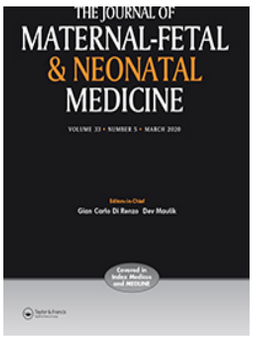

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The association of parental methylenetetrahydrofolate reductase polymorphisms (*MTHFR* 677C > T and 1298A > C) and fetal loss: a case–control study in South Australia

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ORIGINAL ARTICLE

The association of parental methylenetetrahydrofolate reductase polymorphisms (*MTHFR* 677C > T and 1298A > C) and fetal loss: a case–control study in South Australia

Britt J. P. Kos[a,b], Shalem Y. Leemaqz[a], Catherine D. McCormack[c], Prabha H. Andraweera[a], Denise L. Furness[a], Claire T. Roberts[a] and Gustaaf A. Dekker[a,b]

[a]Adelaide Medical School, Robinson Research Institute, University of Adelaide, Adelaide, Australia; [b]Women and Children's Division, Lyell McEwin Hospital, Elizabeth Vale, South Australia, Australia; [c]Women's and Children's Hospital, North Adelaide, Adelaide, Australia

ABSTRACT

Objective: To determine the association between parental MTHFR 677C > T (RS1801133) and 1298A > C (RS1801131), and fetal loss (FL).

Design: Case–control study.

Setting: Department of Obstetrics and Gynecology, Lyell McEwin Hospital (LMH), and the Women's and Children's Hospital (WCH) in Adelaide, Australia.

Patients: A total of 222 couples with FL and 988 couples with uncomplicated pregnancies.

Measurements: The main outcomes were FL and hyperhomocysteinemia (HHcy). All couples were tested for MTHFR 677C > T and 1298A > C. Fasting homocysteine was measured in the women with FL.

Results: The main finding was a significant difference between the FL group and controls in couples with ≥4 abnormal alleles compared to <4 [p=.0232, OR 1.9 (95% CI 1.1–3.3)]. None of the couples with FL had zero abnormal alleles (both parents 677CC/1298 AA). However, this was also rare amongst the controls. Maternal carriage of both 677C > T and the 1298A > C polymorphisms was similar between the FL group and controls. The prevalence of paternal 677TT/1298AA and 677CC/1298AC was significantly higher in the FL group compared with controls. HHcy was significantly more common in the FL group compared with controls.

Conclusion: The presence of parental MTHFR 677C > T and 1298A > C is associated with FL. The association between maternal MTHFR genotypes with FL is less pronounced than in previously published articles investigating first trimester miscarriages. Maternal HHcy is a significant risk factor for FL.

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Fetal loss; hyperhomocysteinemia; methylenetetrahydrofolate reductase; MTHFR; pregnancy loss

Introduction

Pregnancy loss (PL) is a common problem and represents a stressful life event in women [1] and has negative psychological effects on the parents [2]. The term PL is very nonspecific since it includes embryonic loss (PL <10 weeks of gestation) and FL (PL ≥10 weeks of gestation), where FL can be further subdivided into late miscarriage (LM; 10–20 weeks) and stillbirth (≥20 weeks). However, definitions are extremely variable between countries, including high-income countries [3].

One in four women who become pregnant experience a sporadic miscarriage, in which the majority occurs <12 weeks. Miscarriages >12 weeks occur in 1–2% of pregnancies [4]. Chromosomal abnormalities cause 50% of first trimester PL [5]. Causative factors in LM include, among others, antiphospholipid syndrome, cervical weakness, infection, placental insufficiency, congenital uterine anomalies, bacterial vaginosis, and hypothyroidism [6]. The average stillbirth rate in high-income countries is 3.5 per 1000 total births, 2.7 in Australia. Important causes of stillbirth consist of placental pathologies (40%), congenital abnormalities (6–27%), infection (5–22%), and spontaneous preterm birth or preterm rupture of membranes (1–15%) [7]. Important worldwide risk factors of stillbirth consist of maternal age >35 years, adolescent pregnancy, short interpregnancy interval, infections, obesity, chronic hypertension, diabetes, tobacco, (pre-)eclampsia, pregnancy >42 weeks, medical disorders (e.g. thyroid disorders and liver disease), and indoor air pollution [3].

