prevent the consequent morbidity and mortality [6].

MTX enters the cells through an active transport mechanism that is mediated by reduced folate carrier (RFC-1) protein. *RFC*-1 is the dominant, bidirectional, cell membrane transporter molecule for natural folates and folic acid which are essential for a spectrum of biochemical reactions and thus cellular proliferation [7, 8]. The *RFC*-1 gene (OMIM 600424) is located on chromosome 21q22.3. Its product consists of 591 amino acid residues and belongs to the solute carrier (SLC19A1) family of transporters [9].

A common polymorphism at position 80 in exon 2 of RFC-1 results in a change from guanine (G) to an adenine (A), abolishing a Cfo I restriction site [10]. This common nonsynonymous SNP (rs1051266) has a total minor allele frequency of 44 % and is found across all ethnic groups. It has been thoroughly investigated for its function in transport and association with susceptibility to diseases, drug response, and toxicity [11]. A study involving 106 RA patients revealed that the nonwild-type allele of polymorphism in *RFC*-1 -43 T > C was associated with lower *RFC*-1 protein expression, but no association was detected with the treatment response due to smaller sample size [12]. A recent study [13] on South Indian systemic lupus erythematosus (SLE) patients reports that *RFC*-1 80 G > A polymorphism is associated with risk for SLE and also that the expression of *RFC*-1 and HLA DR was downregulated in SLE cases. The aim of the present study was to investigate the association of *RFC*-1 80G > A polymorphism with disease susceptibility, its effect on treatment response, and adverse events to MTX in South Indian RA patients.

## Materials and methods

### Patient selection

The study was conducted at the Department of Clinical Immunology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, a major tertiary referral center for South India. All newly diagnosed, DMARD-naive patients fulfilling the modified ACR criteria for RA [14] were included in the study. A total of 322 age- and gender-matched healthy individuals of Tamil ethnicity, without any family history of autoimmune disease were enrolled as healthy controls (HC). Informed consent was taken from all the study subjects, and the study was approved by the Institute Ethics Committee. The treatment was initiated with an initial MTX dose of 10 mg/week which was escalated by 5 mg every 2 weeks or more based on the disease activity and patient tolerance to a maximum of 25 mg/week at the end of 3 months. The patients were followed up every 2 weeks during the course of MTX dose escalation and subsequently once a month after a stable dose was reached. During each visit, the disease activity was assessed by EULAR DAS28 criteria [15] and evidence of MTX toxicity was evaluated by clinical history and laboratory investigations including hemogram and liver function tests. The response to therapy was assessed at 16 weeks of therapy by EULAR response criteria. The response was classified as "good response," "partial response," and "no response." Nonresponders were offered combination DMARDs consisting of methotrexate, sulfasalazine/leflunomide, and hydroxychloroquine. Partial responders were followed up till 24 weeks and offered additional immunomodulators if they failed to improve further. Complete responders continued with methotrexate.

### *RFC*-1 80G > A genotyping

Five milliliters of venous blood was collected, and DNA was extracted by the salting out procedure [16]. *RFC*-1 80G>A genotyping was performed by PCR-RFLP method. The forward (5′-ATG GTG CCC TCC AGC CCA GC-3′) and reverse (5′-CAT GAA GCC GTA GAA GCA AAG G-3′) primers were used as per published protocols [17]. PCR was carried out in a total reaction volume of 25 μl comprising 17.3 μl of DNase RNase-free water, 2.5 μl of 10× reaction buffer (GeNet Bio), 2.5 μl of 2 mM dNTPs (Fermentas), 0.5 μl each of forward and reverse primer (20 μM) (Sigma), 0.2 μl of Taq DNA polymerase (5 U/μl) (GeNet Bio), and 0.5 μl of magnesium chloride (25 mM) (Genei). PCR was carried out with the following cycling conditions: initial denaturation of 94 °C for 4 min, denaturation of 94 °C for 60 s, extension of 72 °C for 30 s, and a final extension of 72 °C for 7 min. The 114-bp PCR product was digested with *Hha* I enzyme (New England Biolabs) and electrophoresed in 8 % polyacrylamide gel. Individuals with 80GG genotype presented an 114-bp fragment, and individuals with 80AA genotype presented 80- and 34-bp fragments.

