Impact of MTHFR rs1801133, MTHFR rs1801131 and ABCB1 rs1045642 polymorphisms with increased susceptibility of rheumatoid arthritis in the West Algerian population: a case-control study

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ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune disease that results in a chronic systemic inflammation. A few genetic epidemiologic studies found a potential association between genetic polymorphisms C677T (rs1801133) and A1298C (rs1801131) of methylenetatrahydrofolate reductase *(MTHFR)* gene and C3435T (rs1045642) of ATP-Binding cassette *(ABCB1)* gene and the increased risk for RA.

The aim of this case-control study was to determine the relationship between these polymorphisms and RA susceptibility in West Algerian population.

The dataset of the current study is composed of 110 RA patients and 101 healthy controls. All samples were genotyped for theses polymorphisms by TaqMan® allelic discrimination assay. Data were compared between cases and controls by the calculation of the odds ratio (OR) with a confidence interval at 95%.

After age and RA erosion-stratified analyzes, no differences in genotypes or alleles frequencies distribution were found for *MTHFR* C677T (rs1801133) and *ABCB1* C3435T (rs1045642) polymorphisms between RA cases and controls. However, the *MTHFR* A1298C (rs1801131) polymorphism presented a significant distribution in RA with age ≥ 40 (Genotypic data: p=0.007, OR=13.53[1.44-63.31], Allelic data: p=0.001, OR=2.39[1.39-4.1]), and in RA erosive form (Genotypic data: p=0.002, OR=6.92[1.68-30.23], Allelic data: p=0.0001, OR=2.43[1.54-3.85]).These results were confirmed after the Bonferroni correction.

In this study we have showed, for the first time in the West Algerian population, that the *MTHFR* A1298C (rs1801131) polymorphism can be associated with rheumatoid arthritis.

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INTRODUCTION

Rheumatoid arthritis (RA) is the most common form of chronic inflammatory rheumatism. It is an autoimmune and degenerative disease, which is characterized by arthritis often bilateral and symmetrical, relapsing into the deformation and destruction of joints[1].

The prevalence of RA has been estimated approximately at 1% of the world population[2,3] and in Algeria at 0.15 % of the adult population (incidence 30.000)[4].

As most human diseases, the RA has genetic components. Several genes implicated in immune function were involved in RA susceptibility such as: major histocompatibility complex (*MHC*) genes, cytotoxic T lymphocyte antigen 4 (*CTLA4),* protein tyrosine phosphatase non-receptor type 22 (*PTPN22),* interleukin 10 *(L10)*[5]. However, a few studies of RA susceptibility were interested by the polymorphisms of genes involved in folate pathway.

The folate pathway interfere with other pathways such the homocysteine, the *de novo* purine and pyrimidine synthesis. Moreover, the aminoimidazole carboxamide adenosine ribonucleotide (AICAR) transformylase (ATIC) is an enzyme involved in the *de novo* purine synthesis pathway responsible for the conversion of AICAR into formyl-AICAR (FAICAR). In fact, the effect of deficiencies in folate pathway is to lead to adenosine production that will conduct to an increasing of

pro-inflammatory cytokine secretion[6].

The methylenetetrahydrofolate reductase *MTHFR* enzyme is essential for intracellular folate homeostasis and metabolism[7]. It converts the 5 tetrahydrofolate (5-methyl-THF) to the 5,10-methyl tetrahydrofolate (5,10-methyl-THF) which catalyzes the conversion of homocysteine to Methionine, necessary to the methylation reactions of nucleotides in DNA, RNA and proteins[8,9]. The folate deficiency induces a hyper-homocysteine that will lead to an increasing of pro-inflammatory cytokine secretion[7]. On the other hand, the 5,10-methyl-THF is required in the conversion of Uracil to Thymine (pyrimidine synthesis)[10].

The *MTHFR* enzyme has several crucial cellular processes; its deficiency increases the secretion of pro-inflammatory cytokine and the joint lesions in synovial tissues by incorporation of uracil[11]. Many functional polymorphisms were reported in *MTHFR* gene[8] and two of them, C677T (Ala222Val, rs1801133) and A1298C (Glu429Ala, rs1801131) are the most studied because the alleles *MTHFR* 677T and *MTHFR* 1298C reduce the expression of *MTHFR* enzyme[8]. In some previous studies, the effect of *MTHFR* C677T (rs1801133) and *MTHFR* A1298C (rs1801131) polymorphisms on RA susceptibility gave a variable results[11,12].

Another gene implicated in folate metabolism, the ATP-Binding cassette subfamily B, member 1 (*ABCB1*) gene has interested a few researches. The *ABCB1* gene code for a P-glycoprotein (P-gp)[13] that acts as a pump for folate, cytokine (particularly IL-1, IL-2, IL-4 and IFN- [14]) and drug transport[15]. The *ABCB1* C3435T polymorphism (rs1045642) is the most studied because the allele *ABCB1* 3435T decreases the enzymatic activity of P-gp[16], and then reduces the number of the transporters in the cell[16]. This lack of transporters induces secretion disequilibrium of cytokine[15]. In fact, *Tsujimura et al.* have demonstrated that the level of P-gp expression on RA lymphocytes was closely correlated with the disease in each patient[17]. For that, the *ABCB1* C3435T (rs1045642) polymorphism was already studied in RA susceptibility[18,19].

