Received: 28 June 2019 | Revised: 13 August 2019 | Accepted: 13 August 2019

DOI: 10.1002/jcla.23030

RESEARCH ARTICLE

WILEY

Contribution of genetic polymorphism of methylene tetrahydrofolate reductase on the effect of methotrexate in ectopic pregnancy patients

Jie Deng[1] | Li Chen[2] | Heng Xue[3] | Fan-Xiang Zeng[1] | Pei-Guang Niu[1] | Dao-Hua Shi[1]

[1]Department of Pharmacy, Fujian Provincial Maternity and Children's Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, China

[2]Department of Gynecology, Fujian Provincial Maternity and Children's Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, China

[3]Laboratory Medicine, Fujian Provincial Maternity and Children's Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, China

Correspondence
Dao-Hua Shi, Department of Pharmacy, Fujian Provincial Maternity and Children's Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, China.
Email: shidh@yeah.net

Funding information
Natural Science Foundation of Fujian Province, Grant/Award Number: 2017J01169

Abstract

Background: Methotrexate (MTX) is the prior drug in ectopic pregnancy (EP). However, approximately 10% of patients suffer from failure by MTX therapy. Reduced folate carrier 1 (RFC1), methylene tetrahydrofolate reductase (MTHFR), and dihydrofolate reductase (DHFR) are involved in the transport and effects of MTX in vivo. In the present study, we aim to investigate the relationship between the genetic polymorphisms of RFC1, MTHFR, and DHFR and the clinical efficacy of MTX in tubal pregnancies.

Methods: 100 patients of EP were enrolled in this study. Polymorphisms of RFC1 G80A, MTHFR C677T, and DHFR A-317G were genotyped. β-hCG level was detected in day 0, 4, and 7 after MTX injection. Association of MTX efficacy and genetic polymorphisms was analyzed.

Results: Methylene tetrahydrofolate reductase C677T was associated with MTX treatment (P = .017). The success rate of first MTX injection was superior in patients with harboring mutation allele of MTHFR gene than that in patients with wild-type gene (P = .001). However, there was no significant association between the polymorphisms of RFC1 G80A, DHFR A-317G, and surgical treatment (P = .709 and .476, respectively). In addition, β-hCG level decrement was not significantly changed by MTX injection with different polymorphisms of RFC1, MTHFR, and DHFR on either day 4 (P = .214, 0.197 and 0.270, respectively) or day 7 (P = .172, .554, and .726, respectively).

Conclusion: Our results suggested that the reliable indicator was polymorphism of MTHFR C677T in failure by MTX injection.

KEYWORDS

clinical efficacy, dihydrofolate reductase, ectopic pregnancy, genetic polymorphisms, methotrexate, methylene tetrahydrofolate reductase, reduced folate carrier 1

1 | INTRODUCTION

Ectopic pregnancy (EP) is a common acute abdomen occurring in 1% of all pregnancies, and its incidence is increasing in recent years in China.[1] Patients with EP tend to be younger and have more birth demand.[2] Mostly, patients with EP are identified by hCG levels monitoring and ultrasound scans before they have developed clinical symptoms.[3,4] Therefore, conservative medication becomes the predominant treatment in EP, which is reasonable and economical practice.

Methotrexate (MTX), as a folate antagonist, is the priority drug of EP, which binds to the catalytic site of dihydrofolate reductase (DHFR). At a lower intracellular concentration, MTX appears DNA synthesis obstruction by interrupting the synthesis of purine nucleotides, thereby presents an inhibitory effect on gestation trophoblastic.[5] However, effect of MTX is individual difference in EP patients. 5%-30% of patients have a risk of secondary surgery or a supplementary dosage due to failure by first MTX treatment.[6] Several studies had confirmed that genetic factors played an important role in the treatment of acute lymphoblastic leukemia relapse or rheumatoid arthritis with MTX.[7,8] Nevertheless, the relationship between genetic factors and the efficacy of MTX was rarely reported on the EP therapy.

