MTRR A66G, *RFC1* G80A, and *MTHFR* C677T and A1298C Polymorphisms and Disease Activity in Mexicans with Rheumatoid Arthritis Treated with Methotrexate

Mirna Gisel González-Mercado,[1–3] Fernando Rivas,[4] M. Patricia Gallegos-Arreola,[1]
M. Cristina Morán-Moguel,[1,3] Mario Salazar-Páramo,[5,6] Laura González-López,[5,6]
J. Iván Gámez-Nava,[5,6] J. Francisco Muñoz-Valle,[3] Ricardo Medina-Coss y León,[1]
Anahí González-Mercado,[1,3] Mario A. Aceves,[5,6] Nory O. Dávalos,[3]
Agustín Macías-Chumacera,[6] and Ingrid P. Dávalos[1,3]

Aim: To investigate the relationships of polymorphisms in genes whose protein products are related in the metabolic pathway of folic acid, particularly *MTRR* A66G, *RFC1* G80A, and *MTHFR* C677T and A1298C, and disease activity in Mexican patients with rheumatoid arthritis (RA) treated with methotrexate (MTX).
Materials and Methods: Sixty-eight patients with RA were included in the study who were being treated with MTX, either with or without other drugs. In addition to general data, disease activity was measured by the disease activity score 28 (DAS28). Single nucleotide polymorphisms (SNPs) genotyping was performed by allelic discrimination using real-time polymerase chain reaction.
Results: Differences in genotype (homozygotic or heterozygotic for each allele), allele distributions, and phenotype were not statistically different between the RA group and control populations. We did not find any association between the studied polymorphisms and disease activity nor with the intragroup variables (e.g., clinical activity, body mass index, and single- or combined-drug treatment) or between genetic markers; we also did not find any association within the RA group or between the RA group and control populations.
Conclusion: Additional studies of more polymorphisms related to this or other metabolic pathways are required to determine the influence of genetics on disease activity in RA.

Keywords: *MTRR*, *RFC1*, *MTHFR*, rheumatoid arthritis, DAS28, methotrexate

Introduction

R HEUMATOID ARTHRITIS (RA) is a chronic and progressive inflammatory disease that is characterized by cell proliferation and inflammation of the joint synovial membranes (McInnes and Schett, 2011). RA is an autoimmune disease and, thus, its etiology is multifactorial with genetic and environmental components, including diet (Oliver and Silman, 2009).

Disease-modifying anti-rheumatic drugs (DMARDs) are used to decrease inflammation and pain, prevent joint dam-

age, and preserve patient functional capacity. The DMARD methotrexate (MTX) is an antagonist of the essential nutrient folic acid, and it is the most commonly used drug to treat RA, either alone or in combination with other drugs (Calabrese *et al.*, 2001). Although the mechanism of action of MTX in patients with autoimmune diseases is not well understood, it seems to have both antiproliferative and anti-inflammatory effects (Cutolo *et al.*, 2001).

Differences in one or more etiological factors may predispose patients to RA onset or varying degrees of disease

[1]Divisiones de Genética y Medicina Molecular, Centro de Investigación Biomédica de Occidente, IMSS, Guadalajara, México.
[2]División de Biotecnología y Salud, Tecnológico de Monterrey, Guadalajara, México.
[3]Departamento de Biología Molecular y Genómica, DGH, CUCS, Universidad de Guadalajara, Guadalajara, México.
[4]Hospital General de Occidente, Secretaría de Salud Jalisco, Zapopan, México.
[5]División de Investigación, UMAE, HE, CMNO y HGR 110, IMSS, Guadalajara, México.
[6]Departamento de Fisiología, CUCS, Universidad de Guadalajara, Guadalajara, México.

severity (Oliver and Silman, 2009; McInnes and Schett, 2011). Consequently, RA onset, prognosis, and response to MTX could be affected by environmental factors or genetic variations in folic acid metabolism (Inoue and Yuasa, 2014).

