No correlation between MTHFR *c.677 C>T,* MTHFR *c.1298 A>C, and* ABCB1 *c.3435 C>T polymorphisms and methotrexate therapeutic outcome of rheumatoid arthritis in West Algerian population*

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No correlation between *MTHFR* c.677 C > T, *MTHFR* c.1298 A > C, and *ABCB1* c.3435 C > T polymorphisms and methotrexate therapeutic outcome of rheumatoid arthritis in West Algerian population

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Abstract
Context The c.677 C > T and c.1298 A > C polymorphisms of methylenetatrahydrofolate reductase (*MTHFR*) gene and c.3435 C > T polymorphism of ATP-Binding cassette B1 (*ABCB1*) gene are reported as pharmacogenetic markers, influencing the methotrexate (MTX) therapeutic outcome in rheumatoid arthritis (RA) patients.
Objectives The aims of this study were to determine the relationship between these polymorphisms and clinical response and/or adverse drug reaction (ADRs) to MTX treatment.
Materials and methods The cohort of our study was composed of 110 RA patients of the West Algerian population. The clinical response was evaluated using the disease activity score 28 (DAS28) and the ADRs were collected after physical examination of patients. All samples were genotyped for theses polymorphisms by TaqMan® allelic discrimination assay.
Results Based on EULAR criteria, 59.09% RA patients were responders and ADRs were observed in 40.9% patients. The frequency distribution of these three polymorphisms was similar between the responders and the non-responders. The same result was found on ADRs study and no significant difference of distribution between the presence of ADRs group and absence of ADRs group was observed.
Discussion Our study joins the results that found in others population in the world.
Conclusion We have demonstrated, for the first time in the West Algerian population, that these polymorphisms were not predictive for clinical response and/or ADRs to MTX therapeutic outcome.

Keywords Methotrexate · Rheumatoid arthritis · *MTHFR* · *ABCB1* · Polymorphism · Algeria

Introduction

Rheumatoid arthritis (RA) is the most common form of chronic inflammatory rheumatism. It is an autoimmune and chronic disease. It is characterized by inflammation of the synovial membrane with degradation of the cartilage and bone destruction [1]. Methotrexate (MTX) is an anti-folate drug and it is the most used worldwide among disease-modifying anti-rheumatic drugs (DMARDs) for the treatment of RA. The MTX has anti-inflammatory and anti-proliferative effects. It slows the progression of the disease, reduces pain and damage done to the joints, and delays the loss mobility. Several aspects about the MTX actions are not clear. In fact, some patients present a treatment efficacy and the others develop adverse drug reactions (ADRs) which may eventually lead to the interruption of

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the treatment. The mechanisms responsible of the clinical response variability may be due to the polymorphisms in genes which are involved in the MTX cellular pathway [2].

The methylenetetrahydrofolate reductase (MTHFR) enzyme is essential for intracellular folate homeostasis and metabolism. It converts the 5 tetrahydrofolate to the 5,10-methyl tetrahydrofolate (5,10-methyl-THF) which catalyzes the conversion of homocysteine to methionine, necessary to the methylation reactions of DNA, RNA, and proteins [3, 4]. The 5,10-methyl-THF is require for purine and pyrimidine synthesis. The *MTHFR* enzyme has several crucial cellular processes, and its deficiency can have many consequences of folate status, which can influence the clinical response to MTX treatment [3]. Many functional polymorphisms have been reported in the *MTHFR* gene [4]. *MTHFR* c.677 C > T (Ala222Val) and *MTHFR* c.1298 A > C (Glu429Ala) polymorphisms are the most studied in the MTX therapeutic outcome, because they reduce the *MTHFR* enzyme expression [4]. In the previous studies, the effect of *MTHFR* c.677C > T and *MTHFR* c.1298 A > C polymorphisms on the clinical response and/or adverse drug reactions to MTX treatment in RA has produced controversial results [5, 6].

