Genes & Genomics (2021) 43:937–945
https://doi.org/10.1007/s13258-021-01082-3

Online ISSN 2092-9293
Print ISSN 1976-9571

RESEARCH ARTICLE

Folate metabolizing gene polymorphisms and genetic vulnerability to preterm birth in Korean women

Bit Na Kwon[1] · Noo Ri Lee[1] · Hyung Jun Kim[1] · Yun Dan Kang[2] · Jong Soo Kim[2] · Jin Wan Park[2] · Han Jun Jin[1]

Received: 6 May 2020 / Accepted: 6 March 2021 / Published online: 24 May 2021

Abstract

Background The folate metabolism that converts homocysteine to methionine is closely related to the accumulation of homocysteine. Increased homocysteine levels lead to an impaired antithrombotic function of the vascular endothelium and uterine-placental circulation, resulting in abnormal pregnancy outcomes. Previous studies have reported that gene polymorphisms in folate metabolism are associated with the development of preterm birth (PTB) in various populations.

Objective we performed a case–control study to evaluate the association between five polymorphisms in folate metabolic genes (*MTHFR, MTR, MTRR, TCN2*) and PTB.

Methods In this study, a total of 254 subjects were analyzed (111 patients with PTB and 143 women at ≥ 38 weeks of gestation). Genotype and allele frequency differences between patients and control groups and the Hardy–Weinberg equilibrium were assessed using a Chi-square test. For evaluation indicators, odds ratios (ORs) of 95% confidence intervals (CI) were estimated. In addition, we analyzed the combined genotype frequencies of SNPs of folate-metabolizing genes to measure gene–gene interactions for PTB.

Results Our results showed that the *MTR* rs1805087 GG ($p = 0.031$), and *TCN2* rs1801198 CG genotype (OR 0.53, 95% CI 0.288–0.980, $p = 0.042$) were significantly associated with PTB. The *MTHFR* rs4846049 AA showed a marginal trend toward significance (OR 0.15, 95% CI 0.018–1.205, $p = 0.041$). In particular, the combined genotypes, including *MTHFR* rs1537514 CC—*MTRR* rs1801394 GG, *MTHFR* rs1537514 CC—*TCN2* rs1801198 CG, and *MTR* rs1805087 AA—*TCN2* rs1801198 CG, have significant interactions with PTB (OR 0.49, 95% CI 0.248–0.992, $p < 0.05$).

Conclusion The polymorphisms of folate metabolic genes may have a genetic association with the development of PTB in Korean women. A larger sample set and functional studies are required to further elucidate our findings.

Keywords Preterm birth · Folate metabolism · Polymorphisms · Korean women · Genetic association · *MTHFR* · *MTR* · *TCN2*

Introduction

Preterm birth (PTB) is a common pregnancy-related disease defined as delivery before 37 weeks of gestation and is one of the major determinants of neonatal and maternal morbidity (Smith 2012). A previous study has reported that approximately 28% of all neonatal deaths are caused by PTB (Blencowe et al. 2012). In most developed countries, 5–7% of all births were reported as premature, and this figure appears to be increasing (Beck et al. 2010). The proportion of PTBs in the Korean population was 7.2% in 2016, which is 1.5 times more than that in 2006 (http://kostat.go.kr/portal/korea/kor_nw/1/2/1/index.board?bmode=read&aSeq=362574). Various factors, such as maternal anthropometrics, health condition, age, prenatal care, and socioeconomic status, have been associated with PTB (Han et al. 2011). However, the exact etiology of PTB is not yet well understood (Han et al. 2011).