Many roles of these genes make a plausible hypothesis that their polymorphisms may be considered as a predictive factors associated with the increased susceptibility for RA; therefore, the aim of the current study is to determine the relationship between *MTHFR* C677T (rs1801133), *MTHFR* A1298C (rs1801131) and *ABCB1* C3435T (rs1045642) polymorphisms and RA susceptibility in a sample of West Algerian population.

PATIENTS AND METHODS

DATASET

Our dataset was collected from the West Algerian population which is composed of 110 RA patients and 101 healthy controls. Each participant gave his informed consent to participate in the study. The subjects were randomly selected in rheumatology service of Hospital-University Center of Oran (CHUO, Oran, Algeria). The RA patients were diagnosed according to the revised criteria of American College of Rheumatology (ACR) in 1987[20] and reclassified according to ACR/European League Against Rheumatism (EULAR) criteria in 2010[21]. The patients included in this study have been already treated with MTX as monotherapy for at least 6 months (mean dose of MTX =12.67 mg/ /week). The MTX was prescribed as a treatment in the first-line (dose of MTX= 15 mg/week). To prevent its 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). Clinical data were established by the blood analysis, the revision of the medical records and questioning of each patient including age, sex, disease history, smoking habits and family history. The group of controls had no history of RA or any chronic disease.

The patients and controls groups were stratified according age and RA erosion. For age adjustment two groups were set as follow: under 40 years and over 40 years. After the RA erosion stratification, two groups were developed: presence and absence of RA erosive form.

GENOTYPING

DNA was isolated from peripheral white blood cells by a standard manual salting-out method[22]. The allelic discrimination of *MTHFR* C677T (rs1801133), *MTHFR* A1298C (rs1801131) and *ABCB1* C3435T (rs1045642) polymorphisms were assessed using Taq--Man genotyping assays (Applied Biosystems, Foster City, CA). For a reproductive data, two reference samples (from CEPH "Centre d'Etude du Polymorphisme Humain" families) were co-genotyped with all our samples. Moreover, ten percent of randomly chosen samples were genotyped in an independent experiment.

STATISTICAL ANALYSIS

Statistical description for continuous variables of the tested samples was indicated by a mean and a standard deviation (±SD); for categorical variables the statistical data were described in number and frequency.

Chi-square (χ^2) test was performed for each polymorphism to determine whether the control sample demonstrated Hardy-Weinberg equilibrium.

Comparisons of the distribution of the demographic variables (Age, gender) between cases and controls and the polymorphisms frequencies distribution regarding cases and controls study were performed with Program Epi InfoTM version 7. P values were considered as statistically significant when p<0.05. Alleles and genotypes risk were assessed using Odds Ratio (OR) and confidence interval values (CI) at 95%. The Bonferroni correction threshold for a significance of association at 0.0166 was mentioned considering the number of test performed. Multivariate analysis regressions were used to calculate the odds ratios (for RA status, RA with erosive form and controls) and were adjusted for age, gender, BMI and smoking status. These analyses were performed with the SAS version 9.1 software (SAS Institute Inc., Cary, NC, USA).

To test linkage disequilibrium between *MTHFR* C677T (rs1801133), *MTHFR* A1298C (rs1801131) polymorphisms we have calculated the *D*' using the Haploview 4.2 software. The THESIAS 3.1 software[23] was required to determine the haplotypes from genotypes between these polymorphisms and for the analysis of the haplotypes distribution between cases and controls groups.

RESULTS

DATASET DESCRIPTION

We studied 110 RA patients and 101 controls; the characteristics of this dataset are presented in Table I. The distribution of gender and age is similar between cases and controls, which indicate a matched dataset study. In fact, the mean age of RA cases was 48.8 ±13.4 even as mean age of controls 47.3±15.3 (p value=0.6). Furthermore, the sex ratio in RA cases was 99 females per 11 males while in the controls it was 88 females per 13 males (p value=0.5).

MTHFR C677T (RS1801133), *MTHFR* A1298C (RS1801131) AND *ABCB1* C3435T (RS1045642) POLYMORPHISMS AFTER AGE AND RA EROSION STRATIFICATIONS

MTHFR C677T (RS1801133) AND *MTHFR* A1298C (RS1801131) POLYMORPHISMS

All genotypes distribution in control subjects were in Hardy-Weinberg equilibrium for *MTHFR* C677T (rs1801133) and *MTHFR* A1298C (rs1801131) polymorphisms (respectively: p=0.16 and p= 0.30).

The total of the explored healthy group was equal to 89 for *MTHFR* C677T (rs1801133) polymorphism, because twelve controls could not be analyzed. After age and RA erosion adjustments, there was no significant frequencies distribution of the *MTHFR* C677T (rs1801133) polymorphism between cases and controls (Tables II, III).