Previous studies have investigated associations between RA clinical activity and treatment response and the deoxyribonucleic acid (DNA) variants of genes associated with folic acid metabolism such as *MTRR*, *RFC1*, and *MTHFR* (Berkun *et al.*, 2004; Hughes *et al.*, 2006; Wessels *et al.*, 2006; Rubini *et al.*, 2008; Inanir *et al.*, 2013; Salazar *et al.*, 2014; Saad *et al.*, 2015a, 2015b, 2016; Muralidharan *et al.*, 2016; Remuzgo-Martínez *et al.*, 2016). *MTRR* gene is located on chromosome 5p15.3 and encodes for the enzyme methionine synthase reductase, involved in the reductive regeneration of cob(I)alamin (vitamin B12) cofactor required for the maintenance of methionine synthase in a functional state (Jacques, 2003). *MTRR* A66G polymorphism (rs1801394) has been identified with a global minor allele frequency (MAF) G = 36% (https://www.ncbi.nlm.nih.gov/snp).

In the Caucasian population, GG genotype has been associated with an increase in plasma homocysteine (Hcy) levels, having a greater effect than the AG genotype (Gaughan *et al.*, 2001).

RFC1 gene is located on chromosome 21q22.3 and encodes for the reduced folate transporter, which plays an important role in folate metabolism and also works as a transporter of the MTX into the cell (Matherly *et al.*, 2007). *RFC1* G80A polymorphism (rs1051266) has an overall MAF of A = 49% (https://www.ncbi.nlm.nih.gov/snp) and this genetic variant might cause an alteration in the folate transporter, affecting the availability of folate (Dervieux *et al.*, 2004).

MTHFR gene encodes the methylene-tetrahydrofolate reductase enzyme and is located on chromosome 1p36.3. This enzyme catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for Hcy remethylation to methionine (Goyette *et al.*, 1998). *MTHFR* C677T polymorphism (rs1801133) has a global MAF of T = 24% (https://www.ncbi.nlm.nih.gov/snp), which causes a thermolabile variant of the protein, altering the enzymatic function (Van Der Put *et al.*, 1998).

In homozygous individuals, this variant is correlated with a decrease in enzyme activity (35%), elevated Hcy, low levels of folic acid, and reduced formation of 5-methyltetrahydrofolate, a predominant form of folate (Frosst *et al.*, 1995). Otherwise, a global MAF of C = 25% (https://www.ncbi.nlm.nih.gov/snp) has been reported in the *MTHFR* A1298C polymorphism (rs1801131) and causes loss of 40% enzyme activity in individuals homozygous for the mutant allele and only in combination with the C677T polymorphism causes hyperhomocysteinemia (Van Der Put *et al.*, 1998).

In this study, we investigated potential associations between RA severity and the single nucleotide polymorphisms (SNPs) *MTRR* A66G, *RFC1* G80A and *MTHFR* C677T and A1298C.

Materials and Methods

Study population

We analyzed data for 68 Mexican Mestizo patients (67 women and 1 man) with RA diagnosed according to the American College of Rheumatology criteria (ACR, 1987) (Arnett *et al.*, 1988). Patients were seen in the rheumatology services clinic at Regional Hospital 110, Instituto Mexicano del Seguro Social (IMSS) or at the Hospital Civil de Guadalajara ''Fray Antonio Alcalde.'' and they signed a written informed before the sampling of peripheral blood when they accepted their voluntary participation in the study. All of the patients had been previously diagnosed with RA for at least 1 year and had been receiving MTX for at least 3 months. DAS criteria were used to evaluate their RA activity (low activity score, <3.2; moderate, 3.2–5.1; and high, >5.1) (Prevoo *et al.*, 1995; Fransen *et al.*, 2003; Makinen *et al.*, 2005).

Control population

Each polymorphism had a different control population, for *MTRR* A66G $n = 50$ (Shi *et al.*, 2003), *RFC1* G80A $n = 121$ (Rodarte, unpublished data), *MTHFR* C677T $n = 82$ (González-Mercado *et al.*, 2014), and *MTHFR* A1298C $n = 94$ (González-Mercado *et al.*, 2014).

DNA analysis

We extracted DNA from blood samples in accordance with the methods of Miller and Gustincich (Miller *et al.*, 1989; Gustincich *et al.*, 1991). Polymorphisms *MTRR* A66G, *RFC1* G80A and *MTHFR* C677T, and A1298C were typed in an allelic discrimination assay by using TaqMan 5′ exonuclease probes (Applied Biosystems, Foster City, CA) with the ABI 7300 real-time PCR system (Applied Biosystems).

