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Original Article

Impact of methylenetetrahydrofolate reductase (MTHFR) A1298C gene polymorphism on the outcome of methotrexate treatment in a sample of Egyptian rheumatoid arthritis patients

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ABSTRACT

Background: Methotrexate is the most commonly used disease-modifying anti-rheumatic drug (DMARD) and it is considered the first-line treatment in the management of rheumatoid arthritis (RA). MTX treatment outcome regarding response to the drug and adverse effects in RA patients are not universal. Therefore, it would be beneficial if we could predict the response of patients to MTX before starting MTX treatment in order to determine the patient's drug-treatment plan. *Objectives:* The present study aimed to evaluate the impact of *MTHFR* A1298C SNP (rs1801131) on the clinical outcome of MTX treatment as regards treatment efficacy and toxicity in a cohort of Egyptian rheumatoid arthritis patients. *Patients and methods:* Fifty rheumatoid arthritis patients were included in the present study. Data about patient related variables such as age and sex, disease related variables such as disease duration as well as treatment related variables such as treatment duration, dose of MTX, its route of administration and concomitant use of other drugs (NSAIDs) were obtained. DAS28 was calculated to all patients to assess drug response. *MTHFR* A1298C polymorphism was investigated using real time 5′ nuclease allelic discrimination assay. *Results:* Multivariate regression analysis for factors predicting MTX drug response showed that *MTHFR* A1298C SNP and MTX dose were the most significant independent predictors for MTX treatment response (p = .016, OR = 39.113, 95% C.I = 1.970–776.558, p = .003, OR = 1.667, C.I = 1.184–2.348, respectively). Considering clinicopathological variables; longer disease duration, positive anti-CCP, NSAIDs users, higher MTX doses and longer treatment durations were significantly associated with non-response to MTX. Regarding MTX drug toxicity, *MTHFR* 1298 CC genotype, MTX dose and concomitant use of NSAIDs were significantly associated with MTX drug toxicity (^{MC}p = .003, p = .031, p = .029, respectively). *Conclusion:* Our study proved that *MTHFR* A1298C SNP can predict clinical outcome of MTX treatment as regards treatment efficacy and toxicity in Egyptian rheumatoid arthritis patients.

1. Introduction

Rheumatoid arthritis (RA) is a common systemic inflammatory disorder characterized by symmetric arthritis and synovial inflammation leading to progressive joint erosion that end with joint deformity.[1] The prevalence of RA ranges from 0.5% to 1% worldwide. The incidence varies based on gender, population and ethnicity. It is estimated to be 30 cases per 100,000 people per year.[2]

The etiology of RA is unknown. Genetic susceptibility may predispose to RA and it can also be triggered by environmental factors such as smoking and air pollutants.[3]

The synovial inflammation characteristic of RA involves multiple immune cells which act together to form the observed synovial hyperplasia and degradation of cartilage and bone.[4,5]

The management of RA depends mainly on the use of DMARDs. These agents are commonly characterized by their ability to reduce or reverse signs and symptoms and progression of joints damage. So, DMARDs interfere with the entire disease process and these

Abbreviations: RA, rheumatoid arthritis; MTX, methotrexate; MTHFR, methylenetetrahydrofolate reductase; DMARDs, disease-modifying anti-rheumatic drugs; NSAIDs, non-steroidal anti-inflammatory drugs; PCR, polymerase chain reaction; DAS, disease activity score.

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in turn lead to improvement of quality of life.[6] Methotrexate (MTX) is the most commonly used DMARD and it is considered the first-line treatment in the management of RA.[7] Although MTX is one of the best-tolerated DMARDs for the treatment of RA, major drawbacks of MTX therapy are detected such as large inter-patient variability in clinical response and large spectrum of side effects.[8]

Factors influencing disease course and therapeutic outcome include clinicopathological variables such as patient-related variables, disease-related variables and treatment-related variables in addition to genetic factors such as genetic polymorphisms in the key MTX pathway genes.[9]