For the *MTHFR* A1298C (rs1801131) polymorphism, we don't obtain any result concerning four controls and one case; so the total of healthy and cases samples was 97 and 109 subjects respectively. We observed a significant frequencies distribution for the *MTHFR* 1298CC genotype between RA cases with age ≥ 40 and controls (p=0.007, OR=13.53 [1.44-63.31]). This significance was increased when we consider genotypes with at least one C allele (*MTHFR* 1298CT+ 1298CC) (p=0.0003, OR= 4 [1.84-8.69]). The association was confirmed by allelic data analysis (p=0.001, OR=2.39 [1.39 -4.1]) (Table II). Similarly, after RA erosion stratification (Table III), a significant frequencies distribution was found between RA patients with erosion and healthy subject for the *MTHFR* A1298C (rs1801131) polymorphism (Genotypic analysis: p= 0.002, OR=6.92 [1.68-30.23], Allelic analysis: p=0.0001, OR= 2.43 [1.54-3.85]). These results were confirmed after the Bonferroni correction.

Moreover, the haplotype analyses showed that these two polymorphic sites are in high linkage disequilibrium (LD) (D =95). After the analysis of the distribution of differences haplotypes between cases and controls, the results showed that the *MTHFR* 677C/1298C haplotype was significantly under-represented in RA cases with age≥ 40 and RA patients with erosive form (respectively: p=0.00005, OR=10.41 [2.85-42.12] and p= 0.0002, OR= 4.6 [2.07-10.21]) (Tables II, III). The 677T/1298A haplotype also presented a significant frequencies distribution between RA patients with age≥ 40 and controls group (p=0.004, OR= 3.58 [1.45-8.83]) and between RA cases with erosive form and healthy subjects (p=0.001, OR= 3.72 [1.58-8.71]).These significant distributions were found after the Bonferroni test.

ABCB1 C3435T (RS1045642) POLYMORPHISM

All distributions in control subjects were in Hardy-Weinberg equilibrium for *ABCB1* C3435T (rs1045642) polymorphism (p=0.39).

TABLE I. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF RA PATIENTS AND CONTROLS

Variables	RA patients (n=110) n (%)	RA patients with erosive disease (n=72) n (%)	Controls (n=101) n (%)	p value
Patients related				
Gender (female/male)	99/11	65/7	88/13	0.9
Age (years) (mean± SD)	48.8 ±13.4	46.2±14.4	47.3±15.3	0.2
BMI median (IQR) kg/m2	26.12 (10.16-51.11)	26 (10.1-51)	26.3 (11.1- 48.9)	0.5
Current smokers, n (%)	2	1	3	0.1
NPY, median (IQR)	18.6 (0.8–118.0)	18.6 (0.8-118.0)	17.2 (1.1- 101)	0.5
Disease related				
Duration of the disease (years) (mean± SD)	9.2 ±8.9	8.3±8.2		0.2
FR positivity n (%positive)	97 (88.18%)	63 (87.5%)		0.09
Anti-CCP n (%positive)	99 (90%)	66 (91.6%)		0.7
Erosion n (%positive)	72 (65.45%)	72 (100%)		0.06
DAS28 (mean± SD)	3.5± 1.2	3,4 ±1.1		0.5
Individual variable of DAS28				
TJC (out of 28), median (IQR)	3 (0.0-25.0)	3 (0.0-23.5)		0.1
SJC (out of 28), median (IQR)	2 (0.0-23.0)	2 (0.0-26.0)		0.08
ESR, median (IQR), minutes (1st hour)	17.0 (2.0-72.0)	16.0 (2.0–84.0)		0.06
Global health on VAS, median (IQR)	40.0 (0.0-100.0)	41 (0.0-100.0)		0.1
HAQ score, median (IQR)	1.0 (0.0-2.5)	1.1 (0.0-2.3)		0.09
Treatment related				
MTX Dose (mg/week) (mean± SD)	12.67±2.33	12±2.254		0.05
Duration of MTX treatment (months) (mean± SD)	28.5±33.08	25.3 ± 32.1		0.5
Non- Corticosteroid n (%)	52 (47.27%)	32 (44.44%)		Reference
Corticosteroid n (%)	58 (52.72%)	40 (55.55%)		0.2
Non- NSAIDs n (%)	63 (57.27%)	39 (54.16%)		0.5
NSAIDs n (%)	47 (42.72%)	33 (45.83%)		Reference

n: number, %: frequency, NPY: number of cigarettes smoked per day × number of years smoking/20, MTX: methotrexate, NSAID: non steroidal anti-inflammatory drugs, 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

After age adjustment, the genotypes *ABCB1* 3435TT +3435CT were more frequent in the RA group with age <40 than in the healthy group (p=0.03, OR= 3.51 [1.02-12.09]) (Table II); these genotypes were also more frequent in the RA group with age ≥ 40 than in controls group (p=0.03, OR= 2.07 [1.03-4.16]). No difference of frequencies distribution was found when we analyze the allelic data. After the Bonferroni correction, no association was found between the *ABCB1* 3435TT +3435CT genotypes and increased risk for RA.