Statistical analysis

Genotype and allele frequencies were obtained by direct counting. Data analysis included comparisons of allele frequencies and genotypes between the RA patient group and control normal Mexican population distributions (Shi *et al.*, 2003; González-Mercado *et al.*, 2014; Rodarte, unpublished data). All control genotypes were in agreement with Hardy–Weinberg equilibrium (HWE). We performed distribution comparisons by the exact, chi square, and likelihood ratio tests by using SPSS statistical package, v. 22 (IBM®). Quantitative variables were subjected to mean comparisons by Student's *t*-test or one-way analysis of variance.

Results

The general characteristics of the study participants were as follows: The mean age was 53.7 years with a SD ± 10.7 (range 72–76). Most previous studies, including some studies in Mexican populations, have estimated that the female-to-male ratio of patients with RA is ∼3:1 (Rodríguez-Acosta *et al.*, 2001; Spindler *et al.*, 2002). In our study, we identified 67 women and only 1 man who had been diagnosed with RA.

The disease activity score 28 (DAS28) values found in the patients in this study had a mean of 4.7 ± 1.4 SD (range 2.3–7.8). According to the DAS28 (Fransen *et al.*, 2003), disease activity levels were as follows: low, 14 patients; moderate, 28 patients; and high, 27 patients. Forty-one patients were receiving MTX monotherapy, 26 patients were receiving MTX plus an additional DMARD, and 2 patients were receiving MTX plus two additional DMARDs. DMARDs other than MTX were leflunomide ($n = 15$), sulfasalazine ($n = 8$), chloroquine ($n = 4$), and penicillamine ($n = 3$). Since MTX monotherapy is widely recommended in most RA patients as the first line of treatment (Rodríguez-Valverde *et al.*, 2004; Van

TABLE 1. GENOTYPES AND ALLELE FREQUENCIES FOR *MTRR* A66G, *RFC1* G80A, AND *MTHFR* C677T
AND A1298C SINGLE NUCLEOTIDE POLYMORPHISMS IN RHEUMATOID ARTHRITIS PATIENTS
AND CONTROL MEXICAN POPULATIONS

Polymorphism	Group	Genotype, counts (%)				Allele, counts (%)[a]		
		1/1	1/2	2/2	p	1	2	p
MTRR A66G	RA	38 (56)	25 (37)	5 (7)	0.67	101 (74)	35 (26)	0.40
	Control (Shi *et al.*, 2003) (*n* = 50)	32 (64)	15 (30)	3 (6)		79 (79)	21 (21)	
RFC1 G80A	RA	19 (28)	37 (54)	12 (18)	0.99	75 (55)	61 (45)	0.97
	Control (Rodarte, unpublished results) (*n* = 121)	34 (28)	65 (54)	22 (18)		133 (55)	109 (45)	
MTHFR C677T	RA	23 (34)	32 (47)	13 (19)	0.75	78 (57)	58 (43)	0.52
	Control (González-Mercado *et al.*, 2014) (*n* = 82)	23 (28)	42 (51)	17 (21)		88 (53)	76 (47)	
MTHFR A1298C	RA	43 (63)	21 (31)	4 (6)	0.70	107 (79)	29 (21)	0.38
	Control (González-Mercado *et al.*, 2014) (*n* = 94)	54 (57)	32 (34)	8 (9)		140 (74)	48 (26)	

[a]Allele 1: A in *MTRR* 66, G in *RFC-1* 80, C in *MTHFR* 677, and A in *MTHFR* 1298. Allele 2: G in *MTRR* 66, A in *RFC-1* 80, T in *MTHFR* 677, and C in *MTHFR* 1298. Differences in genotype, phenotype (homozygous or heterozygous for each allele), and allele distributions between the RA group and control populations were not statistically significant.
RA, rheumatoid arthritis; SNPs, single nucleotide polymorphisms.

der Heijde *et al.*, 2005; GUIPCAR, 2011), we compared the DAS28 between patients undergoing mono- and combined therapy. The average DAS28 was 4.64 for monotherapy and 4.76 for combined therapy ($p = 0.74$).