Several polymorphisms in genes related to MTX transport across the cell membrane have described. In fact, many studies have evaluated how variations in the ATP-binding cassette, subfamily B, member, 1 (*ABCB1*) gene might impact the MTX therapeutic outcome. The *ABCB1* is a gene that encodes the P-glycoprotein (P-gp) [7] which acts as a pump for drug transport. This gene has an important role in toxic substances and metabolites bioavailability, and is supposed to prevent their intracellular accumulation. The *ABCB1* c.3435 C > T polymorphism (rs1045642) decreases the enzymatic activity of P-gp and consequently reduces the number of carriers; which induces response variability in treatment [8]. This variant in *ABCB1* gene has already been studied in RA patients associated with pharmacogenetic of MTX [5, 9, 10].

The aims of our study were to determine the correlation of the *MTHFR* c.677C > T, *MTHFR* c.1298 A > C, and *ABCB1* c.3435 C > T polymorphisms with clinical response and adverse drug reactions to MTX treatment in RA West Algerian patients.

Materials and methods

Population

Our sample of the West Algerian population was composed of 110 RA patients. Each patient gave informed consent to participate in the study. The subjects were randomly selected in the rheumatology service of Hospital-University Center of Oran (CHU Oran, Algeria). The study was approved by the Institutional Research Ethics Board. The RA patients were diagnosed according to the revised criteria of the American College of Rheumatology (ACR) in 1987 [11] and reclassified according to ACR/European League Against Rheumatism (EULAR) criteria in 2010 [12]. All patients have a low and moderate disease activity profile (DAS28 ≤ 5.1). The patients included in this study were already treated with only MTX (as monotherapy, there was no DMARDs therapy combination) for at least 6 months (mean dose of MTX = 12.67 mg/week). It noted that the MTX was prescribed as a treatment in the first line (dose of MTX = 15 mg/week). To prevent the toxicity, the folic acid supplementation was prescribed once a week for all patients with the same dose of MTX (mean dose folic acid = 12.67 mg/week). The Corticosteroid was prescribed for 58 patients and a non steroidal anti-inflammatory drug for 47 patients. The dose of MTX was reduced to 10 mg/week for the patients, who developed severe MTX-related toxicity (gastrointestinal, liver, pulmonary, cutaneous mucosa, hematopoietic, and renal toxicity). Clinical data were established by the revision of the medical record, blood analysis, and questionnaire of each patient. All patients were physically examined to determine the related adverse reactions and the response to MTX by measuring the disease score activity 28 (DAS28) [12].

Patients were excluded from this study if they are not treated with MTX for at least 6 months and they stopped this therapy for different reasons: recent pregnancy and plans to become pregnant. Finally, we retain 110 RA patients for our study.

Stratification of RA patients regarding clinical response was performed according to EULAR criteria [12]. The evaluation of the MTX treatment response depends on the DAS28. We classified the patients into two groups. The patients with a good and moderate response were considered to be 'responder' and the patients with poor response were considered as 'non-responder's [13].

Methotrexate-related ADRs were defined as one or combination of different events: gastrointestinal (nausea, indigestion or anorexia, diarrhea, mouth ulcers, and abdominal pains), liver (hepatic cytolysis, metabolic hepatic steatosis, cirrhosis, hepatic fibrosis, and rate of transaminase), pulmonary (presence of cough, difficulty breathing, and pneumonia), cutaneous mucosa (hair loss, photosensitivity, erythema of the extremities, and rash), hematopoietic (thrombocytopenia and leukopenia) and renal (kidney failure or renal insufficiency) [5].

Genotyping

DNA was isolated from peripheral white blood cells by a standard manual salting-out method [14]. The allelic

discrimination of *MTHFR* c.677C > T (rs1801133), *MTHFR* c.1298A > C (rs1801131), and *ABCB1* c.3435C > T (rs1045642) polymorphisms was assessed using TaqMan genotyping assays (Applied Biosystems, Foster City, CA). For quality control of the data, two reference samples (from CEPH "Centre d'Etude du Polymorphisme Humain" families) were co-genotyped with all our samples. Moreover, 10% of randomly chosen samples were genotyped in an independent experiment.