Several recent studies have reported that homocysteine is associated with the development of PTB (Tellapragada et al. 2016; Wang et al. 2015). Homocysteine is a sulfur amino acid derived from the demethylation of methionine (Hankey and Eikelboom 1999). It can induce oxidative stress, DNA strand breakage, and apoptosis (Mattson

✉ Han Jun Jin
Jins4658@dankook.ac.kr

[1] Department of Biological Sciences, College of Natural Science, Dankook University, Cheonan, Korea

[2] Department of Obstetrics and Gynecology, Dankook University Hospital, Cheonan, Korea

and Shea 2003). An increased level of homocysteine may also cause trophoblast apoptosis and reduce gonadotropin secretion, which are related with abnormal placentation and PTB (Engel et al. 2006). It is reported that enzymes of the folate metabolism pathway can regulate the blood concentration of homocysteine (Bailey and Gregory 1999; Chen et al. 2016; Stanislawska-Sachadyn et al. 2010; Wu et al. 2017). In the folate metabolism pathway, the transcobalamin II (TCN2) transports vitamin B12 from blood to tissue for folate metabolism (Stanislawska-Sachadyn et al. 2010). Methylenetetrahydrofolate reductase (MTHFR) serves a key role in folate metabolism as it catalyzes the regeneration of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, which transfers the methyl group to the vitamin B12 (Bailey and Gregory 1999). Vitamin B12 is a cofactor for the methylation of homocysteine to methionine by catalysis of the methionine synthase (MTR) and the coenzyme methionine synthase reductase (MTRR) (Wu et al. 2017).

Several studies have previously analyzed the association between the gene polymorphisms of enzymes in folate metabolism and the concentration of homocysteine (Kurzwelly et al. 2010; Mfady et al. 2014; Mohammadpour-Gharehbagh et al. 2018; Oussalah et al. 2017; Wu et al. 2013). Mohammadpour-Gharehbagh et al. (2018) showed that the *MTHFR* rs1537514 G allele is associated with the increased expression of the MTHFR enzyme that might cause downregulation of the homocysteine concentration. Moreover, Wu et al. (2013) showed that the *MTHFR* rs4846049 AA genotype reduces MTHFR protein levels and elevates the level of homocysteine. Kurzwelly et al. (2010) reported that the *MTR* rs1805087 GG genotype caused a decrease in MTR enzyme activity with higher homocysteine levels. Mfady et al. (2014) found that the *MTRR* rs1801394 AA genotype was related to 1.2-fold higher homocysteine concentration. In addition, the *TCN2* rs1801198 polymorphism was significantly associated with increased homocysteine in the European population (Oussalah et al. 2017).

Here, we conducted a case–control study to investigate the genetic association between PTB and folate-metabolizing gene polymorphisms, including *MTHFR* rs4846049 (2572 C>A), rs1537514 (4869 C>G), *MTR* rs1805087 (2756 A>G), *MTRR* rs1801394 (66 A>G), and *TCN2* rs1801198 (776 C>G) in Korean women. In addition, we evaluated the combined interaction among metabolizing gene polymorphisms.

Materials and methods

Subjects

We analyzed a total of 254 women recruited from the Gynecology Department at Dankook University Hospital in Korea. Of these samples, 111 patients with PTB were selected based on their gestational ages being < 37 weeks. The control group consisted of 143 pregnant women with no history of PTB or spontaneous abortion and at a gestation period of at least 38 weeks. Neither the patients with PTB nor those in the control group had any systemic disease, such as hypertension, gestational diabetes, coronary heart disease, placental abruption, chronic nephritis, multiple gestation, and fetal anomalies. All clinical interviews were conducted by an obstetrician, and informed consent was obtained from all the participants of this study. The study protocol was approved by the Ethics Committee of the Dankook University Hospital (date of approval and the project identification code are respectively 16 November 2017 and DKUH 2016-12-003-005).