The study of the associations between genetic variations such as polymorphisms and drug efficacy and toxicity is known as pharmacogenetics. Thus, pharmacogenetic studies can predict the therapeutic outcomes of MTX and this in turn may help clinicians to individualize appropriate treatment for each patient.[10]

The *MTHFR* gene which is located on the short arm of chromosome 1 (1p36.3), consists of 11 exons and 10 introns.[11] This gene codes for a 77-kDa protein.[12] *MTHFR* has a role in conversion of 5, 10-methylenetetrahydrofolate into 5-methyl-tetrahydrofolate (5 methyl-THF). 5 methyl-THF acts as a methyl donor for remethylation of homocysteine to methionine. There are several polymorphisms in *MTHFR* gene but only *C677T* (rs1801133) and *A1298C* (rs1801131) polymorphisms affect enzyme activity. So, they are considered the two most important polymorphisms that may influence the therapeutic response to MTX.[13] The A1298C polymorphism is a missense mutation consisting of A to C transition at base pair 1298 leading to substitution of a glutamate by alanine. This polymorphism results in reduction of the enzyme activity, but to lesser extent than the C677T polymorphism.[14]

2. Aim of the study

The present study aimed to evaluate the impact of *MTHFR* A1298C SNP (rs1801131) on the clinical outcome of MTX treatment as regards treatment efficacy and toxicity in a cohort of Egyptian rheumatoid arthritis patients.

3. Subjects and methods

3.1. Subjects

The study was conducted on fifty Egyptian patients who were diagnosed as having RA based on the American College of Rheumatology (ACR) 2012.

All patients received low-dose MTX for at least 3 months. Non-steroidal anti-inflammatory drugs (NSAIDs) were allowed n the first 8 weeks of the disease and all patients received folic acid supplementation to prevent adverse effects induced by MTX. Any patient received biopharmaceutical therapy or another DMARD concomitantly during the 2-month period prior to enrollment in the study was excluded. This study has been approved by the Ethics Committee of Alexandria University and a written informed consent was obtained from all participants.

Full history was taken from all participants, including patient related variables such as age and sex, disease related variables such as disease duration in addition to treatment related variables such as treatment duration, dose of MTX, its route of administration as well as concomitant use of other drugs such as NSAIDs.

Review of the patients' medical records regarding their MTX weekly dose (mg/week) and some laboratory investigations (RF, anti-CCP).

3.2. Methods

3.2.1. Clinical assessments

Patients' clinical and demographic data were collected at the single study visit. Clinical efficacy was assessed using the disease activity score in 28 joints (DAS 28). The occurrence of side effects was retrospectively recorded at the time of the study visit. MTX related side effects were classified as (1) gastrointestinal complaint such as anorexia, nausea, vomiting and diarrhea. (2) Mucocutaneous complaint such as alopecia, rashes and oral ulcers. (3) Central nervous system toxicity such as insomnia, headache and dizziness. (4) Pulmonary toxicity such as cough and pneumonitis. (5) Hepatotoxicity; elevation of serum transaminases above the upper range of normal. (6) Bone marrow toxicity such as megaloblastic anaemia, leucopenia, thrombocytopenia and pancytopenia. (7) Renal toxicity; elevation of creatinine levels above the upper range of normal.

3.2.2. Routine laboratory investigations

Complete blood count, liver function, renal function and CRP were measured on the day of the study visit using standard laboratory methods.

3.2.3. Molecular studies

- Genomic DNA was extracted from EDTA whole blood samples using QIAamp DNA blood mini Kit 50 (Qiagen, CA, USA, cat. no. 51104). The concentration and purity of extracted DNA was assessed using a nanodrop 2000 spectrophotometer and then stored at −20 °C till genotyping.
- The 1298A > C polymorphism (rs1801131) was detected by 5′ nuclease allele discrimination assay using real-time polymerase chain reaction (PCR). Amplification done according to the following steps: 10 μl of TaqMan® universal PCR Master Mix (2X) was added followed by 0.5 μl of Primer/probe mix (40X). Then 1 μl of extracted DNA (DNA was diluted in nuclease-free water in order to deliver 1–20 ng per well) and sterile water were added to complete a total volume of 20 μl. Rotorgene Q Real time PCR system was employed to obtain specific sequences using the following thermal profile: holding at 95 °C for 10 min followed by 40 cycles of denaturation (92 °C for 15 s) and annealing/extension (60 °C for 1 min).