Polymorphism analysis

Polymorphism distributions for the RA patient group and for the control populations are presented in Table 1. The genetic frequencies of *MTRR* A66G, *RFC1* G80A, and *MTHFR* C677T and A1298C SNPs have been analyzed in previous studies of several Mexican populations; the genotypes were in agreement with HWE (Shi *et al.*, 2003; González-Mercado *et al.*, 2014; Rodarte, unpublished data).

When comparing the allele and genotype frequencies of the four polymorphisms included in this study between patients with RA and various Mexican healthy populations, we found no statistically significant differences between the groups (Table 1). DAS28 values were compared with the genotypes of the polymorphisms studied and there was a trend toward increased activity in the genotypes 2/2 of the polymorphisms *MTRR* A66G, *RFC1* G80A, and *MTHFR* A1298C; however, there were no significant differences within the RA patient group with respect to clinical activity (Fig. 1). There were also no significant differences with body mass index (BMI), and monotherapy versus combined therapy.

Discussion

General characteristics

Although disease onset can occur at any age (Rodríguez-Acosta *et al.*, 2001; Firestein, 2005), the *Diagnóstico y Tratamiento de Artritis Reumatoide del Adulto*, (Diagnosis and Treatment of Adult RA) reports that the mean age at diagnosis is 40 ± 10 SD years in Mexico (Barrera-Cruz *et al.*, 2010). In this series, the mean age at disease onset was 44.7 ± 12.0 SD years, which is similar to that reported in other populations.

In our patients, the mean BMI was 26.9 ± 4.8 SD (range 15–41), which is above the threshold for overweight. Other

studies have reported similar findings with respect to increased BMI in Mexican patients with RA (Puente-Torres *et al.*, 2009). However, the proportion of overweight patients that we observed is comparable to that of the general Mexican population (INEGI, 2016).

Polymorphism analysis

Studies of the genotype and allele frequencies of the polymorphisms that we analyzed have revealed that they vary widely worldwide (Dávalos *et al.*, 2001; Boughrara *et al.*, 2015; Saad *et al.*, 2015a; Li *et al.*, 2016; Remuzgo-Martínez *et al.*, 2016). Several studies have investigated the associations of these polymorphisms with autoimmune diseases, including systemic lupus erythematosus (Summers *et al.*,
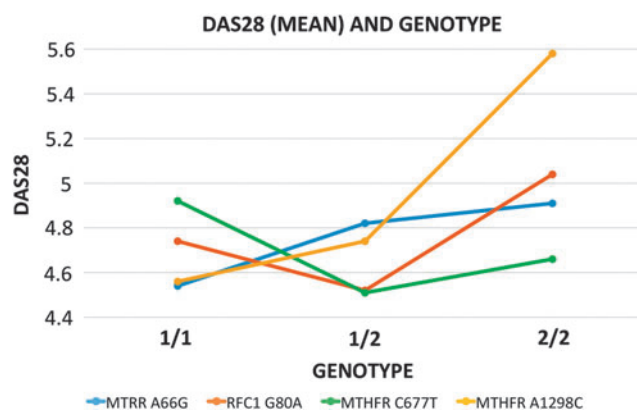

FIG. 1. Comparison between DAS28 mean values and the genotypes of each polymorphism in patients with RA treated with MTX. A slight upward trend in disease activity was observed in genotypes 2/2 of polymorphisms (*MTRR* A66G $p = 0.73$, *RFC1* G80A $p = 0.55$, *MTHFR* A1298C $p = 0.48$) with the exception of *MTHFR* C677T ($p = 0.58$); however, it was not statistically significant. DAS28, disease activity score 28; RA, rheumatoid arthritis.

TABLE 2. ASSOCIATIONS BETWEEN POLYMORPHISMS AND RHEUMATOID ARTHRITIS DISEASE COURSE
AND CLINICAL RESPONSE TO METHOTREXATE TREATMENT IN PREVIOUS STUDIES