CONTACT Britt J. P. Kos britt-kos@hotmail.com Dorpsstraat 781, 1724NM Oudkarspel, Netherlands

In addition to the above mentioned causes and risk factors, hyperhomocysteinemia (HHcy) has been associated with PL since the early 1990s [8]. Unfortunately many cases of PL remain unexplained.

Studies have shown that there is an association between PL and the *MTHFR* single nucleotide polymorphisms (SNPs) 677C > T (RS1801133) [9–12] and 1298A > C (RS1801131) [11,12]. However, the study by Dilley et al. [13], investigating the relationship between recurrent PL and *MTHFR* 677C > T, did not show any association. *MTHFR* is an important enzyme in one-carbon metabolism that plays a part in the generation of methyl groups for methylation of DNA and other molecules [14]. Most of the recent research has focused on the role of these polymorphisms in first trimester miscarriages. A problem with these studies is that, as previously mentioned, chromosome abnormalities will always be the dominant cause in early PL. The focus of this study was therefore to evaluate the involvement of these two common SNPs and HHcy as risk factors for LM and stillbirth.

The majority of previous studies did not include the paternal contribution; in this study, the main objective was therefore to evaluate these two SNPs in couples with FL among a large number of affected couples in South Australia.

Materials and methods

A case–control study was conducted at the Department of Obstetrics and Gynecology of the Lyell McEwin Hospital (LMH) and the Women's and Children's Hospital (WCH) in Adelaide, Australia. This study formed part of the Prediction of Adverse Pregnancy Outcomes (PAPO) study (Clinical Trial No. ACTRN12609000254291). The study was approved by the Women's and Children's Hospital Human Research Ethics Committee in North Adelaide South Australia, REC1481/6/09.

Participant selection

From 2007 to 2012, patients attending the Pregnancy Investigation Unit at the WCH and the Obstetric Counseling Clinic at the LMH after a PL were invited to participate in the PAPO study. All couples who participated in the PAPO study provided informed consent. Those with a clinically diagnosed FL (PL ≥10 weeks of gestation) were selected for the current study. The index FL is defined as the last FL before entering the PAPO study.

Exclusion criteria: FL due to aneuploidy and/or congenital anomalies; couples where one or both parents were known to have a chromosomal translocation; couples where the woman was known to be on high dose folic acid and/or high dose vitamin B12 at the time of the index FL.

The Adelaide and Auckland couples in the Screening for Pregnancy Endpoints (SCOPE) study [15] with uncomplicated pregnancies and for whom *MTHFR* genotypes of both partners were available were used as the control group (i.e. no preeclampsia, preterm birth, history of any PL, small for gestational age, or gestational diabetes mellitus). The women who were originally recruited to the SCOPE study were low-risk nulliparous women.

Data collection

All couples were tested for the maternal and paternal *MTHFR* polymorphisms 677C > T and 1298A > C. Maternal age, BMI, ethnicity, and smoking status were extracted from the medical case notes of the women. Paternal medical case notes were not available for all men. FL was divided into LM (gestation of 10–20 weeks) and stillbirths (gestation of ≥20 weeks).

Fasting homocysteine was measured in the women who had experienced a fetal loss (FL) outside pregnancy and while not taking high dose folic acid and vitamin B12. HHcy was defined as a fasting homocysteine level >9.5 (μmol/l) [16].