### Autoantibody measurement

IgM RF in all cases was measured at baseline using Nephelometry (BN Prospec, Dade Behring, Germany), and anticitrullinated peptide antibody (ACPA) status was identified using second generation commercial ELISA kits (Biosystems, Barcelona, Spain). An antibody level >10 and >20 IU/ml were regarded as RF-positive and ACPA-positive, respectively.

Statistical analysis

Statistical analysis was carried out using GraphPad Instat v 3.0. Allele and genotype frequencies were compared between the study groups by performing chi square test with Yate's continuity correction. Odds ratio and 95 % confidence interval was calculated for each allele and genotype. $p<0.05$ was considered to be statistically significant. Sample size and power of the study (80 %) was calculated using CATS software (Power calculator for genetic studies compiled and published by the Centre for statistical genetics, Michigan University, USA).

## Results

The study included 327 patients with rheumatoid arthritis treated with methotrexate, of which 92.96 % were females. The baseline demographic details of the patients are summarized in Table 1. All patients were DMARD-naive with active disease. The mean age was 42.73± 0.56, and mean disease duration before the start of DMARDS was 3.7±0.2 years. Two hundred thirty-four patients (71.55 %) had deforming disease, and 100 (30.58 %) had extra-articular manifestations. Of all, 255 (77.98 %) patients were RF-positive and 245 (74.92 %) were positive for ACPA. The mean baseline DAS28 score was 5.77±0.05, indicating that majority of the patients had a high disease activity at baseline. The mean dose of methotrexate given was 16.75±4 mg/week. One hundred nineteen (36.39 %) attained good treatment response; 102 (31.19 %) were moderate responders; and 106 (32.41 %) were nonresponders to treatment. Out of 119 good responders, 65 patients attained remission.

Sixty-seven patients (20.48 %) were observed to have MTX-induced adverse effects during the course of treatment. Thirty-six patients (53.73 %) had gastrointestinal side effects like oral ulcers, nausea, vomiting, or diarrhea. Twelve (17.91 %) had hematological manifestations in the form of anemia, leucopenia, or pancytopenia. Five (7.46 %) had hepatotoxicity as evidenced by an increase in amino transferase levels more than twice the upper limit of normal range which improved on MTX discontinuation. Another ten (14.92 %) had infections like herpes zoster, oral candidiasis, cellulitis, or urinary tract infection. Three patients (4.47 %) had pulmonary toxicity and one (1.49 %) MTX-induced nodulosis.

The genotype frequencies of *RFC*-1 80G > A in RA were found to be GG102 (31.19 %), GA 170 (51.98 %), and AA 55 (16.81 %), and in controls, it was found to be GG 84 (26.08 %), GA 196 (60.86 %), and AA 42 (13.04 %). It was found that the heterozygous *RFC*-1 GA genotype was found to be protective ($p=0.02$, OR 0.69, 95 % CI 0.50–0.95). The codominant genetic model also revealed a significant association (Table 2).

**Table 1** Demographic and clinical profile of study subjects

| Patients/controls | 327/322 |
|---|---|
| Sex (female/male) (%) | 92.96/7.03 |
| Mean age (year) ± SEM | 42.73 ± 0.56 years |
| Mean disease duration (year)± SEM | 3.76 ± 0.23 years |
| Young onset disease (YORA) | 307 (93.88 %) |
| Late onset disease (LORA) | 20 (6.11 %) |
| Erosive, deforming disease | 234 (71.55 %) |
| Extra-articular manifestations | 100 (30.58 %) |
| Autoantibody phenotype | |
| RF-positive | 255 (77.98 %) |
| ACPA-positive | 245 (74.92 %) |
| Disease activity | |
| High disease activity | 243 (74.31 %) |
| Moderate disease activity | 77 (23.54 %) |
| Low disease activity | 7 (2.14 %) |
| Baseline DAS28 (mean ± SEM) | 5.77±1.06 |
| Treatment response | |
| Mean dose of MTX (mg/week) | 16.75±4 |
| Good responders | 119 (36.39 %) |
| Moderate responders | 102 (31.19 %) |
| Nonresponders | 106 (32.41 %) |