DNA extraction and genotyping

Genomic DNA was extracted from buccal cells or peripheral blood with a GeneAll Exgene Clinic SV mini kit (GeneAll, Seoul, Korea). We used PCR–RFLP for genotyping the *MTHFR* rs4846049 and rs1537514, *MTR* rs1805087, *MTRR* rs1801394, and *TCN2* rs1801198. Primer sets for the *MTR* rs1805087, *MTRR* rs1801394, and *TCN2* rs1801198 were designed according to previous studies (Cai et al. 2010; Hozyasz et al. 2012). To design primers for the *MTHFR* rs4846049 and rs1537514, Primer3Plus was used (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). The PCR was performed in a total volume of 20 µl, comprising 10 ng of genomic DNA, 10 pM of each primer, 0.2 mM dNTPs, 2.0 mM $MgCl_2$, 10 × PCR buffer, and 1.0 U NV DNA polymerase (NAVI BioTech, Cheonan, Korea). The PCR amplification was conducted with a C1000 Touch thermal cycler (Bio-Rad, California, USA) under the following conditions: 95 °C for 5 min, followed by 35 cycles at 94 °C for 1 min, each annealing temperature for 1 min, and then 72 °C for 1 min, before a final extension at 72 °C for 10 min. Each of the PCR products was digested with 1.0 U *Sty*I-HF (*MTHFR* rs4846049) and *Alw*26I (*MTHFR* rs1537514), *Hae*III (*MTR* rs1805087), *Nde*I (*MTRR* rs1801394), and *Mva*I (*TCN2* rs1801198) restriction enzymes (Enzynomics, Daejeon, Korea) for 6 h at 37 °C. Table 1 shows each of the primer sets and the polymorphic restriction enzyme sites.

Data analyses

An independent t-test was performed to compare the characteristic data (i.e., age, height, weight, pre-gestation weight, systolic blood pressure, diastolic blood pressure, birth weight, and gestational ages) using SPSS 21 Statistics (IBM Korea, Korea). A chi-square test was performed to assess the Hardy–Weinberg equilibrium (HWE). In addition, the

Table 1 PCR primer sequences, annealing temperature and restriction fragments size for each polymorphism

Marker	Region	Primer sequence	Annealing temperature (°C)	Restriction enzyme	Fragments size
MTHFR rs4846049 (2572 C>A)	3′-UTR	F: 5′-TTGCCAACTAAG CCCTCGAAACAA-3′ R: 5′-TGCCACATCTCT TCTACGATGCCA-3′	58	*Sty*I-HF	C allele: 70 bp A allele: 140 bp
MTHFR rs1537514 (4869 C>G)	3′-UTR	F: 5′-AGGCAAGCCCCT CAGCCCTT-3′ R: 5′-TCCAGCCCTGAG CCCAGAGTCT-3′	63	*Alw*26I	C allele: 126 bp G allele: 98, 28 bp
MTR rs1805087 (2756 A>G)	Exon (Asp919Gly)	F: 5′-TGTTCCCAGCTG TTAGATGAAAATC-3′ R: 5′-GATCCAAAGCCT TTTACACTCCTC-3′	60	*Hae*III	A allele: 211 bp G allele: 131, 80 bp
MTRR rs1801394 (66 A>G)	Exon (Ile22Met)	F: 5′-CAGGCAAAGGCC ATCGCAGAAGAC AT-3′ R: 5′-CACTTCCCAACC AAAATTCTTCAAAG-3	55	*Ndes*I	A allele: 151 bp G allele: 124, 27 bp
TCN2 rs1801198 (776 C>G)	Exon (Pro259Arg)	F: 5′-GCATTACAGGTG GGAAAGAGAC-3′ R: 5′-CCAGGGATCTCC ATTTACTGTC-3′	67	*Mva*I	C allele: 363, 118, 24, 17, 3 bp G allele: 481, 24, 17, 3 bp

odds ratio (OR) with 95% confidence intervals (CI) was calculated using the genotype and allele frequencies of cases and controls. A p value of < 0.05 was considered statistically significant. Furthermore, we analyzed the gene–gene interaction among the metabolizing gene polymorphisms using the Generalized Multifactor Dimensionality Reduction (GMDR) analysis (http://www.ssg.uab.edu/gmdr) to construct all possible combinations of the five polymorphisms (Chen et al. 2011). The Bonferroni correction was applied to adjust for multiple comparison tests. Statistical analyses were performed using the web-based statistics tools SISA (http://www.quantitativeskills.com/sisa/) and SNPstats (http://bioinfo.iconcologia.net/SNPstats) and calculated the statistical power using G*Power 3.1 (Faul et al. 2007).