Methotrexate is a polar molecule and difficult to cross cell membranes. Reduced folate carrier 1 (RFC1) has a high affinity for various hydrophilic antifolates, which is the major influx system for MTX.[9] DHFR coverts folates to their active forms included dihydrofolate (DHF) and tetrahydrofolate (THF), which are essential for purine synthesis. The activity of DHFR was inhibited by intracellular MTX and its active metabolites methotrexate polyglutamates (MTX-PGs).[10] Methylene tetrahydrofolate reductase (MTHFR), a key enzyme of folate metabolism, is the competitive protein of folate, MTX and MTX-PGs. Expression level of DHFR and MTHFR impacts the efficacy of MTX.

Genetic polymorphisms may affect the activity and function of RFC1, MTHFR, and DHFR, and then, influent the uptake and efficacy of MTX. In this study, we focus on the association between clinical efficacy of MTX and genetic polymorphisms including RFC1 G80A, MTHFR C677T, and DHFR A-317G, respectively.

2 | MATERIALS AND METHODS

2.1 | Subjects

Hundred patients of tubal EP were collected from 2014 to 2016 in the department of gynecology at Fujian Provincial Maternity and Children's Hospital. Ethical approval was obtained from clinical research ethics committee of Fujian Provincial Maternity and Children's Hospital. The diagnosis of EP was based on the guideline of ACOG.[11] The participant patients treated with MTX should meet the following inclusion criteria: (a) β-hCG < 5000 mIU/mL, (b) diameter of EP mass <3.5 cm by ultrasound scan, (c) unruptured mass, (d) normal liver and renal function, (e) absence of fetal cardiac activity in the adnexa on ultrasound scan. These patients received single-dose MTX treatment with a dose of 50 mg/m^2. β-hCG concentration values were detected at day 0, 4, and 7 after MTX administration, respectively. A repeat dose of MTX was given when a fall of β-hCG concentration was less than 15% from day 4 to day 7. The patients carried out surgical intervention in the event of acute tubal rupture or unsatisfactory β-hCG concentration reduction. Successful treatment of first MTX injection was defined as >15% decrease in β-hCG values between day 4 and day 7 as well as without a second dose and surgery. MTX response failure was defined when further surgical operation was needed after MTX therapy. All participants signed an informed consent form for the study.

2.2 | Genotyping

DNA was isolated from 200 μL whole blood samples by Qiagen blood mini kit (Qiagen, Germany) according to the manufacturer's protocol and then stored at −20°C until analysis. The target fragments of genomic DNA were amplified with specific primers by PCR (GeneAmp PCR system 9700). The positive and reverse primer sequences of RFC1, MTHFR, and DHFR were 5′-GGC GTT TGG TCC TGA GTG-3′ and 5′- GAA GCC GTA GAA GCA AAG GTA −3′, 5′-TGT GGG AGT TTG GAG CA-3′ and 5′-CAC CTG GAT GGG AAA GA-3′, and 5′-ATG GCA ACA GGA AGG AC-3′ and 5′-TGA GGA GAT AGG CAA AGG-3′, respectively. The genotypes were analyzed by DNA pyrosequencing on an ABI Prism 3100 DNA Analyzer (Applied Bio-systems).

2.3 | Statistical analysis

Continuous variables, such as β-hCG level before treatment or day 4, day 7 after the first MTX administration, were compared by Kruskal-Wallis H analysis. Categorical variables, including the successful cases in the whole treatment period and the first MTX injection, were compared by logistic regression analysis. Hardy-Weinberg equilibrium was tested by χ^2 tests to compare expected frequencies

TABLE 1 Characteristics of patients (n = 100)

Characteristics	Values
Age (y)	29.07 ± 5.57
BMI (kg/m^2)	20.70 ± 2.34
Gravidity	2.95 ± 1.51
Parity	0.65 ± 0.73
Pretreatment hCG level (mIU/mL)	950.83 ± 829.80
Spontaneous conception	99 (99%)
Previous EP	16 (16%)
Presence of hemoperitoneum	52 (52%)
Size of hemoperitoneum (cm)	3.00 ± 1.11
Size of EP or inhomogeneous adnexal mass (cm)	2.02 ± 0.67

Note: Values are given as median ± SD or number (percentage).
Abbreviations: EP, ectopic pregnancy.