3.2.4. Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp). Qualitative data were described using number and percent. Quantitative data were described using range, mean, standard deviation and median. Significance of the obtained results was judged at the 5% level. Chi-square test or Fisher's Exact and Monte Carlo correction was used to compare between different groups. For normally quantitative variables, to compare between two studied groups Student *t*-test was applied. Odd ratio was used to calculate the ratio of the odds and 95% Confidence Interval of an event occurring in one risk group to the odds of it occurring in the non-risk group.

4. Results

A total of 50 patients with RA were enrolled to the study (43 females and 7 males) with a mean age of 43.12 ± 9.66 years and a mean disease duration of 4.52 ± 3.82 years. Considering MTX treatment, the mean treatment duration was 3.66 ± 3.25 years with a mean MTX dose of 17.08 ± 6.84 mg/week. Furthermore, 46 patients (92%) administered MTX by IM administration route and 4 (8%) by PO administration route. The mean DAS28 was

4.10 ± 1.47. Seventeen patients (34%) have DAS28 ≤3.2 and they are considered responders while 33 patients (66%) have DAS28 >3.2 and they are considered non-responders. Regarding MTX adverse effects, 34% of patients developed drug toxicity during treatment period.

Concerning disease-related variables, our results demonstrated a statistically significant association between disease duration and MTX drug response (p = .028). Also patients with positive anti-CCP were significantly associated with non-response to MTX (^{FE}p = .008). Considering treatment related variables, our results revealed a significant association between concomitant use of NSAIDs, higher MTX doses and MTX non response (p = .033, p < .001, respectively). In addition, we observed that longer treatment durations were significantly associated with MTX non-response (p = .027).

The distribution of *MTHFR* A1298C genotypes were 24 AA (48%), 20 AC (40%) and 6 CC (12%). The major allele was A and the minor allele was C. The genotype frequency of *MTHFR* A1298C was in Hardy-Weinberg equilibrium (p = .567).

A statistically significant association was found between *MTHFR* A1298C genotype and MTX drug response (^{MC}p = .047); 50% of the patients with AA genotype showed good clinical response compared to 25% and 00% with AC and CC, respectively. It was concluded that AA genotype was associated with better drug response. Table 1, Fig. 1

Binary logestic regression study proved that *MTHFR* A1298C genotype and MTX dose were the most significant independent predictors for MTX treatment response (p = .016, OR = 39.113, 95% C.I = 1.970–776.558, p = .003, OR = 1.667, C.I = 1.184–2.348, respectively) Table 2.

As regards MTX drug toxicity, a statistically significant relation was observed between the dose of MTX and drug toxicity (p = .031). Moreover, concomitant use of NSAIDs was significantly associated with MTX drug toxicity (p = .029).

Regarding the relation between *MTHFR* A1298C SNP and MTX drug toxicity, our results showed a significant association between *MTHFR* 1298 CC genotype and MTX drug toxicity (^{MC}p = .003) Table 3, Fig. 2.

5. Discussion

MTX represents an interesting target for pharmacogenetic studies. This helps identification of response predictors to achieve maximal response with minimal toxicity.[15]

The present study investigated the relation between *MTHFR* A1298C and MTX drug response. It was observed that *MTHFR*

Table 1
Relation between MTHFR A1298C genotype and MTX drug response.

	MTHFR genotype						χ^2	^{MC}p
	AA (n = 24)		AC (n = 20)		CC (n = 6)			
	No.	%	No.	%	No.	%		
MTX drug response								
Responder	12	50.0	5	25.0	0	0.0	6.178*	.047*
Non responder	12	50.0	15	75.0	6	100.0		

χ^2: Chi square test.
MC: Monte Carlo for Chi square test.
* Statistically significant at p ≤ .05.