| Disease | Population | n | Polymorphism | | References |
			Allele frequency	Association	
RA and MTX response	Spain	61 RA patients responders 16 RA patients no responders	*MTHFR* C677T RA responders C 0.68 T 0.32 RA no responders C 0.75 T 0.25	N.A.	Salazar *et al.* (2014)
			MTHFR A1298C RA responders A 0.67 C 0.33 RA no responders A 0.56 T 0.46	N.A.	
RA and MTX response	Netherlands	205 RA patients	*MTRR* A66G Not available	N.A.	Wessels *et al.* (2006)
RA and MTX response	India	327 RA patients 322 controls	*RFC1* G80A RA patients G 0.57 A 0.43 Controls G 0.57 A 0.43	N.A. but polymorphism confers protection for RA response	Muralidharan *et al.* (2016)
RA and MTX toxicity	Mexico	57 RA patients without toxicity 13 RA patients with toxicity	*MTHFR* C677T RA without toxicity C 0.55 T 0.45 RA with toxicity C 0.58 T 0.42	N.A.	Mena *et al.* (2011)
			MTHFR A1298C RA without toxicity A 0.79 C 0.21 RA with toxicity A 0.58 C 0.42	Associated with elevation of transaminases	
RA and MTX toxicity	Tunisia	141 RA MTX tolerant/MTX intolerant	*MTRR* A66G MTX tolerant A 0.51 G 0.49 MTX intolerant A 0.52 G 0.48	N.A.	Chaabane *et al.* (2016)
			MTHFR C677T MTX tolerant C 0.75 T 0.25 MTX intolerant C 0.65 T 0.35	Associated with MTX toxicity	
			MTHFR A1298C MTX tolerant A 0.70 C 0.30 MTX intolerant A 0.74 C 0.26	N.A.	

(continued)

TABLE 2. (CONTINUED)

| Disease | Population | n | Polymorphism | | References |
			Allele frequency	Association	
RA and serum MTX levels	Japan	100 RA patients	RFC1 G80A G 0.37 A 0.63	N.A.	Fukino et al. (2007)
			MTHFR C677T C 0.57 T 0.43	N.A.	
			MTHFR A1298C A 0.80 C 0.20	N.A.	
RA and MTX	Japan	170 RA patients	RFC1 G80A G 0.48 A 0.52	G allele may be associated with lower intracellular MTX uptake and poor efficacy	Hayashi et al. (2013)
RA and DAS28	Japan	55 RA patients	MTRR A66G A 0.75 G 0.25	N.A.	Kato et al. (2012)
			RFC1 G80A G 0.47 A 0.53	N.A.	
			MTHFR C677T C 0.6 T 0.4	N.A.	
			MTHFR A1298C A 0.86 C 0.14	AA genotype had lower mean DAS28 than 1298AC/CC genotypes	

DAS28, disease activity score 28; MTX, methotrexate; N.A., no association.

2008), multiple sclerosis (Naghibalhossaini et al., 2015), and thyroiditis (Arakawa et al., 2012). Previous studies of RA patients have focused on disease evolution (Brambila-Tapia et al., 2012; Remuzgo-Martínez et al., 2016) and severity or the therapeutic response to MTX. Associations of these polymorphisms with MTX treatment response seem to be consistently significant, but studies of their association with disease onset and clinical severity have yielded conflicting results (Table 2).

The role of polymorphisms MTRR A66G, RFC1 G80A, and MTHFR C677T and A1298C in RA and other autoimmune diseases remains unclear.

Our failure to find an association between RA and these SNPs could be due to the small sample size included in this study or to the weak contribution of these genes to RA onset and evolution, either in Mexico or worldwide.

Polymorphisms and disease activity

The patients of this study showed the three levels of disease activity, with the most frequent being moderate activity. Although there are clinical or laboratory measures used in clinical practice, a good individual performance in the assessment of disease activity has not been demonstrated, so it has been decided to use DAS28 for a better classification of the disease (Prevoo et al., 1995; Fransen et al., 2003), which is used as the basis for the evaluation of the response to treatment established

by the European League Against Rheumatism. Although we found no statistical differences between polymorphisms and disease activity, there was a trend in three of the polymorphisms studied (MTRR 66GG, RFC1 80AA, and MTHFR 1298CC) and we concluded that perhaps a larger sample size might have yielded data with statistical significance.

This study has some limitations to be discussed: Because this study was exploratory, there was no previous information related with our main objective of investigating potential associations between RA severity and the SNPs MTRR A66G, RFC1 G80A, and MTHFR C677T and A1298C in the Mexican population from Western Mexico. In the results of the comparisons (described in Table 1) of allele and genotype frequencies observed in RA cases versus controls, we were not able to exclude the probability of an insufficient statistical power to identify differences between these two groups (type II error). Nevertheless, we consider that this study might help future investigations based in our data on the computation of the sample size required to perform these comparisons.