Statistical analysis

Statistical description of the sample tested was made and indicated by a mean and a standard deviation (±SD). All statistical data were described in number and frequency. Comparisons of the polymorphisms frequency distributions regarding clinical response and adverse drug reactions were performed using X^2 tests (Program Epi InfoTM version 7). *p* values were considered statistically significant at $p < 0.05$. Allele and genotype risk were assessed using odds ratio (OR) and 95% confidence interval values (CI).

The constitution of the haplotypes from the *MTHFR* c.677C > T and *MTHFR* c.1298 A > C polymorphisms and the analysis of the distribution between responders/non-responders and presence/absence of ADRs were done by the THESIAS 3.1 software [15].

Results

Description of the population

We studied 110 RA patients; the characteristics of RA group are presented in Table 1. All patients were treated with only MTX (as monotherapy, there was no DMARDs therapy combination) for at least 6 months. Our results showed that there was any difference according to the age, duration of the disease, erosion, DAS28, individual variable of DAS28, MTX dose, and corticosteroid and non steroidal anti-inflammatory drugs between the responders and non-responders group as shown in Table 1.

Among the 110 RA patients, 65 (59.09%) responded and 45 (40.9%) did not respond to MTX therapy. Among the

Table 1 Demographic and clinical characteristics of RA patients

Characteristics	RA patients ($n = 110$) *n* (%)	Responders ($n = 65$) *n* (%)	Non-responders ($n = 45$) *n* (%)	*p* value
Patients related				
Gender (female/male)	99/11	45/5	54/6	1
Age (years) (mean ± SD)	48.8 ± 13.44	42.4 ± 11.65	55.2 ± 15.23	0.6
BMI median (IQR) kg/m^2	26.12 (10.16–51.11)	28.32 (9.8–49.1)	23.92 (10.52–53.12)	0.07
Disease related				
Duration of the disease (years) (mean ± SD)	9.28 ± 8.936	8.3 ± 8.2	10.26 ± 9.672	0.09
Factor rheumatoid positivity *n* (%positive)	97 (88.18%)	54 (83.07%)	43 (95.55%)	0.7
Erosion *n* (%positive)	72 (65.45%)	42 (64.61%)	32 (66.66%)	0.08
DAS28 (mean ± SD)	3.5 ± 1.2	3.9 ± 1.1	3.1 ± 1.3	0.9
Individual variable of DAS28				
TJC (out of 28), median (IQR)	3 (0.0–25.0)	3 (0.0–26.5)	3 (0.0–23.5)	0.3
SJC (out of 28), median (IQR)	2 (0.0–23.0)	2 (0.0–20.0)	2 (0.0–26.0)	0.07
ESR, median (IQR), minutes (1st hour)	17.0 (2.0–72.0)	18.0 (2.0–60.0)	16.0 (2.0–84.0)	0.05
Global health on VAS, median (IQR)	40.0 (0.0–100.0)	39 (0.0–100.0)	41 (0.0–100.0)	0.52
HAQ score, median (IQR)	1.25 (0.0–2.5)	1.0 (0.0–2.7)	1.5 (0.0–2.3)	0.06
Treatment related				
MTX dose (mg/week) (mean ± SD)	12.67 ± 2.33	12 ± 2.254	13.34 ± 2.406	0.9
Non-corticosteroid *n* (%)	52 (47.27%)	29 (44.61%)	23 (51.11%)	1[a]
Corticosteroid *n* (%)	58 (52.72%)	35 (53.84%)	23 (51.11%)	0.62
NSAIDs *n* (%)	47 (42.72%)	37 (56.92%)	26 (57.77%)	1[a]
Non-NSAIDs *n* (%)	63 (57.27%)	28 (43.07%)	19 (42.22%)	0.9

n number, % frequency, *NSAID* non steroidal anti-inflammatory drugs, *MTX* methotrexate, *IQR* interquartile range, *Anti-CCP* anti-cyclic citrullinated peptide, *BMI* body mass index, *DAS28* disease activity score 28, *HAQ* health assessment questionnaire, *RF* rheumatoid factor, *SD* standard deviation, *SJC* swollen joints count, *TJC* tender joints count, *VAS* visual analog scale, *ESR* erythrocyte sedimentation rate