TABLE 2 Relationship between genetic polymorphisms and successful treatment after first MTX injection (n = 100)

Genotypes	Successful first MTX injection (n = 64)	Failed first MTX injection (n = 36)	*P* value	OR(95% CI)
RFC1				
AA	19	8	.661	1
AG	31	18		1.379 (0.502-3.786)
GG	14	10		1.696 (0.533-5.401)
MTHFR				
CC	13	14	.001	1
CT	29	20		0.640 (0.249-1.649)
TT	22	2		0.084 (0.016-0.432)
DHFR				
GG	33	19	.238	1
AG	30	14		0.811 (0.347-1.895)
AA	1	3		5.211 (0.506-53.687)

Note: ORs were determined by logistic regression analysis.

TABLE 3 Relationship between genetic polymorphisms and successful MTX treatment (n = 100)

Genotypes	Successful MTX injection (n = 77)	Failed MTX injection (n = 23)	*P* value	OR(95% CI)
RFC1				
AA	21	6	.709	1
AG	39	10		0.897 (0.286-2.814)
GG	17	7		1.441 (0.407-5.102)
MTHFR				
CC	19	8	.017	1
CT	35	14		0.950 (0.338-2.668)
TT	23	1		0.103 (0.012-0.901)
DHFR				
GG	41	11	.476	1
AG	34	10		1.096 (0.416-2.891)
AA	2	2		3.727 (0.470-29.534)

Note: ORs were determined by logistic regression analysis.

of genotypes and observed values. *P* values <.05 were considered statistically significant. Data were performed by SPSS (Version 19.0; SPSS Inc).

3 | RESULTS

3.1 | Characteristics of patients

The patient characteristics of EP were described in Table 1, including age, BMI, gravidity, parity, pretreatment β-hCG level, spontaneous conception, previous EP, presence of hemoperitoneum, size of hemoperitoneum and size of EP, or inhomogeneous adnexal mass. Genotype frequencies of polymorphisms of RFC1, MTHFR, and DHFR were found to be in Hardy-Weinberg equilibrium ($P > .05$).

3.2 | Association between genetic polymorphisms and successful MTX treatment in ectopic pregnancy

The relationship between the genotypes and treatment success was shown in Table 2 and Table 3. MTHFR C677T was associated with successful MTX treatment ($P = .017$). Meanwhile, a higher success rate of first MTX injection in patients with harboring mutation allele of MTHFR gene was observed than that in patients with wild-type gene ($P = .001$). However, no significant association was found between surgical treatment and polymorphisms of RFC1 and DHFR ($P = .709$ and 0.476, respectively). In addition, there was also no significant relationship between the polymorphisms of RFC1, DHFR, and success rate in the first MTX injection ($P = .661$ and .238, respectively).

TABLE 4 Relationship between genetic polymorphisms and β-hCG reduction

Genotypes	Pretreatment β-hCG (n = 100)	*P* value	Day 4 β-hCG (n = 97)	*P* value	△ 0-4 d (n = 97)	*P* value	Day 7 β-hCG (n = 95)	*P* value	△ 0-7 d (n = 95)	*P* value
RFC1										
AA	979.55 ± 739.25		999.59 ± 1122.22	.144	−20.04 ± 825.77		856.11 ± 1024.98	.063	123.44 ± 816.89	.172
AG	877.28 ± 836.63	.463	720.28 ± 1048.94		140.54 ± 619.14	.214	390.00 ± 467.74		429.04 ± 748.64	
GG	1068.67 ± 926.46		1280.82 ± 1724.26		−184.86 ± 1006.45		607.98 ± 497.57		368.22 ± 579.40	
MTHFR										
CC	1085.03 ± 773.80		1223.96 ± 1536.42	.009	−165.84 ± 974.19		760.28 ± 889.38	.090	191.46 ± 740.56	.554
CT	984.91 ± 960.03	.207	1028.73 ± 1308.91		−30.84 ± 728.18	.197	601.34 ± 660.82		357.69 ± 789.72	
TT	730.27 ± 537.72		406.76 ± 487.46		323.51 ± 581.74		320.96 ± 484.97		409.31 ± 644.02	
DHFR										
GG	937.47 ± 849.84		931.39 ± 1225.26	.436	−1.93 ± 786.02		563.31 ± 725.46	.943	366.16 ± 872.79	.726
AG	890.61 ± 710.68	.458	770.56 ± 987.06		120.05 ± 660.84	.270	531.77 ± 542.88		313.86 ± 548.49	
AA	1786.97 ± 1489.07		2549.38 ± 2959.59		−762.41 ± 1563.52		1248.23 ± 1871.94		−69.21 ± 931.59	