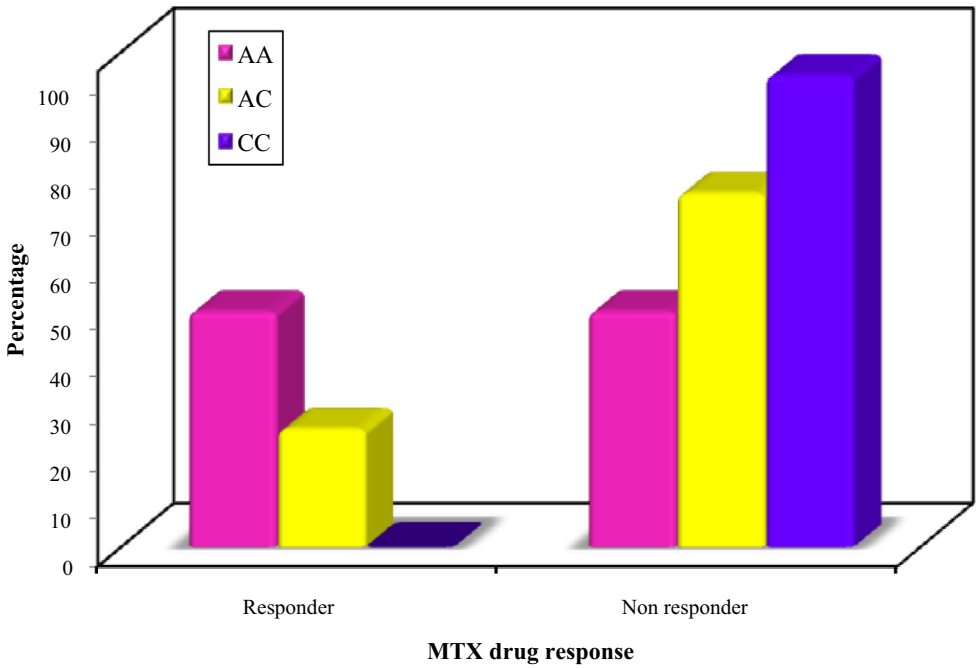

Fig. 1. Relation between MTHFR A1298C genotype and MTX drug response.

Table 2
Binary logistic regression for the parameters that affecting MTX drug response.

	B	S.E.	Sig.	OR	95% C.I	
					L.L	U.L
Dose of MTX	0.511	0.175	0.003*	1.667*	1.184	2.348
Anti CCP	2.177	1.309	0.096	8.823	0.678	114.845
Disease duration (years)	−0.154	0.529	0.771	0.857	0.304	2.417
NSAIDs	−1.117	1.693	0.510	0.327	0.012	9.045
Treatment duration (years)	0.194	0.582	0.739	1.214	0.388	3.799
MTHFR A1298C (AC + CC genotypes)	3.666	1.525	0.016*	39.113*	1.970	776.558

Table 3
Relation between MTHFR A1298C genotype and MTX drug toxicity.

	MTHFR genotype						χ^2	^{MC}p
	AA (n = 24)		AC (n = 20)		CC (n = 6)			
	No.	%	No.	%	No.	%		
Toxicity								
No	14	58.3	18	90.0	1	16.7	12.040*	.003*
Yes	10	41.7	2	10.0	5	83.3		

χ^2: Chi square test.
MC: Monte Carlo for Chi square test.
* Statistically significant at $p \leq .05$.