[a]Saved as reference

responders, 19 were good responders and 46 were moderate responders.

During the MTX therapy, 45 among 110 (40.9%) RA patients developed adverse drug reactions as illustrated in Table 2. Gastrointestinal reactions were the most frequent events (32/44 patients, 72.72%), however the hepatic reaction was less frequent (2/44 patients, 4.54%). The adverse drug events were observed before 3 months (mean duration to observed a ADRs = 0.22 year).

Analysis of MTHFR c.677C > T, MTHFR c.1298A > C and ABCB1 c.3435C > T polymorphisms related to the clinical response to MTX therapeutic outcome

Table 3 presents the comparison of *MTHFR* c.677C > T, *MTHFR* c.1298 A > C, and *ABCB1* c.3435C > T polymorphisms distribution between responders and non-responders groups. Our results showed no significant difference in the frequency distribution of genotypes, alleles, and haplotypes.

It should be noted that we have not obtained results for *MTHFR* c.1298 A > C polymorphism genotyping in one case.

Analysis of MTHFR c.677C > T, MTHFR c.1298 A > C, and ABCB1 c.3435C > T polymorphisms related to adverse drug reactions to MTX therapeutic outcome

In Table 4, the frequency distribution of *MTHFR* c.677C > T, *MTHFR* c.1298 A > C, and *ABCB1* c.3435C > T polymorphisms in patients with and without adverse drug reactions was similar. No significant difference of frequency distribution of genotypes, alleles, and haplotypes regarding the presence or absence of adverse drug reactions was observed.

It is noteworthy that we have not obtained results for *MTHFR* c.1298 A > C polymorphism genotyping in one case. Finally, the number of total explored patients for this polymorphism is 109 ($n = 109$).

Discussion

Description of the population

Since 30 years ago, methotrexate (MTX) has been the most used worldwide among DMARDs for RA treatment. The clinical response to MTX treatment was observed in 37.1–96% of patients [16] and the possibility to develop an adverse drug reaction was observed in 10–60% [17]. Similar results were observed in our cohort, with 59.09% of responders and 40.9% of adverse drug reactions. This observation may be due to the window of opportunity, because that there are superior clinical responses and the potential for remission when RA patients with are managed early and aggressively with disease-modifying anti-rheumatic drugs (DMARDs) [18, 19].

In the present investigation, we explore the impact of *MTHFR* c.677C > T, *MTHFR* c.1298 A > C, and *ABCB1* c.3435C > T polymorphisms on the MTX therapeutic outcome. First, in clinical response study, by the comparison of frequencies between responders group and non-responders

Table 2 Adverse drug reactions (ADRs) observed in 110 PR patients

	Adverse drug reactions (ADRs)	Frequency *n* (%)
None		65 (59.09)
General	Tiredness	1 (33.33)
	Pain	1 (33.33)
	Vertigo	1 (33.33)
Gastrointestinal	Nausea	26 (54.16)
	Abdominal pain	8 (16.66)
	Mouth ulcers	3 (6.25)
	Indigestion or anorexia	9 (18.75)
	Diarrhea	1 (2.08)
	Stomatitis	1 (2.08)
Liver	Hepatic cytolysis	2
Pulmonary	Presence of cough	3 (60)
	Difficulty breathing	1 (20)
	Pneumonia and immune-allergy	1 (20)
Cutano mucosa symptoms	Photosensitivity	3 (15.78)
	Erythema of the extremities	3 (15.78)
	Hair loss	13 (11.81)

n number, % frequency, *ADRs* adverse drug reactions

Table 3 Distribution of genotypes, frequency of alleles, and haplotypes of the *MTHFR* c.677C > T, *MTHFR* c.1298A > C, and *ABCB1* c.3435C > T polymorphisms and its correlation with clinical response of MTX