YORA young onset RA (onset <55 years); LORA late onset RA (onset 55 years or above); high disease activity (DAS28 score >5.1), moderate disease activity (DAS28 3.2≤5.1); good response (an improvement of >1.2 in DAS28 score and a DAS of ≤2.6 (remission) on follow-up); no response (≤0.6 change in DAS score or a change between 0.6 and 1.2 with DAS score >3.7 on follow-up); moderate response (an improvement 0.6–1.2 in DAS score with overall DAS between 2.6–3.7)

There were no significant differences between allele and genotype frequencies among the good responder and the non-responder groups (Table 3). We also performed dominant, recessive, and codominant genetic models to find the association of *RFC*-1 80 G > A polymorphism with treatment response; however, no significance was detected (Table 3).

The results were corrected for other important determinants of treatment response like age, gender, disease duration, and ACPA status. When performing a multinomial logistic regression, age, gender, and ACPA status did not seem to influence the results. Though 68 % of the patients in the current study presented with a disease duration of >1 year, the disease duration also did not influenced the MTX treatment response.

The homozygous wild-type genotype *RFC*-1 GG was more frequent in patients with adverse effects than in patients without adverse effects (40.29 vs 28.84 %); however, the results were not statistically significant ($p=0.09$, OR 1.66, 95 % CI 0.95–2.90) (Table 4). Thus, *RFC*-1 80G > A gene polymorphism was not found to be associated with MTX-induced adverse events in the South Indian cohort of patients with RA.

**Table 2** Genotype and allele frequencies of *RFC*-1 80G>A in RA and healthy controls

| Genotype | RA ($n$=327) | Healthy controls ($n$=322) | Pc | OR (95 % CI) |
|---|---|---|---|---|
| GG | 102 (31.19 %) | 84 (26.08 %) | 0.17 | 1.28 (0.91–1.80) |
| GA | 170 (51.98 %) | 196 (60.86 %) | 0.02 | 0.69 (0.50–0.95) |
| AA | 55 (16.81 %) | 42 (13.04 %) | 0.21 | 1.34 (0.87–2.08) |
| Allele | | | | |
| G | 374 (57.18 %) | 364 (56.52 %) | 0.85 | 1.02 (0.82–1.28) |
| A | 280 (42.81 %) | 280 (43.47 %) | 0.85 | 0.97 (0.78–1.21) |
| Dominant model analysis | | | | |
| AA + GA | 225 (68.80 %) | 238 (73.91 %) | 0.17 | 0.77 (0.55–1.09) |
| GG | 102 (31.19 %) | 84 (26.08 %) | 0.17 | 1.28 (0.91–1.80) |
| Recessive model analysis | | | | |
| AA | 55 (16.81 %) | 42 (13.04 %) | 0.21 | 1.34 (0.87–2.08) |
| GA + GG | 272 (83.18 %) | 280 (86.95 %) | 0.21 | 0.74 (0.48–1.14) |
| Codominant model analysis | | | | |
| GA | 170 (51.98 %) | 196 (60.86 %) | 0.02 | 0.69 (0.50–0.95) |
| AA + GG | 157 (48.01 %) | 126 (39.13 %) | 0.02 | 1.43 (1.05–1.96) |
| Homozygotic model analysis | | | | |
| AA | 55 (35.03 %) | 42 (33.33 %) | 0.86 | 1.07(0.65–1.76) |
| GG | 102 (64.96 %) | 84 (66.66 %) | 0.86 | 0.92 (0.56–1.52) |