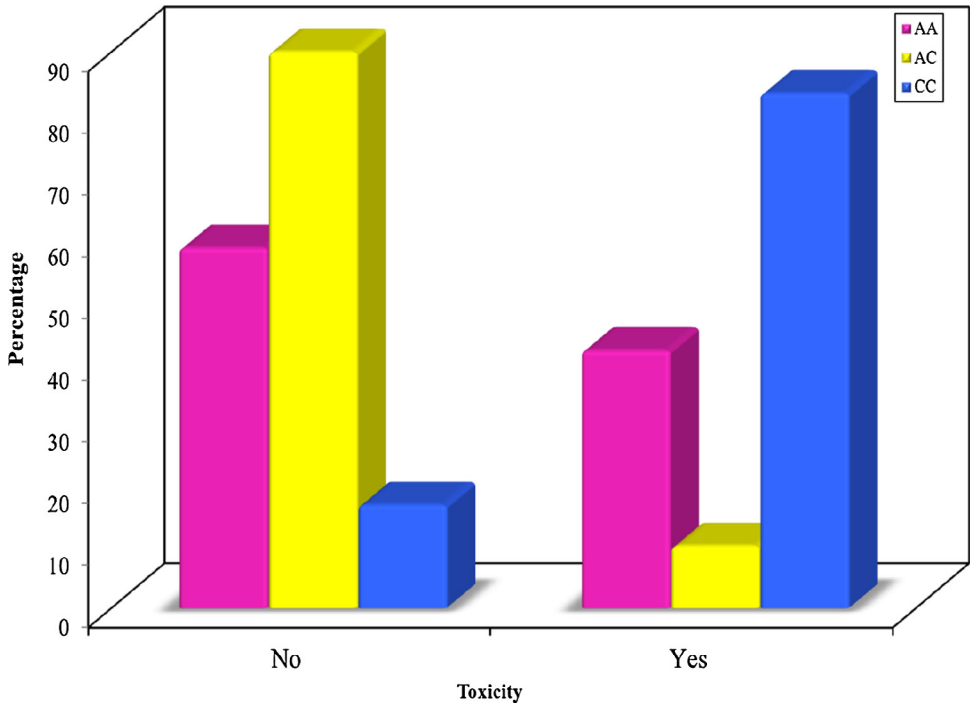

Fig. 2. Relation between MTHFR A1298C genotype and MTX drug toxicity.

1298 AC/CC genotypes were significantly associated with MTX non-response ($^{MC}p = .047$). Carriers of C allele are significantly associated with non-response to MTX than non carriers ($p = .008$). This may be attributed to reduction of MTHFR activity leading to reduced 5-MTHF and other folate cofactors levels which in turn leads to decreased adenosine release that partially explain MTX non-response.[16,17]

Similar results were detected by Kato et al.[18] and Wessels et al.[19] They observed that *MTHFR* 1298AA was associated with good response relative to 1298C allele carriers. In addition, Ghodke-Puranik et al.[20] evaluated the influence of 12 genetic polymorphisms in folate pathway genes in Indian rheumatoid arthritis patients receiving MTX. It was concluded that patients carrying at least one *MTHFR* 1298A allele (AA-AC) were more likely to have better MTX efficacy relative to those with *MTHFR* 1298 CC.

In contrast to our results, Kurzawski et al.[21] suggested that *MTHFR* 1298C alleles may be associated with an increased rate of RA remission in patients treated with MTX receiving high doses of folic acid supplementation. These results were explained by down regulation of 5-methyl-THF synthesis through MTHFR and subsequently reduced methionine production from homocysteine and 5-methyl-THF through methionine synthase and decreased

synthesis of S-adenosylmethionine. S-adenosylmethionine is a main donor of methyl group in numerous biochemical pathways and reactions of DNA methylation. The limited availability of S-adenosylmethionine may affect expression of many genes, being a postulated mechanism of immunosuppressive properties of MTX.[21]

Similarly, Urano et al.[22] examine whether there was a correlation between *MTHFR* gene and the efficacy or toxicity of MTX in the treatment of RA. They concluded that improvement in ESR and CRP was significantly associated with increasing numbers of 1298C alleles. This was attributed to exertion of MTX efficacy through inhibition of folate metabolism. So, the reduction in the activity of a key enzyme in folate metabolism is likely to enhance the effects of MTX.

Taraborelli et al.[23] conducted their study to investigate the role of genetic screening of *MTHFR* polymorphisms (C677T, A1298C) in planning the treatment of rheumatoid patients. Against our results, they did not find any significant association between *MTHFR* A1298C genotype/allele and MTX response.