Considering the RA erosion-stratified analysis, results showed a significant difference of frequencies distribution of the *ABCB1* 3435TT +3435CT genotypes between RA patients with erosive form and controls group (p=0.02, OR=2.11 [1.11-4.01] (Table III). However, after the Bonferroni correction this significant distribution was lost.

DISCUSSION

In the present work, we explore the impact of *MTHFR* C677T (rs1801133), *MTHFR* A1298C (rs1801131) and *ABCB1* C3435T (rs1045642) polymorphisms on RA susceptibility, in a case-control study performed on a West Algerian population.

TABLE II . DISTRIBUTION OF GENOTYPES AND FREQUENCY OF ALLELES OF THE MTHFR C677T, MTHFR A1298C AND ABCB1 C3435T POLYMORPHISMS AND ITS ASSOCIATION WITH RISK OF RA AFTER AGE ADJUSTMENT

	Age < 40				Age≥ 40			
	Cases n (%)	Controls n (%)	p value	OR (95% CI)	Cases n (%)	Controls n (%)	p value	OR (95% CI)
MTHFR C677T, rs1801133 Genotypes	(*n=38*)	(*n=39*)			(*n=72*)	(*n=50*)		
677CC	19 (50)	24 (61.5)		1[b]	22 (30.5)	24 (48)		1[b]
677CT	15 (39.4)	14 (35.8)			48 (66.6)	24 (48)		
677TT	4 (10.5)	1 (2.5)	0.17	5.05 [0.43-258.96][a]	2 (2.7)	2 (4)	1	1.09 [0.07-16.22][a]
677CT+677TT	19 (50)	15 (38.4)	0.30	1.60 [0.64-3.95]	50 (69.4)	26 (52)	0.05	2.09 [0.99-4.43]
Alleles								
677C	53 (69.7)	62 (79.4)		1[b]	92 (63.8)	72 (72)		1[b]
677T	23 (60.5)	16 (20.5)	0.16	1.68 [0.8-3.51]	52 (36.1)	28 (28)	0.18	1.45 [0.83-2.52]
MTHFR A1298C, rs1801131 Genotypes	(*n=38*)	(*n=41*)		(*n=71*)	(*n=56*)			
1298AA	10 (26.3)	21 (51.2)		1[b]	15 (21.1)	29 (51.7)		1[b]
1298AC	24 (63.1)	16 (39.02)		49 (73.8)	26 (46.4)			
1298CC	4 (10.5)	4 (9.7)	0.42	2.1 [0.43-10.16][a]	7 (9.8)	1 (1.7)	**0.007***	13.53 [1.44-63.31][a]
1298AC+1298CC	28 (73.6)	20 (48.7)	0.05	2.94 [1.14-7.5]	56 (78.8)	27 (48.2)	**0.0003***	4 [1.84-8.69]
Alleles								
1298A	44 (57.8)	58 (70.7)		1[b]	79 (55.6)	84 (75)		1[b]
1298C	32 (42.1)	24 (29.2)	0.09	1.75 [0.9-3.39]	63 (44.3)	28 (25)	**0.001***	2.39 [1.39-4.1]
Haplotypes C677T/A1298C	(*n=38*)	(*n=39*)		(*n=71*)	(*n=50*)			
677C/1298A	16 (42.1)	21 (53.8)		1[b]	12 (16.9)	25 (50)		1[b]
677C/1298C	19 (50)	15 (38.4)	0.20	1.66 [0.65-4.25]	25 (35.2)	5 (10)	**0.00005***	10.41[2.85-42.12][a]
677T/1298A	2 (5.2)	1 (2.5)	0.50	2.62 [0.12-162.1][a]	31(43.6)	18 (36)	**0.004***	3.58 [1.45-8.83]
677T/1298C	1 (2.6)	2 (5.1)	1	0.65 [0.05-7.89][a]	3 (4.2)	2 (4)	0.31	3.12 [0.45-21.25][a]
ABCB1 C3435T, rs1045642 Genotypes	(*n=38*)	(*n=41*)		(*n=72*)	(*n=60*)			
3435CC	4 (10.5)	12 (29.2)		1[b]	29 (40.2)	35 (58.3)		1[b]
3435CT	32 (84.2)	24 (58.5)		39 (54.1)	17 (28.3)			
3435TT	2 (5.2)	5 (12.1)	1	1.2 [0.08-12.08][a]	4 (5.5)	8 (13.3)	0.53	0.60 [0.12-2.55][a]
3435CT+3435TT	34 (89.4)	29 (70.7)	0.03	3.51 [1.02-12.09][a]	43 (59.7)	25 (41.6)	0.03	2.07 [1.03-4.16]
Alleles								
3435C	40 (52.6)	48 (58.5)		1[b]	97 (67.3)	87 (72.5)		1[b]
3435T	36 (47.3)	34 (41.4)	0.45	1.27 [0.67-2.38]	47 (65.2)	33 (27.5)	0.36	1.27 [0.75-2.17]

n: number, %: frequency, OR: odds ratio, CI: confidence interval, p: significance, b: genotype saved as reference category, RA: rheumatoid arthritis, p: p value and considered as statistically significant at $p<0.05$, *: p still statistically significant after Bonferroni correction, a: fisher exact test.