Statistical analysis

To compare the frequencies of the *MTHFR* alleles for the FL group and controls, a proportions test was used. Fisher's exact test, with Haldane correction in contingency tables containing 0 cells counts, were then performed for each category/combinations of genotypes compared to the normal wild type, and the corresponding odds ratios and 95% confidence intervals were also obtained. These statistical tests were performed using R version 3.1.0. Any missing data were omitted from all analyses. Variables are considered significant at 5% significance level (p values <.05).

All of the above mentioned analyses on *MTHFR* 677C > T and 1298A > C were conducted separately and together for individual parents. Because of the published importance [17–19] of the embryonic/fetal genotype, we also analyzed the total number of abnormal alleles per couple. However, due to a low number of couples who had no mutant alleles, the

Table 1. Demographics maternal cases and controls.

	Control (n= 988)	Fetal loss (n= 222)		Late miscarriage (n= 150)		Stillbirth (n= 72)	
	Mean ± SEM/n (%)	Mean ± SEM/n (%)	p	Mean ± SEM/n (%)	p	Mean ± SEM/n (%)	p
Age	27.8 ± 0.2	30.0 ± 0.4	***<.001***	30.8 ± 0.5	***<.001***	28.3 ± 0.7	.4935
<35	898 (90.9%)	163 (73.4%)	Ref	104 (69.3%)	Ref	59 (81.9%)	Ref
≥35	90 (9.1%)	59 (26.6%)	***<.001***	46 (30.7%)	***<.001***	13 (18.1%)	***.0214***
BMI							
<18.5	13 (1.3%)	9 (4.2%)	***.0015***	7 (4.9%)	***.0036***	2 (2.9%)	.2856
18.5–24.9	586 (59.3%)	74 (34.7%)	Ref	54 (37.8%)	Ref	20 (28.6%)	Ref
25–29.9	263 (26.6%)	64 (30.0%)	***.0018***	39 (27.3%)	.1059	25 (35.7%)	***.0028***
≥30	126 (12.8%)	66 (31.0%)	***<.001***	43 (30.1%)	***<.001***	23 (32.9%)	***<.001***
Ethnicity							
Caucasian	877 (88.8%)	188 (84.7%)	Ref	130 (86.7%)	Ref	58 (80.6%)	Ref
Asian	68 (6.9%)	18 (8.1%)	.9288	11 (7.3%)	1.0000	7 (9.7%)	.6440
Other	43 (4.4%)	16 (7.2%)	.1621	9 (6.0%)	.7932	7 (9.7%)	.0811
Smoker							
No	906 (91.7%)	171 (80.3%)	Ref	112 (78.9%)	Ref	59 (83.1%)	Ref
Yes	82 (8.3%)	42 (19.7%)	***<.001***	30 (21.1%)	***<.001***	12 (16.9%)	***.0271***

The bold italics values are the variables that are considered significant at 5% significance level (p values < .05).

risk of FL was compared between ≥4 (i.e. the embryo will have at least two abnormal alleles) and <4 mutant alleles using logistic regression, corrected for maternal BMI, age, and smoking. A global F test was added for the logistic regression to indicate the overall significance of the coefficients. Since the paternal demographics of these factors were not collected it was not possible to correct for paternal BMI, age, or smoking.

Results

The current study includes 222 couples with FL. The genetic analysis for the *MTHFR* 677C > T and 1298A > C SNPs was completed on 222 women and 158 men. The SCOPE control group consisted of 988 controls (n= 352 from Adelaide and n= 636 from Auckland) with complete data on *MTHFR* genotypes.

Table 1 shows the maternal demographics of the FL group and controls. In the FL group significantly more women were ≥35 years, had a BMI <18.5 and ≥25 and were smokers. The majority of both the FL and control groups were of Caucasian ethnicity reflecting the community in which they reside.