*OR* odds ratio, *95% CI* 95 % confidence interval

$P < 0.05$ is considered significant

## Discussion

The *RFC*-1 transports methotrexate and subsequently MTX is converted to its polyglutamate form within the cells. The polyglutamated methotrexate acts by inhibiting purine and pyrimidine synthesis required for cellular proliferation, inhibits the production of proinflammatory cytokines, suppresses the lymphocyte proliferation, and promotes the release of anti-inflammatory molecule, adenosine [18, 19]. *RFC*-1 transports reduced folates like methotrexate and folinic acid

**Table 3** Genotype and allele frequencies of *RFC*-1 80G > A in responders and non responders

| Genotype | Good responders ($n$=119) | Nonresponders ($n$=106) | Pc | OR (95 % CI) |
|---|---|---|---|---|
| GG | 34 (28.57 %) | 35 (33.01 %) | 0.56 | 0.81 (0.46–1.43) |
| GA | 64 (53.78 %) | 59 (55.66 %) | 0.88 | 0.92 (0.54–1.56) |
| AA | 21 (17.64 %) | 12 (11.32 %) | 0.25 | 1.67 (0.78–3.60) |
| Allele | | | | |
| G | 132 (55.46 %) | 129 (60.84 %) | 0.28 | 0.80 (0.55–1.16) |
| A | 106 (44.53 %) | 83 (39.15 %) | 0.28 | 1.24 (0.85–1.81) |
| Dominant model analysis | | | | |
| AA + GA | 85 (71.42 %) | 71 (66.98 %) | 0.56 | 1.23 (0.69–2.17) |
| GG | 34 (28.57 %) | 35 (33.01 %) | 0.56 | 0.81 (0.46–1.43) |
| Recessive model analysis | | | | |
| AA | 21 (17.64 %) | 12 (11.32 %) | 0.25 | 1.67(0.78–3.60) |
| GA + GG | 98 (82.35 %) | 94 (88.67 %) | 0.25 | 0.59 (0.27–1.27) |
| Codominant model analysis | | | | |
| GA | 64 (53.78 %) | 59 (55.66 %) | 0.88 | 0.92 (0.54–1.56) |
| AA + GG | 55 (46.21 %) | 47 (44.33 %) | 0.88 | 1.07 (0.63–1.82) |
| Homozygotic model analysis | | | | |
| AA | 21 (38.18 %) | 12 (25.53 %) | 0.25 | 1.80 (0.76–4.22) |
| GG | 34 (61.81 %) | 35 (74.46 %) | 0.25 | 0.55 (0.23–1.30) |

*OR* odds ratio, *95% CI* 95 % confidence interval

$p<0.05$ is considered significant

**Table 4** Genotype and allele frequencies of *RFC*-1 80G > A in patients with MTX-induced adverse effects and without adverse effects

| Genotype | Patients with adverse effects ($n$=67) | Patients without adverse effects ($n$=260) | Pc | OR (95 % CI) |
|---|---|---|---|---|
| GG | 27 (40.29 %) | 75 (28.84 %) | 0.09 | 1.66 (0.95–2.90) |
| GA | 32 (47.76 %) | 138 (53.07 %) | 0.52 | 0.80 (0.47–1.38) |
| AA | 8 (11.94 %) | 47 (18.07 %) | 0.31 | 0.61 (0.27–1.37) |
| Allele | | | | |
| G | 86 (64.17 %) | 288 (55.38 %) | 0.08 | 1.44 (0.97–2.13) |
| A | 48 (35.82 %) | 232 (44.61 %) | 0.08 | 0.69 (0.46–1.02) |
| Dominant model analysis | | | | |
| AA + GA | 40 (59.70 %) | 185 (71.15 %) | 0.09 | 0.60 (0.34–1.04) |
| GG | 27 (40.29 %) | 75 (28.84 %) | 0.09 | 1.66 (0.95–2.90) |
| Recessive model analysis | | | | |
| AA | 8 (11.94 %) | 47 (18.07 %) | 0.31 | 0.61(0.27–1.37) |
| GA + GG | 59 (88.05 %) | 213 (81.92 %) | 0.31 | 1.62 (0.72–3.63) |
| Codominant model analysis | | | | |
| GA | 32 (47.76 %) | 138 (53.07 %) | 0.52 | 0.80 (0.47–1.38) |
| AA + GG | 35 (52.23 %) | 122 (46.92 %) | 0.52 | 1.23 (0.72–2.11) |
| Homozygotic model analysis | | | | |
| AA | 8 (22.85 %) | 47 (38.52 %) | 0.13 | 0.47 (0.19–1.12) |
| GG | 27 (77.14 %) | 75 (61.47 %) | 0.13 | 2.11 (0.88–5.04) |