TABLE III. DISTRIBUTION OF GENOTYPES AND FREQUENCY OF ALLELES OF THE MTHFR C677T, MTHFR A1298C AND ABCB1 C3435T POLYMORPHISMS AND ITS ASSOCIATION WITH RISK OF RA AFTER RA EROSION ADJUSTMENT

	Erosion	Controls			Non--erosion		
	n (%)	n (%)	p value	OR (95% CI)	n (%)	p value	OR (95% CI)
MTHFR C677T, rs1801133 Genotypes	**(*n*=72)**	**(*n*=89)**		**(*n*=38)**			
677CC	28 (38.8)	48 (53.9)		1[b]	13 (34.2)		1[b]
677CT	41 (56.9)	38 (42.6)			22 (57.8)		
677TT	3 (4.1)	3 (3.3)	0.66	1.71 [0.21-13.6]	3 (7.8)	0.14	3.69 [0.66-20.48][a]
677CT+677TT	44 (61.11)	41 (46.06)	0.06	1.83 [0.97-3.45]	25 (65.7)	0.05	2.25 [1.02-4.95]
Alleles							
677C	97 (67.3)	134 (75.2)		1[b]	48 (63.1)		1[b]
677T	47 (32.6)	44 (24.7)	0.06	1.57 [0.96-2.55]	28 (36.8)	0.05	1.77 [0.99-3.16]
MTHFR A1298C rs1801131 Genotypes	**(*n*=71)**	**(*n*=97)**		**(*n*=38)**			
1298AA	13 (18.3)	50 (51.5)		1[b]	12 (31.5)		1[b]
1298AC	49 (69.01)	42 (43.2)			24 (63.1)		
1298CC	9 (12.6)	5 (5.1)	**0.002***	6.92 [1.68-30.23][a]	2 (5.2)	0.60	1.66 [0.14-11.72][a]
1298AC+1298CC	58 (81.6)	47 (48.4)	**0.00001***	4.7 [2.3-9.76]	26 (68.4)	0.05	2.3 [1.04-5.08]
Alleles							
1298A	75 (52.8)	142 (73.1)		1[b]	48 (63.1)		1[b]
1298C	67 (47.1)	52 (26.8)	**0.0001***	2.43 [1.54-3.85]	28 (36.8)	0.13	1.59 [0.9-2.8]
Haplotypes C677T/A1298C	**(*n*=71)**	**(*n*=87)**		**(*n*=38)**			
677C/1298A	16 (22.5)	46 (52.8)		1[b]	12 (31.5)		1[b]
677C/1298C	32 (45.07)	20 (22.9)	**0.0002***	4.6 [2.07-10.21]	12 (31.5)	0.08	2.3 [0.88-5.98]
677T/1298A	22 (30.9)	17 (19.5)	**0.001***	3.72 [1.58-8.71]	11 (28.9)	0.06	2.4 [0.92-6.67]
677T/1298C	1 (1.4)	4 (4.5)	1	0.71 [0.01-8.03][a]	3 (7.8)	0.30	2.8 [0.36-19.27][a]
ABCB1 C3435T, rs1045642 Genotypes	**(*n*=72)**	**(*n*=101)**		**(*n*=38)**			
3435CC	21 (29.1)	47 (46.5)		1[b]	12 (31.5)		1[b]
3435CT	49 (68.05)	41 (40.5)			22 (57.8)		
3435TT	2 (2.7)	13 (12.8)	0.24	0.34 [0.03-1.75][a]	4 (10.5)	0.70	1.2 [0.33-4.36][a]
3435CT+3435TT	51 (70.8)	54 (53.4)	0.02	2.11 [1.11-4.01]	26 (68.4)	0.11	1.88 [0.85-4.14]
Alleles							
3435C	91 (63.1)	135 (66.8)		1[b]	46 (60.5)		1[b]
3435T	53 (36.8)	67 (33.1)	0.42	1.17 [0.74-1.83]	30 (39.4)	0.33	1.31 [0.76-2.26]

n: number, %: frequency, OR: odds ratio, CI: confidence interval, p: significance, b: genotype saved as reference category, RA: rheumatoid arthritis, p: p value and considered as statistically significant at p<0.05, ,*: p still statistically significant after Bonferroni correction, a: fisher exact test.

DATASET DESCRIPTION

According to the univariate and multivariate analyses, our results demonstrated no association between clinic--pathological variables and increased risk for RA. However, some studies have shown a potential effect of patient's related variable on RA susceptibility beyond gender and age. In previous study, the immunosuppressive and anti-inflammatory effects of cigarette nicotine were demonstrated[24,25]. In other study, it was founded that this variable seems to reduce radiogra-

phic progression[26]. Also, the alcohol and caffeine impact on RA susceptibility was shown[27,28,29]. Moreover, a correlation between many patients' related variables and response to Methotrexate therapy on RA patients was demonstrated such as tobacco and anti-CCP[30]. However, in multivariate analyses these two variants in the *MTHFR* gene were not associated with response to MTX therapy[31].