	Responders *n* (%)	Non-responders *n* (%)	*p* value	OR (95% CI)
MTHFR c.677C > T	(*n* = 65)	(*n* = 45)		
Genotypes				
677CC	24 (36.92)	17 (37.77)		1[b]
677CT	36 (55.38)	27 (60)		
677TT	5 (7.69)	1 (2.22)	0.38	0.28 [0.005–2.92][a]
677CT + 677TT	41 (63.07)	28 (62.22)	0.92	0.96 [0.43–2.11]
Allele				
677C	84 (64.61)	61 (67.77)		1[b]
677T	46 (35.38)	29 (32.22)	0.62	0.86 [0.49–1.53]
MTHFR c.1298A > C				
Genotypes	(*n* = 65)	(*n* = 44)		
1298AA	16 (14.67)	10 (22.72)		1[b]
1298AC	39 (60)	33 (75)		
1298CC	10 (15.38)	1 (2.27)	0.11	0.16 [0.003–1.51][a]
1298AC + 1298CC	49 (75.38)	34 (77.27)	0.82	1.11 [0.45–2.73]
Allele				
1298A	71 (54.61)	53 (60.22)		1[b]
1298C	59 (45.38)	35 (39.77)	0.41	0.79 [0.45–1.37]
Haplotypes	(*n* = 65)	(*n* = 44)		
C677T/A1298C				
677C/1298A	15 (23.07)	13 (29.54)		1[b]
677C/1298C	27 (41.53)	17 (38.63)	0.51	0.72 [0.27–1.89]
677T/1298A	21 (32.3)	13 (29.54)	0.51	1.13 [0.25–1.97]
677T/1298C	2 (3.07)	1 (2.27)	1	0.57 [0.009–12.51]
ABCB1 c.3435C > T	(*n* = 65)	(*n* = 45)		
Genotypes				
3435CC	20 (30.76)	13 (28.88)		1[b]
3435CT	42 (64.61)	29 (64.44)		
3435TT	03 (4.61)	03 (6.66)	0.67	1.53 [0.17–13.1][a]
3435CT + 3435TT	45 (69.23)	32 (71.11)	0.83	1.09 [0.47–2.51]
Allele				
3435C	82 (63.07)	55 (61.11)		1[b]
3435T	48 (36.92)	35 (38.88)	0.76	1.08 [0.62–1.89]

n number, % frequency, *OR* odds ratio, *CI* confidence interval, *p* significance, *RA* rheumatoid arthritis

[a]Fisher exact test

[b]Genotype/allele /haplotype saved as reference category

group second in adverse drug reactions study, by the comparison of frequencies between the presence and absence of adverse drug reactions.

Analysis of MTHFR c.677C > T and MTHFR c.1298A > C polymorphisms related to the clinical response and to adverse drug reactions to MTX therapeutic outcome

The current study showed first that the genetic polymorphisms of *MTHFR* c.677C > T and *MTHFR* c.1298 A > C do not influence a clinical response to MTX treatment in RA patients of our study. Two recent studies on the RA Indian and Italian patients agreed that there was no association between *MTHFR* c.677C > T and *MTHFR* c.1298 A > C polymorphisms and efficacy of MTX treatment in RA patients [20, 21]. Two recent meta-analyses on the Spanish and UK populations suggest that the *MTHFR* genetic variants can be considered as no pharmacogenetic markers of MTX outcome in RA patients [22, 23]. Another study of 150 RA patients in the Indian population suggested that *MTHFR* c.677C > T polymorphism was not predictor of the MTX efficacy even with folate supplementation [24]. However, Wessels et al. showed that the *MTHFR* 1298AA