Abnormal allele counts for each couple were obtained. Couples with both parents having the wild type for both SNPs (*MTHFR* 677CC/1298 AA) will have a zero abnormal allele count. While, for example, a couple with paternal 677TT/1298AA and maternal 677CT/1298AA would be given a count of three abnormal alleles. In theory, a couple could have a maximum of eight abnormal alleles. When analyzing couples with ≥4 abnormal alleles to <4 (Table 2), a significant difference was seen between the FL group (21.5%) and control group (14.5%) [p=.023, OR 1.9 (95% CI 1.1–3.3)]. When separating the FL group into LM and stillbirths a significant difference was only seen in the LM group [p= .041, OR 1.9 (95% CI 1.0–3.7)]. All couples in the FL group had one or more abnormal alleles, while 23 couples amongst the controls had a zero abnormal allele count (2.3%). Only one of the couples in the control group and none in the FL group had five mutant alleles. The control couple with the five mutant allele count was incorporated in the ≥4 mutant allele group.

When separating maternal and paternal *MTHFR* genotypes there was no difference in the frequency of the maternal *MTHFR* genotypes between the FL group and controls. The frequency of the paternal 677TT/1298AA genotype was significantly higher [p= .026, OR 2.4 (95% CI 1.1–5.1)] in the FL group compared with controls, 13.3% versus 9.6%, respectively. When dividing the FL group into LM and stillbirths, significance for this genotype was only seen in the LM group [p= .026, OR 2.6 (95% CI 1.1–6.2)]. A significant difference between the FL group and controls was also found for the paternal 677CC/1298AC genotype, respectively 25.9% versus 21.9% [p= .036, OR 2.0 (95% CI 1.1–3.9)], where now this difference was only seen in the stillbirth group [p= .042, OR 3.3 (95% CI 1.0–10.2)].

Fasting homocysteine was measured in 202 of the 222 women in the FL group. The rate of HHcy was significantly higher in the FL group when compared to the expected frequency (based on the 97.5th centile) in the control group [p<.0001, OR =7.1 (95% CI 4.1–12.3)], respectively 15.3% versus 2.5%. None of the maternal *MTHFR* genotypes in the FL group were associated with HHcy.

Discussion

The importance of *MTHFR* in PL is still a matter of significant debate. The main finding in this large case–control study was a significant difference in the number of couples with ≥4 abnormal *MTHFR* alleles

Table 2. Risks of FL for couples with >4 mutant alleles in *MTHFR* 677/1298 genotypes combined.

Number of mutant alleles	Control *n* (%)	FL *n* (%)	adjOR (95% CI)a	adj *p*a	Late miscarriage *n* (%)	adjOR (95% CI)a	adj *p*a	Stillbirth *n* (%)	adjOR (95% CI)a	adj *p*a
<4	845 (85.5)	124 (78.5)	1	Ref	82 (77.4)	1	Ref	42 (80.8)	1	Ref
≥4	143 (14.5)	34 (21.5)	1.9 (1.1–3.3)	***.0232***	24 (22.6)	1.9 (1.01–3.7)	***.0408***	10 (19.2)	1.6 (0.6–3.7)	.2682

FL, fetal loss.
[a]Adjusted for maternal BMI, age, and smoking.
The bold italics values are the variables that are considered significant at 5% significance level (*p* values < .05).

compared to <4 between the FL group and controls (Table 2). The emphasis of the more recent pregnancy literature on *MTHFR* has shifted to the potential role of epigenetic processes and DNA methylation throughout pregnancy [19,20]. In contrast, most earlier studies focused on HHcy and adverse pregnancy outcomes where the mutant *MTHFR* alleles were considered to be part of the so-called thrombophilias.

Of course, *MTHFR* polymorphisms and HHcy, particularly in individuals with low folate and/or vitamin B6/B12 status, among other factors potentially affect DNA methylation and associate with DNA damage [20–22]. Currently, women with abnormal *MTHFR* alleles are often treated with high dose vitamin B12 and folic acid.