*OR* odds ratio, *95% CI* 95 % confidence interval

$p<0.05$ is considered significant

with a relatively high affinity ($K_m$=1–10 μM) and folic acid with a low affinity ($K_m$ 200–400 μM) [20, 21]. Membrane transport via *RFC*-1 is an important determinant of antifolate therapeutics used in cancer chemotherapy, and impaired uptake of antifolates by *RFC*-1 is a frequent mode of drug resistance [22]. The *RFC*-1 80G > A mutation affects residue 27 of the protein and substitutes a histidine with an arginine [23]. The change of a strongly basic amino acid arginine to a weak base histidine influences folate substrate binding, and the rate of uptake might be expected to alter human *RFC* transport properties [22]. Polymorphisms in the folate metabolic pathway and transporters are of paramount importance as they modify MTX transport and influence MTX metabolic effect [24].

In the present study on South Indian patients with rheumatoid arthritis, *RFC*-1 80 G > A polymorphism was found to be associated with disease susceptibility. The observed mutant allele (A) frequency in the present study on South Indian healthy subjects was 43 % which is lower than in the Western Indian population (57 %) [25] but higher compared to the North Indian population (28 %) [26]. In a recent study involving 414 south Indian healthy subjects [13], the *RFC*-1 mutant allele frequency was reported to be 36.5 %. To the best of our knowledge, there are no studies carried out in investigating the contribution of genetic polymorphisms in one carbon metabolism with disease susceptibility to RA. However, a study from Polish population [27] found that methionine synthase (*MTR*) 2756 A > G polymorphism is associated with the risk for SLE. Similarly, Rupasree et al. [13] elucidated the effect of genetic variations in one carbon metabolism on the epigenetic regulation of MHC2TA, RFC-1, and HLA-DR in SLE. It was observed that ***RFC***-1 80G > A polymorphism was associated with the risk for SLE, and also, the expression of HLADR and ***RFC***-1 was downregulated in SLE cases. The present finding of how RFC-1 80G > A gene polymorphism confers that protection in RA could be interpreted by the shared chromosomal location of RFC-1 and autoimmune regulator (AIRE) genes at 21q22. AIRE is a transcriptional regulator primarily involved in regulating the expression of autoantigens and negative selection of autoreactive T cells in the thymus. Recent evidences from Japanese and Chinese cohort of RA patients [28, 29] shows that AIRE genes are genetic risk factors in RA. The other functional SNPs in the AIRE gene in linkage disequilibrium with RFC-1 80G > A SNP could be responsible for the observed protection against RA. Further studies exploring the role of AIRE and RFC gene interactions may help to clarify the protective effect observed in our study.

Our results are in agreement with other studies which report that *RFC*-1 80G > A polymorphism is not associated with MTX treatment response. A study on 205 Caucasians [30] with RA reported that *RFC*-1 80G > A polymorphism was not associated with methotrexate treatment response or with methotrexate-induced toxicity. Another study [12] from Caucasians on *RFC*-1 with three polymorphic variants *RFC*-1 80G > A, 696C > T, and -43 T > C in the 5′ flanking sequence to the ATG transcription start site reported no significant difference in the distribution of these genotypic combinations between responders and nonresponders to MTX

monotherapy. In a retrospective study from North India [26] involving 281 patients with RA, SNPs in genes involved in MTX transporter pathway and polyglutamation deconjugation pathway including *RFC-1*, *FOLR1*, *FPGS*, and *GGH* were analyzed. No significant allelic/genotypic/haplotypic associations were observed for five SNPs in the RFC gene with MTX treatment response.