Otherwise, we have observed that although the n of controls increases, the allelic frequencies of the polymorphisms are maintained, since a minimum of 100 alleles is required. Our results represent relevant information demonstrating that these polymorphisms might not be related with disease severity in RA. These findings support the importance of seeking other genetic factors that might predispose to the observed phenotype differences in the severity of this disease among these patients.

In conclusion, in this study, we did not find any significant associations between RA or RA characteristics such as activity disease and polymorphisms *MTRR* A66G, *RFC1* G80A, and *MTHFR* C677T and A1298C. Additional studies that include greater numbers of patients and more polymorphisms related to this or other metabolic pathways are required to determine the influence of genetics on disease activity in RA in Mexican populations, and thus provide greater knowledge about individualized pharmacological therapies for a better response to treatment.

Author Disclosure Statement

No competing financial interests exist.

References

Arakawa Y, Watanabe M, Inoue N, *et al.* (2012) Association of polymorphisms in DNMT1, DNMT3A, DNMT3B, MTHFR and MTRR genes with global DNA methylation levels and prognosis of autoimmune thyroid disease. Clin Exp Immunol 170:194–201.

Arnett FC, Edworthy SM, Bloch DA, *et al.* (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31:315–324.

Barrera Cruz A, Beltran Castillo J, Blanco Favela F, *et al.* (2010) Diagnóstico y Tratamiento de Artritis Reumatoide del Adulto. México: Secretaria de Salud.

Berkun Y, Levartovsky D, Rubinow A, *et al.* (2004) Methotrexate related adverse effects in patients with rheumatoid arthritis are associated with the A1298C polymorphism of the MTHFR gene. Ann Rheum Dis 63:1227–1231.

Boughrara W, Aberkane M, Fodil M, *et al.* (2015) 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. Acta Reumatol Port 40:363–371.

Brambila-Tapia AJ, Durán-González J, Sandoval-Ramírez L, *et al.* (2012) MTHFR C677T, MTHFR A1298C, and OPG A163G polymorphisms in Mexican patients with rheumatoid arthritis and osteoporosis. Dis Markers 32:109–114.

Calabrese DC, Konop R, Cannon E, *et al.* (2001) Discussion. Treatment algorithm: managing rheumatoid arthritis. Manag Care 10:19–24.

Chaabane S, Marzouk S, Akrout R, *et al.* (2016) Genetic determinants of methotrexate toxicity in tunisian patients with rheumatoid arthritis: a study of polymorphisms involved in the MTX metabolic pathway. Eur J Drug Metab Pharmacokinet 41:385–393.

Cutolo M, Sulli A, Pizzorni C, *et al.* (2001) Anti-inflammatory mechanisms of methotrexate in rheumatoid arthritis. Ann Rheum Dis 60:729–735.

Dávalos IP, Olivares N, Castillo MT, *et al.* (2001) The C677T polymorphism of the methylenetetrahydrofolate reductase gene in Mexican mestizo, neural tube defects parents, control Mestizo and native populations. Ann Genet 43:89–92.

Dervieux T, Furst D, Lein DO, *et al.* (2004) Polyglutamation of methotrexate with common polymorphisms in reduced folate carrier, aminoimidazole carboxamide ribonucleotide transformylase, and thymidylate synthase are associated with methotrexate effects in rheumatoid arthritis. Arthritis Rheum 50:2766–2774.

Firestein G (2005) Pathogenesis of rheumatoid arthritis: how early is easy? Arthritis Res Ther 7:157–159.

Fransen J, Stucki G, van Riel PLCM (2003) Rheumatoid arthritis measures. Disease activity score (DAS), disease activity score 28 (DAS28), rapid assessment of disease activity in rheumatology (RADAR), and rheumatoid arthritis disease activity index (RADAI). Arthritis Rheum 49:214–224.

Frosst P, Blom Hj, Milos R, *et al.* (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nature Genet 10:111–113.

Fukino K, Kawashima T, Suzuki M, *et al.* (2007) Methylenetetrahydrofolate reductase and reduced folate carrier-1 genotypes and methotrexate serum concentrations in patients with rheumatoid arthritis. J Toxicol Sci 32:449–452.