MTHFR C677T (RS1801133), *MTHFR* A1298C (RS1801131) AND *ABCB1* C3435T (RS1045642) POLYMORPHISMS AFTER AGE AND RA EROSION STRATIFICATIONS

MTHFR C677T (RS1801133) AND *MTHFR* A1298C (RS1801131) POLYMORPHISMS

According to our results, the minor allele frequency (MAF) of *MTHFR* A1298C (rs1801131) polymorphism is *MTHFR* 1298C allele (26.8 %) and it is in line with the published reports [32, 33]. Regarding the frequency of *MTHFR* C677T (rs1801133) polymorphism, the minor allele *MTHFR* 677T (24.7%) is slightly different than the one found by *Hambaba L et al*. study[34] on the East Algerian population; this variation could be due to ethnic and geographic differences in Algerian dataset. However, our MAF of *MTHFR* 677T allele is equivalent to those reported in Moroccan, Turkish and Spanish populations[35,32,36].

Our case-control study (Table II) showed that the *MTHFR* A1298C polymorphism were significantly associated with RA with age ≥40 and erosive RA. Previous studies have showed that, the homozygous 1298 CC confers an increased risk for RA in Italian and Jewish populations[11,12]. On Turkish population, the *MTHFR* C677T (rs1801133) was associated with susceptibility of a person for development of RA[37]. However, these associations were not confirmed by Hughes's study on American population. In fact, the allele frequencies were founded similar between patients with rheumatoid arthritis and controls of the same racial (Caucasian, African-Americans) or ethnic group[38]. The Genome-wide association study of rheumatoid arthritis on the Spanish population reported no association between these *MTHFR* polymorphisms and susceptibility to RA[39].

Moreover, haplotype analyses showed that these two polymorphic sites are in high linkage disequilibrium (LD), as showed in previous study[40]. The same results were found in the previous study[19]. However, a large-scale epidemiological study has evidenced that the haplotype-based approach (the combined effect of *MTHFR* C677T and *MTHFR* A1298C polymorphisms) is more predictive than the single *MTHFR* polymorphism for total plasma homocysteine level[41]. In fact, based on a specific combination of these polymorphisms, there was proposed a possible model of the MTHFR enzyme dimmer arranged[40]. In our study we showed that, the *MTHFR* 677C/1298C and 677T/ /1298A haplotypes may present a significant association with this disease with age ≥40 and erosive form (Tables II, III).

ABCB1 C3435T (RS1045642) POLYMORPHISM

The distribution of *ABCB1* C3435T (rs1045642) polymorphism in control group shows that the minor allele is *ABCB1* 3435T (33.1%). Our result is in accordance with the study realized on Turkish population which found that the *ABCB1* 3435T frequency is the same as our findings[42], but not with the one on English population[43].

We have also studied the impact of *ABCB1* C3435T (rs1045642) polymorphism on RA susceptibility (Table II). The result of this case-control study suggests no association between *ABCB1* C3435T (rs1045642) polymorphism and susceptibility to RA. *Pawlik and al*. concluded in his study on Poland population, that the *ABCB1* C3435T (rs1045642) polymorphism is not an important factor that increase susceptibility for RA[18]. In the same way, in another study conducted on a Chinese population, the *ABCB1* C3435T (rs1045642) was not related to the RA occurring[44].

The difference in results between this present study and the studies describing associations between these polymorphisms explored and the occurrence of RA may be due to differences of allele frequencies between ethnic groups, genetic heterogeneity in pathogenesis of RA and different factors such as infectious, psychology and hormonal factors. The most important limitation of our study could be due to the small sample size. In fact a small sample size affects the precision of the statistical results. Another limitation of this study was that the cases were selected from just one hospital. Actually, the identified risk factors could be unique to that single hospital.

It will be interesting to explore other polymorphisms that affect the proteins implicated in folate metabolism such as the transporter solutes carriers (SLC) that were reported previously as having an important impact on MTX metabolism[30,45], can also influence the

increase the RA susceptibility. Moreover, many studies have explored the impact of *MTHFR* C677T, *MTHFR* A1298C and *ABCB1* C3435T polymorphisms on Methotrexate (MTX) therapeutic outcome in RA patients[30,45,46], because the MTX acts as a folate antagonist. It will be necessary to perform a pharmacogenetic study on our population.

CONCLUSION

In conclusion, for the first time in the West Algerian population, we have showed that the *MTHFR* A1298C (rs1801131) polymorphism was associated with rheumatoid arthritis with age ≥40 and RA erosive form. The *MTHFR* C677T (rs1801133) and *ABCB1* C3435T (rs1045642) polymorphisms were identified as having a null effect. These results have to be replicated on a larger sample to confirm the involvement of these polymorphisms in RA genetic and it probably will be interesting to explore other polymorphisms involved in folate metabolism.