Table 4 Distribution of genotypes, frequency of alleles, and haplotypes of the *MTHFR* c.677C > T, *MTHFR* c.1298A > C, and *ABCB1* c.3435C > T polymorphisms and its correlation with drug adverse reactions (ADRs) of MTX

	Presence of ADRs *n* (%)	Absence of ADRs *n* (%)	*p* value	OR (95% CI)
MTHFR c.677C > T	(*n* = *45*)	(*n* = *65*)		
Genotypes				
677CC	16 (35.55)	17 (37.77)		1[b]
677CT	28 (62.22)	27 (60)		
677TT	1 (2.22)	1 (2.22)	0.28	3.2 [0.34–29.96]
677CT + 677TT	29 (64.44)	28 (62.22)	0.75	0.88 [0.40–1.94]
Allele				
677C	60 (66.66)	85 (65.38)		1[b]
677T	30 (33.33)	45 (34.61)	0.84	1.05 [0.6–1.87]
MTHFR c.1298A > C	(*n* = 45)	(*n* = 64)		
Genotypes				
1298AA	7 (15.55)	19 (29.68)		1[b]
1298AC	33 (73.33)	39 (60.93)		
1298CC	5 (11.11)	6 (9.37)	0.27	0.44 [0.10–1.92]
1298AC + 1298CC	38 (84.44)	45 (70.31)	0.08	0.43 [0.16–1.14]
Allele				
1298A	47 (52.22)	77 (60.15)		1[b]
1298C	43 (47.77)	51 (39.84)	0.24	0.72 [0.42–1.24]
Haplotypes	(*n* = 45)	(*n* = 64)		
C677T/A1298C				
677C/1298A	16 (35.55)	25 (39.06)		1[b]
677C/1298C	14 (31.11)	17 (26.56)	0.6	0.77 [0.30-2]
677T/1298A	8 (17.77)	13 (20.31)	0.94	0.84 [0.35–3.06]
677T/1298C	7 (15.55)	9 (14.06)	0.74	0.82 [0.25–2.65]
ABCB1 c.3435C > T	(*n* = 45)	(*n* = 65)		
Genotypes				
3435CC	13 (28.88)	20 (30.76)	1[b]	
3435CT	27 (60)	44 (67.69)		
3435TT	5 (11.11)	1 (1.53)	0.07	0.13 [0.002–1.42][a]
3435CT + 3435TT	32 (71.11)	45 (69.23)	0.91	0.95 [0.41–2.19]
Allele				
3435C	53 (58.88)	84 (64.61)		1[b]
3435T	37 (41.11)	46 (35.38)	0.38	0.78 [0.45–1.36]

n number, % frequency, *OR* odds ratio, *CI* confidence interval, *p* significance, *RA* rheumatoid arthritis

[a]Fisher exact test

[b]Genotype/allele /haplotype saved as reference category

genotype was correlated with efficacy to MTX outcome [25]; this observation could be explained by the presence of another therapy combined to MTX treatment. The study performed on the RA Portuguese patients demonstrated that the *MTHFR* 677TT genotype was associated with over fourfold increased risk for non-response [26]. Moreover, the haplotype *MTHFR* 677C/1298A was associated with efficacy of MTX treatment in the RA USA patients [27], but the haplotype 677T/1298A was correlated with inefficacy in the RA Japanese patients [28].