Gaughan DJ, Kluijtmans LAJ, Barbaux S, *et al.* (2001) The methionine synthase reductase (MTRR) A66G polymorphism is a novel genetic determinant of plasma homocysteine concentrations. Atherosclerosis 157:451–456.

González-Mercado MG, Rivas-Solis F, Salazar-Páramo M, *et al.* (2014) Homocisteína, polimorfismos MTHFR C677T y A1298C y variables clínico/bioquímicas en población mexicana. Acta Bioq Clin Lat 48:23–31.

Goyette P, Pai A, Milos R (1998) Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). Mamm Genome 9:652–656.

GUIPCAR (2011) Guía de práctica clínica para el manejo de la Artritis Reumatoidea (Sociedad Española de Reumatologia 2011). http://portal.guiasalud.es/web/guest/home (last accessed September 3, 2016).

Gustincich S, Manfioletti G, Del Sal G, *et al.* (1991) A fast method for high quality genomic-DNA extraction from whole human blood. Biotechniques 11:300–302.

Hayashi H, Tazoe Y, Tsuboi S, *et al.* (2013) A single nucleotide polymorphism of reduced folate carrier 1 predicts methotrexate efficacy in Japanese patients with rheumatoid arthritis. Drug Metab Pharmacokinet 28:164–168.

Hughes LB, Beasley TM, Patel H, *et al.* (2006) Racial or ethnic differences in allele frequencies of single-nucleotide polymorphisms in the methylenetetrahydrofolate reductase gene and their influence on response to methotrexate in rheumatoid arthritis. Ann Rheum Dis 65:1213–1218.

Inanir A, Yigit S, Tekcan A, *et al.* (2013) IL-4 and MTHFR gene polymorphism in rheumatoid arthritis and their effects. Immunol Lett 152:104–108.

INEGI (2016) Instituto Nacional de Estadística y Geografía. Available at www.beta.inegi.org.mx (last accessed September 3, 2016).

Inoue K, Yuasa H (2014) Molecular basis for pharmacokinetics and pharmacodynamics of methotrexate in rheumatoid arthritis therapy. Drug Metab Pharmacokinet 29:12–19.

Jacques P (2003) Effects of polymorphisms of methionine synthase and methionine synthase reductase on total plasma homocysteine in the NHLBI Family Heart Study. Atherosclerosis 166:49–55.

Kato T, Hamada A, Mori S, *et al.* (2012) Genetic polymorphisms in metabolic and cellular transport pathway of methotrexate impact clinical outcome of methotrexate monotherapy in Japanese patients with rheumatoid arthritis. Drug Metab Pharmacokinet 27:192–199.

Li X, Hu M, Li W, *et al.* (2016) The association between reduced folate carrier-1 gene 80G/A polymorphism and methotrexate efficacy or methotrexate related-toxicity in rheumatoid arthritis: a meta-analysis. Int Immunopharmacol 38:8–15.

Makinen H, Kautiainen H, Hannonen P, *et al.* (2005) Is DAS28 an appropiate tool to assess remission in rheumatoid arthritis? Ann Rheum Dis 64:1410–1413.

Matherly LH, Hou Z, Deng Y (2007) Human reduced folate carrier: translation of basic biology to cancer etiology and therapy. Cancer Metastasis Rev 26:111–128.

McInnes IB, Schett G (2011) The pathogenesis of rheumatoid arthritis. N Engl J Med 365:2205–2219.

Mena JP, Salazar-Páramo M, González-López L, *et al.* (2011) Polymorphisms C677T and A1298C in the MTHFR gene in Mexican patients with rheumatoid arthritis treated with methotrexate: implication with elevation of transaminases. Pharmacogenomics J 11:287–291.

Miller SA, Dikes DD, Polesky HP (1989) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 16:1215.

Muralidharan N, Mariaselvam CM, Mithun CB, *et al.* (2016) Reduced folate carrier-1 80G>A gene polymorphism is not associated with methotrexate treatment response in South Indian Tamils with rheumatoid arthritis. Clin Rheumatol 35: 879–885.