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REFERENCES

1. Hurkmans EJ, van der Giesen FJ, Bloo H, Boonman DC, van der Esch M, Fluit M. Physiotherapy in rheumatoid arthritis: development of a practice guideline. Acta Reumatol Port 2011;36(2):146-158.
2. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med 2011;365(23):2205-2219.
3. Moreland L. Unmet needs in rheumatoid arthritis. Arthritis Res Ther 2005;7:S2-S8.
4. Slimani S, Kharfallah B, Ladjouze-Rezig A. Prevalence of rheumatoid arthritis in Barika—Algeria. Rheumatology (Oxford) 2014; 53(3):571–573.
5. Rego-Pérez I, Fernández-Moreno M, Blanco F J. Gene Polymorphisms and Pharmacogenetics in Rheumatoid Arthritis. Current Genomic 2008; 9: 381-393.
6. Genestier L, Paillot R, Quemeneur L et al. Mechanisms of Methotrexate. Immunopharmacology 2000 ; 47 : 247–257.
7. Lim U, Wang SS, Hartge P et al. Gene-nutrient interactions among determinants of folate and one-carbon metabolism on the risk of non-Hodgkin lymphoma: NCI-SEER. Blood 2007 ; 109: 3050-3059.
8. Rozen R. Molecular genetics of methylenetetrahydrofolate reductase deficiency. J Inherit Metab Dis 1996; 19: 589-594.
9. Daniel Leclerc, Rima Rozen. génétique moléculaire de MTHFR les polymorphismes ne sont pas tous bénins. Medecine sciences 2007; 23 : 297-302.
10. Van ede AE, Laan RF, Blom HG, DeAbreu RA, Van de Putte LBA. Methotrexate in rheumatoid arthritis: an update with focus on mechanisms involved in toxicity, Seminars in Arthritis and Rheumatism 1998;27 (5): 277–292.
11. Rubini M, Padovan M, Baricordi O, Fotinidi M, Govoni M, Trotta F. The c.1298A>C polymorphism in the methylenetetrahydrofolate reductase gene is associated with rheumatoid arthritis susceptibility in Italian patients. Clin Exp Rheumatol 2008;26(1):163.
12. Berkun Y, Levartovsky D, Rubinow A et al. Methotrexate related adverse effects in patients with rheumatoid arthritis are associated with the A1298C polymorphism of the MTHFR gene. Ann Rheum Dis 2004;63:1227-1231.
13. Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 1976;455:152-162.
14. Marchetti S, Mazzanti R, Beijnen JH, Jan Schellensa HM. Concise review: clinical relevance of drug–drug and herb–drug interactions mediated by the ABC transporter ABCB1 (MDR1,P-glycoprotein). Oncologist 2007;12:927–941.
15. Pawlik A, Baskiewicz-Masiuk M, Machalinski B, Kurzawski M, Gawronska-Szklarz B. Involvement of C3435T and G2677T multidrug resistance gene polymorphisms in release of cytokines from peripheral blood mononuclear cells treated with methotrexate and dexamethasone. European Journal of Pharmacology 2005;5(5):821-828.
16. Hoffmeyer S, Burk O, von Richter O, Arnold H P, Brockmoller J, Johne A. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci U S A 2000; 97: 3473-3478.
17. Tsujimura S, Saito K, Nawata M, Nakayamada S, Tanaka Y. Overcoming drug resistance induced by P-glycoprotein on lymphocytes in patients with refractory rheumatoid arthritis. Ann Rheum Dis 2008; 67: 380–388.
18. Pawlik A, Wrzesniewska J, Fiedorowicz-Fabrycy I, Gawronska-Szklarz B. The MDR1 3435 polymorphism in patients with rheumatoid arthritis. Int J Clin Pharmacol Ther 2004;42: 496--503.
19. Urano W, Taniguchi A, Yamanaka H et al. Polymorphisms in the methylenetetrahydrofolate reductase gene were associated with both the efficacy and the toxicity of methotrexate used for the treatment of rheumatoid arthritis, as evidenced by single locus and haplotype analyses. Pharmacogenetics 2002 ; 12(3): 183--190
20. Arnett FC, Edworthy SM, Bloch DA. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1998; 3, 315-324.
21. Aletaha D, Neogi T, Silman A J. 2010 rheumatoid arthritis classification criteria: an AmericanCollege of Rheumatology/European League Against Rheumatism collaborative initiative. Annals of the Rheumatic Diseases 2010; 69(9): 1580–1588.
22. Miller SA, Dykes D D, Polesky H F. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 1988; 16(3): 1215.
23. Tregouet DA, Tiret L. Cox proportional hazards survival regression in haplotype-based association analysis using the Stochastic-EM algorithm. Eur J Hum Genet 2004; 12: 971–974.