Results

We analyzed a total of 254 pregnant women. There were no meaningful differences between the mean values of the ages, heights, pre- and post-pregnancy weights, systolic blood pressures, and diastolic blood pressures of the patients with PTB and those in the control group ($p > 0.05$). However, there were significant differences in the birth weights and gestational ages between the patients with PTB and those in the control group ($p < 0.05$) (Table 2).

The genotyping data of *MTHFR* rs4846049, rs1537514, *MTR* rs1805087, *MTRR* rs1801394, and *TCN2* rs1801198 polymorphisms for the 111 patients with PTB and the 143 patients in the control group are presented in Table 3. The genotype frequencies of the five SNPs had no deviation from the HWE in patients with PTB and the control group patients (Table 3). *MTR* rs1805087 GG genotype ($p = 0.031$), and *TCN2* rs1801198 CG genotype (OR 0.53, 95% CI 0.288–0.980, $p = 0.042$). In the genetic models of each polymorphism, we found significant differences between the patients with PTB and those in the control group (Table 3). In particular, the *TCN2* rs1801198 polymorphism was observed as a protective factor for PTB in the dominant model (OR 0.55, 95% CI 0.310–0.982, $p = 0.042$). And we observed a marginal trend toward significance in *MTHFR* rs4846049 AA genotype (OR 0.15, 95% CI 0.018–1.205, $p = 0.041$).

We constructed possible genotype combinations of the folate-metabolizing gene polymorphisms (Table 4). As a result, *MTHFR* rs4846049/rs1537514, *MTHFR* rs1537514/*MTRR* rs1801394, *MTHFR* rs1537514/*TCN2* rs1801198, and *MTR* rs1805087/*TCN2* rs1801198 models were selected by GMDR analysis (cross-validation consistency $\geq 7/10$). We found that the combination of the *MTHFR* rs1537514 CC genotype and the *MTRR* rs1801394 GG genotype is a risk factor for PTB (OR 3.14, 95% CI 1.155–8.555, $p = 0.021$). Meanwhile, the protective interactions were identified in the combination of the *MTHFR* rs1537514 CC genotype and the *TCN2* rs1801198 CG genotype (OR 0.52, 95% CI 0.269–0.996, $p = 0.047$) and the *MTR* rs1805087 AA genotype and the *TCN2* rs1801198 CG genotype (OR 0.49, 95% CI 0.248–0.992, $p = 0.046$). The p values were

Table 2 Characteristics data of patients with PTB (preterm-birth) (n = 111) and control groups (n = 143)

Characteristics	Patients with PTB (n = 111)	Controls (n = 143)	*p* value[a]
Age (years) (mean ± SD)	30.59 ± 4.59	30.41 ± 4.90	0.779
Height (cm) (mean ± SD)	160.29 ± 4.59	160.56 ± 5.56	0.697
Pre-pregnant weight (g) (mean ± SD)	56.82 ± 9.92	56.10 ± 10.41	0.599
Post-pregnant weight (g) (mean ± SD)	67.37 ± 10.51	69.46 ± 10.45	0.140
SBP (mmHg) (mean ± SD)	120.24 ± 14.97	121.32 ± 14.47	0.585
DBP (mmHg) (mean ± SD)	74.76 ± 12.82	75.79 ± 11.87	0.535
Birth weight (g) (mean ± SD)	2354.51 ± 643.04	3158.46 ± 463.68	< 0.001*
Gestational age at delivery (weeks) (mean ± SD)	33.19 ± 3.06	38.74 ± 1.06	< 0.001*

*$p < 0.05$

[a]The p value is for t test

[b]SBP systolic blood pressure

[c]DBP diastolic blood pressure

not significant after these differences were adjusted by the Bonferroni correction (threshold; $p < 0.005$).