Our work also suggested that the *MTHFR* c.677C > T and *MTHFR* c.1298A > C polymorphisms were not predicted to adverse drug reactions to MTX treatment in RA patients of our study. In fact, our results are in line with findings of other studies on RA Indian and Italian patients [17, 20]. Interestingly, the *MTHFR* 677TT genotype was associated with reactions for neural system in the RA American population [27]. In Poland population, the MTHFR 677CC genotype was associated with a reduction in the number of MTX-related adverse events [28]. In the RA Japanese patients, the correlation between *MTHFR* 677T allele and MTX-related side effects was also reported [29, 30]. The same, in Jordanian population, there was an association between *MTHFR* c.677 C > T polymorphism

and any MTX toxicity [31]. In another study, the *MTHFR* c.677C > T polymorphism was not related with MTX response, but *MTHFR* c.1298A > C polymorphism was related with MTX toxicity [32]. In RA Israel patients, the homozygous *MTHFR* 1298CC genotype was associated with the presence of adverse events [33]. A meta-analysis reported by Song GG et al. suggested that the *MTHFR* c.677C > T and *MTHFR* c.1298A > C polymorphisms are associated with MTX toxicity in a South Korean population [6]. In contrast, a study performed on the RA Slovenian patients showed that the *MTHFR* c.1298A > C polymorphism had a protective effect on overall MTX toxicity [34].

Analysis of ABCB1 c.3435C > T polymorphism-related polymorphisms related to the clinical response and to adverse drug reactions to MTX therapeutic outcome

Second, the present study showed that the *ABCB1* c.3435C > T polymorphism did not present any significant distribution of genotypes and alleles frequencies between the two groups: responders and non-responders in RA patients of our study. Two studies on the RA Dutch and Jordanian populations founded similar results [35, 36]. In RA Japanese patients, Tatkatori et al. estimated that patients with genotype homozygous *ABCB1* 3435TT were more non-responders than patients with genotype *ABCB1* 3435CC [37]. In contrast, the correlation between the *ABCB1* 3435CT + 3435CC genotypes and ineffectiveness to MTX treatment was reported in the RA German patients [38]. In Indian population, the *ABCB1* c.3435C > T polymorphism was conferred with a poor response [39]. Another study on the RA Chinese patients suggests that this polymorphism in *ABCB1* gene may influence the efficacy of some anti-rheumatic drugs [9]. The same, in RA Polish patients, Pawlik et al. showed that *ABCB1* c.3435C > T polymorphism may have an influence on response to therapy with DMARDs [38].

Our findings also suggested that the *ABCB1* c.3435C > T polymorphism was not predicted to adverse drug reactions of MTX therapeutic outcome in RA patients. This result was similar to the previous study reported on the RA Dutch and Japanese patients [35, 37]. While, in another study, this polymorphism was associated with methotrexate toxicity in RA Slovenian patients [34]. In RA Jordanian patients, the *ABCB1* c.3435C > T polymorphism seems to affect the toxicity [36]. The effect of this variant on toxicity was confirmed by a recent study performed in the RA Spanish patients [10]. In another hand, many study analyses confirmed that the *ABCB1* c.3435C > T polymorphism is associated with other polymorphisms of the ABCB1 gene [40–42]. The most frequently studied haplotype consists of this polymorphism combined with the *ABCB1* c. 2677G > T/A polymorphism and/or the *ABCB1* c. C1236C > T polymorphism which have a strong linkage imbalance between these SNPs and have demonstrated in several studies [41].

Multiple factors such as RA disease duration, autoantibody rheumatoid factor (RF), or smoking status can influence the response to different medications in RA patients [43]. These can explain why the genetic variances do not correlate to outcomes.

The most important limitation of our study could be due to the small sample size. The main influence of a small sample size in one it has on statistical power: as sample size is increased, power increases.

Conclusion

In conclusion, our results represent the first report in West Algerian population that demonstrate the absence of correlation between *MTHFR* c.677C > T and *MTHFR* c.1298A > C and *ABCB1* c.3435 C > T polymorphisms and the MTX response in RA patients. These results have to be replicated in a larger sample to confirm their involvement on the MTX therapeutic outcome in RA. On the other hand, it will be interesting to study other polymorphisms involved in the pharmacogenetic of MTX that might be responsible for the variability of clinical response.

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