There are other studies which report an association of *RFC*-1 80G > A polymorphism with MTX treatment response. A recent meta-analysis on recessive and additive genetic model analysis reported that *RFC*-1 80 G > A polymorphism is associated with MTX efficacy but not toxicity [31]. A strong association of *RFC*-1 80G > A polymorphism was found in Polish RA patients, where the probability of remission of RA was 3.3-fold higher in the patients carrying AA genotype when compared to those with GG genotype [32]. Among 170 Japanese patients with RA, it was found that *RFC*-1 80 G allele frequency was higher in patients who required biologicals due to nonresponse to MTX [33].

In our study, patients with MTX-induced side effects had higher frequency of *RFC*-1 80 G allele and GG genotype although, the difference failed to reach statistical significance. Despite an adequate sample size, the reduced frequency of adverse effects in our study could be due to the fact that all patients were receiving currently recommended folic acid supplementation or South Indian population may be inherently less susceptible to MTX-induced adverse effects. Some studies have reported associations of *RFC*-1 80G > A polymorphism with MTX-induced adverse events. Grabar et al. [24] reported that patients with *RFC*-1 80GG genotype had a 2.3-fold higher risk for MTX toxicity as compared to patients with one *RFC*-1 80 A allele. Similarly, in a recent study involving 159 Jordanian RA patients, it was found that patients with *RFC*-1 80 GG genotype were at a higher risk of developing gastrointestinal toxicity [34]. In 233 Portuguese patients with RA, 23 SNPs in methotrexate transporter genes namely *SLC19A1* (*RFC-1*), *SLC46A1*, and *SLCO1B1* were analyzed. A toxicogenetic risk index was created, and it was observed that patients carrying the *SLC19A1* G allele were at an increased risk of developing gastrointestinal toxicity [35]. The probability of increase in liver enzymes aminotransferase activity (ALT) and asparagine transaminase (AST) during MTX therapy was 5-fold higher in carriers of 80AA genotype when compared with 80 GG genotype patients though the results were not statistically significant [32].

In Japanese and Caucasian patients, the *RFC*-1 80 G > A polymorphism is known to influence the methotrexate polyglutamate levels [36, 37]. In a cross-sectional study by Dervieux et al. [36], *RFC*-1 80G > A and *GGH* -401C > T polymorphisms were analyzed and MTXPG concentrations were measured by HPLC. It was found that both *RFC*-1 and *GGH* polymorphisms affected the polyglutamate levels of MTX. Another study by Ando et al. [37] from Japan reported that *RFC*-1 80G > A polymorphism was significantly associated with the detectability of MTXPGs which was lower in patients with the mutant A allele of *RFC*.

The limitation of the present study includes lack of measurement of methotrexate polyglutamates and its association with *RFC*-1 80G > A polymorphism. The lack of association of *RFC*-1 80G > A gene polymorphism with MTX treatment response and adverse effects could be due to interethnic differences in the folate metabolic and transporter genes. Other genetic polymorphisms in the *RFC*-1 gene or methotrexate transporters like folate receptor beta [38] could possibly be influencing the treatment response in South Indian Tamils.

**Acknowledgments** The study was supported by an intramural research grant by JIPMER, Puducherry, India. In addition, the infrastructure and other support provided by DST-FIST and ICMR-INSERM programs are also acknowledged.

**Author Contributions** VSN conceived and planned the study. NM performed the laboratory work with the precious help of CMM. NM performed statistical analysis.VSN and MCB recruited the patients, collected, organized, and interpreted the clinical data. VSN and NM wrote the manuscript. VSN coordinated the research and critically reviewed the manuscript and takes the primary responsibility for the article.

**Conflict of interest** All authors declare that there are no conflicts of interest.

**Ethical standards** The study was carried out in compliance with international, national, and institutional regulations. Institute Ethics Committee has approved the study. All persons gave informed consent prior to the inclusion in the study.

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