Note: P value were determined by Kruskal-Wallis H analysis.

3.3 | Association between genetic polymorphisms and β-hCG level after MTX treatment in ectopic pregnancy

The relationship between genotypes and β-hCG level changes was showed in Table 4. There was no correlation between β-hCG levels and genotypes of RFC1, MTHFR, and DHFR before treatment (*P* = .463, .207, and .458, respectively). β-hCG level at day 4 was significant association with MTHFR C677T (*P* = .009), whereas the decrement of β-hCG level in 4 days was indifference (*P* = .197). There were no significant differences on β-hCG level at day 4 (*P* = .144 and .436, respectively) and day 7 (*P* = .063 and .943, respectively) among polymorphisms of RFC1 and DHFR.

4 | DISCUSSION

As a transporter of folacin, RFC1 plays a critical role in membrane transport of MXT and its metabolites. RFC1 participates in regulating cellular uptake of MTX and cellular efflux of MTX-PGs.[12,13] RFC1 G80A impairs transporting of MTX into cells caused a lower level of MTX.[14] Intercellular MTX is converted to more active MTX-PGs with up to seven glutamyl residues (MTX-PG_1 to MTX-PG_7). Longer chain polyglutamates have longer antifolate effects, with better retained in cells.[13] Dervieux et al concluded that there was a higher level of intracellular MTX-PG_5 in patients with RFC1 80AA genotype.[15] In fact, there is correlation between RFC1 G80A and efficacy of MTX in several diseases. A meta-analysis demonstrated that odd of MTX efficacy is increased by 49% for those carrying AA genotype in rheumatoid arthritis patients.[8] Laverdière et al showed that children harboring A allele of RFC1 had a significantly worse prognosis than that harboring G allele in acute lymphoblastic leukemia.[16] The survival was also associated with the genetic polymorphism of RFC1 G80A in osteosarcoma patients treated with MTX.[17] However, our results showed that there was no association with RFC1 G80A on either failure treatment with MTX or β-hCG level decrement after MTX treatment. The absence of correlation maybe due to the lower dosage of MTX for tubal EP therapy, implying that the dosage is a critical factor for genetic polymorphisms of RFC1 in modifying the inhibitory effect of MTX on proliferation of trophocytes.

C677T is one of the most common single nucleotide polymorphism in MTHFR gene. Enzyme activity is reduced by 30% and 65% in genotypes CT and TT carriers, respectively.[18] Patients harboring T allele were almost accompanied by an increased toxicity and decreased efficacy in several studies, while those results are still contradictory.[19] Chen Y et al concluded that C677T polymorphism of MTHFR predicts nonresponse and adverse effects of MTX in juvenile idiopathic arthritis.[20] However, two studies implied that MTHFR C677T was not associated with MXT failure and β-hCG concentration changes during MTX treatment in patients with tubal EP.[21,22] Interestingly, our results showed that CC carriers had a lower success rate than CT and TT carriers by first MTX injection and another dosage of MTX treatment. This result may be partially attributed to

the characteristic of MTHFR function. A recent study showed that TT carriers needed a lower MTX dose compared to CC carriers in order to achieve an equivalent efficacy, due to MTHFR function affecting transmethylation flux and S-adenosylmethionine homeostasis rather than nucleotide biosynthesis.[23] Despite no significant variance in β-hCG reduction between different polymorphisms carrier, our results found significant differences in β-hCG level on day 4. Meanwhile, mean β-hCG level on day 4 has a rising trend in CC carriers, which may be linked with increased enzyme activity caused by CC homozygote.