The relevance of the findings of this current study that 21.5% of the FL group had ≥4 mutant alleles compared to 14.5% of controls is that in the case of a couple with four mutant alleles the embryo will inevitably have two abnormal alleles. Reported in the paper by Zetterberg et al. [18] one or more fetal 677T and 1298C alleles were significantly more prevalent in spontaneously aborted embryos compared to the wild type genotype 677CC/1298 AA, which indicates that *MTHFR* polymorphisms may have substantial impact on fetal survival. This effect was not seen in the stillbirth group when dividing the FL group into LM and stillbirth. Our finding concurs with the study by Silver et al. [23] that found no association between *MTHFR* mutations and stillbirth in mothers and fetuses. However, their research did not include paternal genotypes. Both stillbirth and LM were associated with maternal age, BMI, and smoking in the current study.

Due to the fact that no couples in the FL group had zero abnormal alleles, a reliable estimate of odds ratios and confidence intervals could not be obtained when separating the number of mutant alleles and comparing them to the wild type. Therefore, we chose to analyze the number of mutant alleles by grouping them together as ≥4 versus <4.

No couples in the FL group in the current study had more than four mutant alleles. In the study by Isotalo et al. [17] combined *MTHFR 677CT/1298CC* and *677TT/1298CC* genotypes, containing three and four mutant alleles, respectively, were not identified in the neonatal group. This suggests decreased viability among fetuses with these combined mutations and a possible selection disadvantage among fetuses with a high number of mutant *MTHFR* alleles. In theory, a couple may have eight mutant alleles. Isotalo et al. [17] demonstrated that one would typically not expect more than two abnormal alleles per individual, i.e. not more than four abnormal alleles per couple. Surprisingly, we did find one couple with five mutant alleles in the control group.

Interestingly, not one of the couples in the FL group had a combined parental genotype with a zero count for abnormal *MTHFR* alleles, but it should be noted that this was also an infrequent finding (2.3%) amongst the control couples due to the high prevalence of these SNPs in the normal population. The high frequency of these two SNPs has also been described previously [24], where 677C > T homozygosity amongst Caucasians ranged from 8% to 18% and 1298A > C homozygosity was reported as approximately 9%. Compound heterozygosity (677CT/1298AC) ranged from 15% to 20%, which is consistent with the *MTHFR* SNP rate in our large control group.

A limitation of our study is the discrepancy in the number of women (*N*= 222) and men (*N*= 158) tested for *MTHFR*, which is due to unknown paternity at the time of the index PL and a variety of other factors (male partner absent or not willing to participate, test/laboratory issues, unknown). Another limitation regarding paternal participants is that demographics were not collected.

While the maternal genotypes were not different between the FL group and controls, we did find a significantly higher frequency of the 677TT/1298AA and 677CC/1298AC genotypes amongst the men in the FL group. The paternal frequency of 677TT/1298AA was significantly higher in the FL group compared with controls, 13.3% versus 9.6%, respectively. The paternal frequency of 677CC/1298AC was 25.9% versus 21.9%, respectively. The effect of paternal 677TT/1298AA was not seen in the subgroup stillbirth and for the paternal 677CC/1298AC the effect was not seen in the subgroup LM. There are several reports of an association of 677TT with male infertility, particularly in Asian men. The 1298A > C polymorphism has not been

associated with male infertility [25–28]. Due to the fact that, in the current study, fasting homocysteine levels were not measured in the men, it is currently uncertain whether the effect of paternal *MTHFR* polymorphisms on FL is due to a possible elevated fasting homocysteine or another effect of the paternal *MTHFR* genotype on sperm quality. Both genotype and HHcy have been described to affect sperm quality [29]. These interesting findings need to be confirmed in future studies but appear to suggest that it may be beneficial to also prescribe folic acid and vitamin B12 for the male partner as part of preconception planning.