24. Nouri-Shirazi M and Guinet E. Evidence for the immunosuppressive role of nicotine on human dendritic cell functions. Immunology 2003; 109 (3): 365–373.
25. Kalra R, Singh S P, Pena-Philippides J et al. Immunosuppressive and anti-inflammatory effects of nicotine administered by patch in an animal model. Clinical and Diagnostic Laboratory. Immunology 2004; 11(3): 563–568.
26. Visser K, Goekoop-Ruiterman YPM, De Vries- Bouwstra JK et al. A matrix risk model for the prediction of rapid radiographic progression in patients with rheumatoid arthritis receiving different dynamic treatment strategies: post hoc analyses from the Best study. Annals of the Rheumatic Diseases 2010; 69 (7): 1333–1337.
27. Bergström U, Jacobsson LT, Nilsson JÅ, et al. Smoking, low formal level of education, alcohol consumption, and the risk of rheumatoid arthritis. Scand J Rheumatol 2013; 42(2): 123-130.
28. Mikuls TR, Cerhan JR, Criswell LA et al. Coffee, tea, and caffeine consumption and risk of rheumatoid arthritis: results from the Iowa Women's Health Study 2002; 46(1): 83-91.
29. Szarc Vel Szic K, Ndlovu MN, Haegeman G, Vanden Berghe W. Nature or nurture: Let food be your epigenetic medecin in chronic inflammatory disorders. Biochem Pharmacol 2010; 80(12):1816-1836.
30. Lima A, Monteiro J, Bernardes M et al. Prediction of méthotrexate clinical response in Portuguese rheumatoid arthritis patients: implication of MTHFR rs 180113 and ATIC rs4673993 polymorphisms. BioMed Res Int 2014: ID368681:11.
31. Salazar J, Moya P, Altés A et al. Polymorphisms in genes involved in the mechanism of action of methotrexate: are they associated with outcome in rheumatoid arthritis patients? Pharmacogenomics 2014 : 15(8) :1079–1090.
32. Karaa I, Sazcib A, Ergulb E, Kayaa G, Kilic G. Association of the C677T and A1298C polymorphisms in the 5,10 methylenetetrahydrofolate reductase gene in patients with migraine risk. Molecular Brain Research 2003; 111 (1-2): 84–90.
33. Bernal ML, Sinues B, Fanlo A, Mayayo E. Frequency distribution of C3435T mutation in exon 26 of the MDR1 gene in a Spanish population. Ther Drug Monit 2003; 25, 107–111.
34. Hambaba L, Abdesamed S, Yahia M, Laraoui S, Rouabah F. Relation entre l'homocysteimie et polymorphisme C677T gene de la Méthyléne tetrahydrofolate réductase dans la population algérienne saine. Annales de biologie clinique 2008 ; 66 (6) : 637-641.
35. Guéant-Rodriguez R M, Guéant J L, Debard R et al. Prevalence of methylenetetrahydrofolate reductase 677T and 1298C alleles and folate status: a comparative study in Mexican, West African, and European populations. Am J Clin Nutr 2006; 83: 701–707.
36. Paluku They-They T., Hamzi K., Mazabraud A., Nadifi S. Fréquence du polymorphisme C677T du gène de la méthylène tetrahydrofolate réductase (MTHFR) dans les populations arabe et berbère du Maroc. Antropo 2009 ; 20 : 11-17.
37. Inanir A, Yigit S, Tekcan A et al. IL-4 and MTHFR gene polymorphism in rheumatoid arthritis and their effects. Immunol Lett 2013; 152(2): 104-108.
38. Hughes LB, Beasley TM, Patel H et al. Racial Or ethnic differences in allele frequency of single –nucleotide polymorphisms in the methylenetetrahydrofolate Redeuctase gene and their infl uence On response to methotrexate in rheumatoid arthritis. Ann Rheum Dis 2006; 65: 1213-1218.
39. Julia A, Ballina J, Canete J D et al. Genome-Wide Association Study of Rheumatoid Arthritis in the Spanish Population. Arthritis & Rheumatism 2008; 58(8): 2275-2286.
40. Chen J, Ma J, Stampfer MJ, Palomeque C, Selhub J, Hunter DJ. Linkage disequilibrium between the 677C > T and 1298A > C polymorphisms in human methylenetetrahydrofolate reductase gene and their contributions to risk of colorectal cancer. Pharmacogenetics 2002;12: 339–342.
41. Ulvik A, Ueland PM, Fredriksen A, et al. Functional inference of the methylenetetrahydrofolate reductase 677C > T and 1298A > C polymorphisms from a large-scale epidemiological study. Hum Genet 2007;121:57–64.
42. Turgut S, Turgut G, Atalay EO. Genotype and allele frequency of human multidrug resistance (MDR1) gene C3435T polymorphism in Denizli province of Turkey. Mol Biol Rep 2006; 33: 295–300.
43. Ameyaw MM, Regateiro F, Li T et al. MDR1 pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity. Pharmacogenetic 2001; 11: 217–221.
44. Chen J, Chen L, Mao N, Liu Y. Association of the MDR1 3435 polymorphism in patients with refractory rheumatoid arthritis in a Chinese population. Rheumatol Int 2012; 32: 3127–3130.
45. Lima A, Bernardes M, Azevedo R et al. SLC19A1, SLC46A1 and SLCO1B1 Polymorphisms as Predictors of Methotrexate-Related Toxicity in Portuguese Rheumatoid Arthritis Patients. Toxicol Sci 2014; 15 (6): 807-820.
46. Song GG, Bae SC, Lee YH. Association of the MTHFR C677T and A1298C polymorphisms with methotrexate toxicity in rheumatoid arthritis: a meta-analysis; Polymorphisms in genes involved in the mechanism of action of méthotrexate. Clin Rheumatol 2014;33(12):1715-24.