Naghibalhossaini F, Ehyakonandeh H, Nikseresht A, *et al.* (2015) Association between MTHFR genetic variants and multiple sclerosis in a Southern Iranian population. Int J Mol Cell Med 4:87–93.

Oliver JE, Silman AJ (2009) What epidemiology has told us about risk factors and aetiopathogenesis in rheumatic diseases. Arthritis Res Ther 11:223.

Prevoo MLL, van't Hof MA, Kuper HH, *et al.* (1995) Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 38:44–48.

Puente-Torres L, Hurtado-Torres GF, Abud-Mendoza C, *et al.* (2009) Evaluación del estado nutricio en una población mexicana de pacientes adultos con artritis reumatoide. Nutr Hosp 24:233–238.

Remuzgo-Martínez S, Genre F, López-Mejías R, *et al.* (2016) Decreased expression of methylene tetrahydrofolate reductase (MTHFR) gene in patients with rheumatoid arthritis. Clin Exp Rheumatol 34:106–110.

Rodríguez-Acosta R, Arévalo-Tan CE, Pérez-García D, *et al.* (2001) Factores sociodemográficos y severidad radiológica de la artritis reumatoide. Rev Cubana Med Gen Integr 17:423–428.

Rodríguez-Valverde V, García-Alvaro JM, Tornero-Molina J (2004) En representación de los médicos del panel. Segunda actualizacion del consenso de la Sociedad Española de Reumatologia sobre la terapia biologica en la Artritis Reumatoidea. Rev Esp Reumatol 31:394–401.

Rubini M, Padovan M, Baricordi O, *et al.* (2008) The c.1298A>C polymorphism in the methylenetetrahydrofolate reductase gene is associated with rheumatoid arthritis susceptibility in Italian patients. Clin Exp Rheumatol 26:163.

Saad MN, Mabrouk MS, Eldeib AM, *et al.* (2015a) Genetic case-control study for eight polymorphisms associated with rheumatoid arthritis. PLoS One 10:e0131960.

Saad MN, Mabrouk MS, Eldeib AM *et al.* (2015b) Effect of MTHFR, TGFbeta1, and TNFB polymorphisms on osteoporosis in rheumatoid arthritis patients. Gene 568:124–128.

Saad MN, Mabrouk MS, Eldeib AM, *et al.* (2016) Identification of rheumatoid arthritis biomarkers based on single nucleotide polymorphisms and haplotype blocks: a systematic review and meta-analysis. J Adv Res 7:1–16.

Salazar J, Moya P, Altés A, *et al.* (2014) Polymorphisms in genes involved in the mechanism of action of methotrexate: are they associated with outcome in rheumatoid arthritis patients? Pharmacogenomics 15:1079–1090.

Shi M, Caprau D, Romitti P, *et al.* (2003) Genotype frequencies and linkage disequilibrium in the CEPH human diversity panel for variants in folate pathway genes MTHFR, MTHFD, MTRR, RFC1, and GCP2. Birth Defects Res A Clin Mol Teratol 67:545–549.

SNP [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2004. Available at https://www.ncbi.nlm.nih.gov/snp (last accessed date July 27, 2017).

Spindler A, Bellomio V, Berman A (2002) Prevalence of rheumatoid arthritis in Tucuman, Argentina. J Rheumatol 29:1166–1170.

Summers CM, Cucchiara AJ, Nackos E, *et al.* (2008) Functional polymorphisms of folate-metabolizing enzymes in relation to homocysteine concentrations in systemic lupus erythematosus. J Rheumatol 35:2179–2186.

Van der Heijde D, Landewé R, Klareskog L, *et al.* (2005) Presentation and analysis of data on radiographic outcome in clinical trials: experience from the TEMPO study. Arthritis Rheum 52:49–60.

Van Der Put NM, Gabreels F, Stevens EM, *et al.* (1998) A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet 62:1044–1051.

Wessels JA, Kooloos WM, De Jonge R, *et al.* (2006) Relationship between genetic variants in the adenosine pathway and outcome of methotrexate treatment in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 54:2830–2839.

Address correspondence to:
Ingrid P. Dávalos, MD, PhD
Centro de Investigación Biomédica de Occidente
IMSS, DGH, CUCS
Universidad de Guadalajara Sierra Mojada 800
Guadalajara 44340
México

E-mail: ingriddavalos@hotmail.com