DFHR is the major target of MTX, a key enzyme participating in the conversion of dihydrofolate to tetrahydrofolate. Polymorphism of DHFR A-317G is linked with the enzyme activity and results in dysfunction of purine synthesis. Ceppi F et al concluded the role of DHFR variants in predicting the outcome of MTX treatment on acute lymphoblastic leukemia in children.[24] Another study revealed that patients harboring AA alleles had a poor response to methotrexate in patients with rheumatoid arthritis.[25] However, our results showed there was no association between the polymorphisms of DHFR, failure treatment with MTX, and β-hCG level decrement. A lower dosage of MTX in EP might reduce the contribution of DHFR A-317G on curative effect.

β-hCG level over 5000 mIU/mL before treatment is a crucial factor to indicate failure treatment with MTX.[11] Therefore, those patients were excluded to avoid the interference in the present study. The dosage of MTX for efficacy on EP is likely to weaken the impact of heredity factors. In addition, some limitations may be ruled out by a number of samples.

In conclusion, our results suggest that MTHFR C677T polymorphism be a reliable indicator of failure treatment with MTX. There is no association among genetic polymorphisms (RFC1 and DHFR), successful MTX treatment, and β-hCG reduction. However, large randomized prospective studies will be needed to effectively replicate and validate these findings.

ACKNOWLEDGMENTS

This work was supported by the Natural Science Foundation of Fujian Province, China (No: 2017J01169).

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

FUNDING INFORMATION

Natural Science Foundation of Fujian Province, China, Grant/Award Number: 2017J01169.