In the current study, HHcy was seen in 15.3% of the women in the FL group versus the expected 2.5% in the control group. As mentioned above, there was no difference in the frequency of the maternal *MTHFR* genotypes between the FL group and controls. This discrepancy appears to indicate that from a maternal perspective, HHcy might have a stronger association with FL than the maternal *MTHFR* genotype. It is important to note that these days many pregnant women consume various "pregnancy vitamin B preparations" which may "camouflage" the effect of the *MTHFR* genotypes [30]. This perhaps is in contrast to men who typically do not take vitamin B/folic acid supplements. The current study did not include the vitamin B/folic acid supplementation status in the men. Although we did try to exclude women with prescribed high dose folic acid and vitamin B12, we cannot exclude the possibility that patients were taking over the counter doses of potent vitamin B/folic acid preparations without specifically mentioning this to their care provider.

In conclusion, the presence of parental *MTHFR* 677C > T and 1298A > C SNPs is associated with FL. The association between the maternal *MTHFR* genotype with FL is less pronounced than in previously published articles investigating first trimester miscarriages. Maternal HHcy is a significant risk factor for FL.

Disclosure statement

The authors report no conflicts of interest.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, BK. The data are not publicly available due to restrictions in ethics approval, where there was no specific consent from women to make the data publically available. This is to preserve the privacy of research participants.

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ORCID

Catherine D. McCormack http://orcid.org/0000-0002-6209-6904
Prabha H. Andraweera https://orcid/org/0000-0003-2434-8370
Denise L. Furness https://orcid.org/0000-0002-1075-0257
Claire T. Roberts https://orcid.org/0000-0002-9250-2192

References

[1] Janssen HJ, Cuisinier MC, Hoogduin KA, et al. Controlled prospective study on the mental health of women following pregnancy loss. Am J Psychiatry. 1996;153:226–230.
[2] Heazell AE, Siassakos D, Blencowe H, et al. Stillbirths: economic and psychosocial consequences. Lancet. 2016;387:604–616.
[3] Lawn JE, Blencowe H, Waiswa P, et al. Stillbirths: rates, risk factors, and acceleration towards 2030. Lancet. 2016;387:587–603.
[4] Regan L, Rai R. Epidemiology and the medical causes of miscarriage. Baillieres Best Practice Res Clin Obstet Gynaecol. 2000;14:839–854.
[5] Goddijn M, Leschot NJ. Genetic aspects of miscarriage. Best Practice Res Clin Obstet Gynaecol. 2000;14:855–865.
[6] McNamee KM, Dawood F, Farquharson RG. Mid-trimester pregnancy loss. Obstet Gynecol Clin North Am. 2014;41:87–102.
[7] Flenady V, Wojcieszek AM, Middleton P, et al. Stillbirths: recall to action in high-income countries. Lancet. 2016;387:691–702.
[8] Wouters MG, Boers GH, Blom HJ, et al. Hyperhomocysteinemia: a risk factor in women with unexplained recurrent early pregnancy loss. Fertil Steril. 1993;60:820–825.
[9] Wu X, Zhao L, Zhu H, et al. Association between the MTHFR C677T polymorphism and recurrent pregnancy loss: a meta-analysis. Genet Test Mol Biomark. 2012;16:806–811.
[10] Rai V. Methylenetetrahydrofolate reductase C677T polymorphism and recurrent pregnancy loss risk in