Also, Kumagai et al.[24] and Bohanec et al.[25] investigated the influence of common SNPs in genes of the folate metabolic pathway on the efficacy and toxicity of MTX treatment in a group of RA patients. Regarding treatment response, they did not find any significant association between *MTHFR* A1298C genotype and MTX response.

Moreover, the present study investigated the relation between multiple clinicopathological variables and MTX drug response. Regarding disease related variables, longer disease duration and positive anti-CCP were significantly associated with non response to MTX ($p = .028$, $^{FE}p = .008$, respectively).

In agreement with our results, Lee et al.[26] studied the relation between three candidate alleles in the *ATIC*, *ITPA* and *MTHFR* genes and DAS28-CRP as a marker of disease activity. It was concluded that disease duration and anti-CCP were significantly associated with MTX drug response ($p = .002$, $p = .01$, respectively).

In contrast to our results, Iqpal et al.[27] investigated the association between SNPs in *MTHFR* gene (C677T and A1298C) with response to MTX therapy in Pakistani RA patients. It was proved that disease duration was not significantly associated with MTX treatment response ($p = .53$).

Regarding treatment related variables, the present study concluded that NSAIDs users, higher MTX doses and longer treatment durations were significantly associated with non-response to MTX ($p = .033$, $p < .001$, $p = .027$, respectively).

In concordance with our results, Lima et al.[9] studied the role of *MTHFR* C677T and ATIC T675C polymorphisms and clinicopathological variables in clinical response to MTX in RA patients. It was found that MTX dose and concomitant use of NSAIDs were significantly associated with MTX drug response. However, no significant relation was detected between treatment duration and MTX response ($p = .204$).

Contrary to our results, Świerkot et al.[28] evaluated the effect of gene polymorphisms on the efficacy of therapy and side effects in RA patients treated with MTX. It was observed that MTX dose and concomitant use of NSAIDs were not associated with MTX drug response ($p = 1$, $p = .76$, respectively).

In addition, our study revealed also a significant association between MTHFR 1298 CC genotype and MTX drug toxicity ($^{MC}p = .003$). This could be explained by low drug response with MTHFR 1298CC genotype. Therefore those with CC genotype need higher doses of MTX to achieve response which lead to exposure to more side effects.

In accordance to our results, Choe et al.[29] observed that the proportion of rheumatoid patients with *MTHFR* 1298CC genotype who experienced at least one drug toxicity was significantly greater than the proportion of patients with 1298AA. However, Davis et al.[30] and Dervieux et al.[31] observed that *MTHFR* 1298AC/CC genotypes were associated with an increased risk of toxicity.

On the other hand, Cáliz et al.[7] assessed the involvement of the C677T and A1298C polymorphisms in the *MTHFR* gene in the toxicity of MTX in a group of RA patients. As regards *MTHFR* A1298C, they observed that *MTHFR* A1298C polymorphism was not significantly associated with increased MTX toxicity ($p = .761$).

Similarly, Taraborelli et al.[23] concluded that *MTHFR* A1298C polymorphism was not significantly associated with MTX drug toxicity.

As regards the relation between clinicopathological variables and MTX drug toxicity, the present study detected a significant association between concomitant use of NSAIDs, MTX dose and MTX drug toxicity ($p = .029$, $p = .031$, respectively).

Jenko et al.[32] investigated predictors of MTX induced adverse events that may lead to treatment discontinuation. They observed a significant association between dose of MTX and drug toxicity ($p = .03$).

On the other hand, Lima et al.[33] found no association between co-administration of NSAIDs and MTX drug toxicity ($p = .053$).

The discrepancy between our results and previous studies may be attributed to different ethnic populations, larger sample size compared to our study, the prescription of folate supplementation, different methods of MTX drug response evaluation and different techniques used for SNPs genotyping.

6. Conclusion

Our study found that *MTHFR* A1298C SNP (rs1801131) can predict clinical outcome of MTX treatment as regards treatment efficacy and toxicity in Egyptian rheumatoid arthritis patients. Thus pharmacogenetics can help clinicians to take appropriate treatment decisions.

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