ORCID

Dao-Hua Shi https://orcid.org/0000-0002-3383-5640

REFERENCES

1. Committee on Practice B-G. ACOG practice bulletin no. 191: Tubal ectopic pregnancy. *Obstet Gynecol*. 2018;131:e65-e77.
2. Malacova E, Kemp A, Hart R, Jama-Alol K, Preen DB. Long-term risk of ectopic pregnancy varies by method of tubal sterilization: a whole-population study. *Fertil Steril*. 2014;101:728-734.
3. Chen CY, Hwu YM, Chen CP, Chang CC. Quantitative analysis of total beta-subunit of human chorionic gonadotropin concentration in urine by immunomagnetic reduction to assist in the diagnosis of ectopic pregnancy. *Int J Nanomedicine*. 2015;10:2475-2483.
4. Lee R, Dupuis C, Chen B, Smith A, Kim YH. Diagnosing ectopic pregnancy in the emergency setting. *Ultrasonography*. 2018;37:78-87.
5. Stika CS. Methotrexate: the pharmacology behind medical treatment for ectopic pregnancy. *Clin Obstet Gynecol*. 2012;55:433-439.
6. Barnhart KT, Gosman G, Ashby R, Sammel M. The medical management of ectopic pregnancy: a meta-analysis comparing "single dose" and "multidose" regimens. *Obstet Gynecol*. 2003;101:778-784.
7. Niedzielska E, Weclawek-Tompol J, Matkowska-Kocjan A, Chybicka A. The influence of genetic RFC1, MS and MTHFR polymorphisms on the risk of acute lymphoblastic leukemia relapse in children and the adverse effects of methotrexate. *Adv Clin Exp Med*. 2013;22:579-584.
8. Li X, Hu M, Li W, et al. 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*. 2016;38:8-15.
9. Sirotnak FM, Tolner B. Carrier-mediated membrane transport of folates in mammalian cells. *Annu Rev Nutr*. 1999;19:91-122.
10. Sramek M, Neradil J, Veselska R. Much more than you expected: the non-DHFR-mediated effects of methotrexate. *Biochim Biophys Acta*. 2017;1861:499-503.
11. ACOG Practice Bulletin No. 193: Tubal ectopic pregnancy. *Obstet Gynecol*. 2018;131:e91-e103.
12. Wang SM, Sun LL, Wu WS, Yan D. MiR-595 suppresses the cellular uptake and cytotoxic effects of methotrexate by targeting SLC19A1 in CEM/C1 cells. *Basic Clin Pharmacol Toxicol*. 2018;123:8-13.
13. Ando Y, Shimada H, Matsumoto N, et al. Role of methotrexate polyglutamation and reduced folate carrier 1 (RFC1) gene polymorphisms in clinical assessment indexes. *Drug Metab Pharmacokinet*. 2013;28:442-445.
14. Ghodke-Puranik Y, Puranik AS, Shintre P, et al. Folate metabolic pathway single nucleotide polymorphisms: a predictive pharmacogenetic marker of methotrexate response in Indian (Asian) patients with rheumatoid arthritis. *Pharmacogenomics*. 2015;16:2019-2034.
15. Dervieux T, Furst D, Lein DO, et al. Pharmacogenetic and metabolite measurements are associated with clinical status in patients with rheumatoid arthritis treated with methotrexate: results of a multicentred cross sectional observational study. *Ann Rheum Dis*. 2005;64:1180-1185.
16. Laverdiere C, Chiasson S, Costea I, Moghrabi A, Krajinovic M. Polymorphism G80A in the reduced folate carrier gene and its relationship to methotrexate plasma levels and outcome of childhood acute lymphoblastic leukemia. *Blood*. 2002;100:3832-3834.
17. Jabeen S, Holmboe L, Alnaes GI, Andersen AM, Hall KS, Kristensen VN. Impact of genetic variants of RFC1, DHFR and MTHFR in osteosarcoma patients treated with high-dose methotrexate. *Pharmacogenomics J*. 2015;15:385-390.
18. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet*. 1995;10:111-113.
19. Giletti A, Esperon P. Genetic markers in methotrexate treatments. *Pharmacogenomics J*. 2018;18:689-703.
20. Chen Y, Zou K, Sun J, Yang Y, Liu G. Associations between gene polymorphisms and treatment outcomes of methotrexate in

patients with juvenile idiopathic arthritis. *Pharmacogenomics*. 2018;19:529-538.
21. Zafer E, Kahraman Cetin N, Turan OD, Kardelen E, Kurt Omurlu I, Yuksel H. Common methylenetetrahydrofolate reductase polymorphisms (A1298C & C677T) in ectopic trophoblasts and methotrexate treatment failure in tubal pregnancies. *Ginekol Pol*. 2018;89:536-540.
22. Fedota O, Roschenyuk L, Tyzhnenko T, Merenkova I, Vorontsov V. Pharmacogenetic effects of methotrexate (Mtx) in ukrainian patients depending on the mthfr genotypes (Clinical Cases). *Georgian Med News*. 2018;111-117.
23. Wang YC, Wu MT, Tang FY, et al. MTHFR C677T polymorphism increases MTX Sensitivity via the inhibition of S -adenosylmethionine and de novo purine synthesis. *Clin Sci (Lond)*. 2019;133:253-267.
24. Ceppi F, Gagne V, Douyon L, et al. DNA variants in DHFR gene and response to treatment in children with childhood B ALL: revisited in AIEOP-BFM protocol. *Pharmacogenomics*. 2018;19:105-112.
25. Milic V, Jekic B, Lukovic L, et al. Association of dihydrofolate reductase (DHFR) -317AA genotype with poor response to methotrexate in patients with rheumatoid arthritis. *Clin Exp Rheumatol*. 2012;30:178-183.

How to cite this article: Deng J, Chen L, Xue H, Zeng F-X, Niu P-G, Shi D-H. Contribution of genetic polymorphism of methylene tetrahydrofolate reductase on the effect of methotrexate in ectopic pregnancy patients. *J Clin Lab Anal*. 2020;34:e23030. https://doi.org/10.1002/jcla.23030