Asian population: a meta-analysis. Ind J Clin Biochem. 2016;31:402–413.
[11] Yang Y, Luo Y, Yuan J, et al. Association between maternal, fetal and paternal MTHFR gene C677T and A1298C polymorphisms and risk of recurrent pregnancy loss: a comprehensive evaluation. Arch Gynecol Obstet. 2016;293:1197–1211.
[12] Farahmand K, Totonchi M, Hashemi M, et al. Thrombophilic genes alterations as risk factor for recurrent pregnancy loss. J Matern Fetal Neonatal Med. 2016;29:1269–1273.
[13] Dilley A, Benito C, Hooper WC, et al. Mutations in the factor V, prothrombin and MTHFR genes are not risk factors for recurrent fetal loss. J Maternal-Fetal Neonatal Med. 2002;11:176–182.
[14] Furness DL, Fenech MF, Khong YT, et al. One-carbon metabolism enzyme polymorphisms and uteroplacental insufficiency. Am J Obstet Gynecol. 2008;199:276.e1–276.e8.
[15] Kenny LC, Black MA, Poston L, et al. Early pregnancy prediction of preeclampsia in nulliparous women, combining clinical risk and biomarkers: the Screening for Pregnancy Endpoints (SCOPE) international cohort study. Hypertension. 2014;64:644–652.
[16] Fenech M, Aitken C, Rinaldi J. Folate, vitamin B12, homocysteine status and DNA damage in young Australian adults. Carcinogenesis. 1998;19:1163–1171.
[17] Isotalo PA, Wells GA, Donnelly JG. Neonatal and fetal methylenetetrahydrofolate reductase genetic polymorphisms: an examination of C677T and A1298C mutations. Am J Hum Genet. 2000;67:986–990.
[18] Zetterberg H, Regland B, Palmér M, et al. Increased frequency of combined methylenetetrahydrofolate reductase C677T and A1298C mutated alleles in spontaneously aborted embryos. Eur J Hum Genet. 2002;10:113–118.
[19] van Mil NH, Bouwland-Both MI, Stolk L, et al. Determinants of maternal pregnancy one-carbon metabolism and newborn human DNA methylation profiles. Reproduction. 2014;148:581–592.
[20] McKay JA, Groom A, Potter C, et al. Genetic and non-genetic influences during pregnancy on infant global and site specific DNA methylation: role for folate gene variants and vitamin B12. PLoS One. 2012;7:e33290.
[21] Furness D, Fenech M, Dekker G, et al. Folate, vitamin B12, vitamin B6 and homocysteine: impact on pregnancy outcome. Matern Child Nutr. 2013;9:155–166.
[22] La Merrill M, Torres-Sánchez L, Ruiz-Ramos R, et al. The association between first trimester micronutrient intake, MTHFR genotypes, and global DNA methylation in pregnant women. J Matern Fetal Neonatal Med. 2012;25:133–137.
[23] Silver RM, Saade GR, Thorsten V, et al. Factor V Leiden, prothrombin G20210A, and methylene tetrahydrofolate reductase mutations and stillbirth: the Stillbirth Collaborative Research Network. Am J Obstet Gynecol. 2016;215:468.e1–468.e17.
[24] Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol. 2000;151:862–877.
[25] Lee HC, Jeong YM, Lee SH, et al. Association study of four polymorphisms in three folate-related enzyme genes with non-obstructive male infertility. Hum Reprod. 2006;21:3162–3170.
[26] Li SS, Li J, Xiao Z, et al. Prospective study of MTHFR genetic polymorphisms as a possible etiology of male infertility. Genet Mol Res. 2014;13:6367–6374.
[27] Safarinejad MR, Shafiei N, Safarinejad S. Relationship between genetic polymorphisms of methylenetetrahydrofolate reductase (C677T, A1298C, and G1793A) as risk factors for idiopathic male infertility. Reprod Sci. 2011;18:304–315.
[28] Wei B, Xu Z, Ruan J, et al. MTHFR 677C > T and 1298A > C polymorphisms and male infertility risk: a meta-analysis. Mol Biol Rep. 2012;39:1997–2002.
[29] Singh K, Jaiswal D. One-carbon metabolism, spermatogenesis, and male infertility. Reprod Sci. 2013;20:622–630.
[30] Murphy RP, Donoghue C, Nallen RJ, et al. Prospective evaluation of the risk conferred by factor V Leiden and thermolabile methylenetetrahydrofolate reductase polymorphisms in pregnancy. Arterioscler Thromb Vasc Biol. 2000;20:266–270.