Discussion

In this study, we evaluated the effects of folate-metabolizing gene polymorphisms on PTB. The results of this study show that folate-metabolizing gene polymorphisms, including *MTHFR* rs4846049, rs1537514, *MTR* rs4805087, *MTRR* rs1801394, and *TCN2* rs1801198, are significantly associated with the development of PTB in Korean women ($p < 0.05$) (Table 3).

The GG genotype frequency of *MTR* 2576 A>G (rs1805087) was significantly different between patients with PTB and those in the control group ($p = 0.031$) (Table 2). The *MTR* rs1805087 polymorphism converts aspartic acid to glycine at codon 919, and the SNP is possibly associated with lower MTR activity, followed by increased homocysteine level (Haghiri et al. 2016). Previous studies have indicated that the *MTR* rs1805087 polymorphism has a significant association with gestational disorders (Kim et al. 2013; Sata et al. 2012; Shi et al. 2017). Kim et al. (2013) and Sata et al. (2012) both showed that the *MTR* rs1805087 is significantly associated with recurrent pregnancy loss (Kim et al. 2013; Sata et al. 2012). Similarly, the meta-analysis study observed that the *MTR* rs1805087 polymorphism is related with thrombophilia (Shi et al. 2017). These studies support our results, revealing the association between *MTR* rs1805087 and PTB.

The A allele of *MTRR* 66 A>G (rs1801394) polymorphism in the exon 2 of *MTRR* is associated with decreased homocysteine levels (Jones et al. 2018). For the *MTRR* rs1801394, our results indicate no association with PTB ($p > 0.05$) (Table 2). However, the *MTRR* rs1801394 GA genotype was reported as a risk factor of PTB (Engel et al. 2006), and Song et al. (2018) suggested that the *MTRR* rs1801394 polymorphism is associated with increased plasma folate and homocysteine in pregnant women. The *TCN2* gene is located in 22q12.2, including 9 exons and 8 introns, and encodes the transcobalamin II (Regec et al. 1995). The CC genotype of *TCN2* 776C>G (rs1801198) polymorphism is a factor for higher B12 levels and a lower concentration of homocysteine (Stanislawska-Sachadyn et al. 2010). However, in this study, the CG genotype protected against PTB in Korean women (OR 0.53, 95% CI 0.288–0.980, $p = 0.042$) (Table 2). Previous studies have indicated that the *TCN2* rs1801198 polymorphism has no association with recurrent spontaneous abortion in Iranian women (Hashemi et al. 2018) and recurrent implantation failure in Korean women (Kim et al. 2014; Park et al. 2019). To verify these inconsistencies, a larger sample set and functional studies are required.

The results of the *MTHFR* 2572 C>A (rs4846049) polymorphism showed that the AA genotype was not statistically significant, yet marginal tendency (OR 0.15, 95% CI 0.018–1.205, $p = 0.041$) (Table 2). It is reported that haplotypes of rs4846049 polymorphism and other *MTHFR* SNPs showed an association with recurrent pregnancy loss in Korean women (Kim et al. 2017). The rs4846049 polymorphism in the 3′ UTR region of the *MTHFR* is reported to be associated with the MTHFR protein level through modifying miRNA binding (Wu et al. 2013). The AA genotype has led a reduced MTHFR protein level compared with that of the CC genotype (Salehi et al. 2018). A decreased level of *MTHFR* was found to be associated with the development of hyperhomocysteinemia that is related with placental abruption (Ferguson et al. 2001).

MTHFR 4869 C>G (rs1537514) causes *MTHFR* mRNA overexpression (Mohammadpour-Gharehbagh et al. 2018). Increased MTHFR activity in vivo is associated with down-regulation of plasma homocysteine and a protective factor

Table 3 Genotype and allele frequencies of *MTHFR, MTR, MTRR, TCN2* gene polymorphisms in the patients with PTB (preterm-birth) and control group

Marker			Patients with PTB n (%) (n = 111)	Control n (%) (n = 143)	*p value*	OR (95% CI)	Statistical power (%)
MTHFR rs4846049 (2572 C>A)	Genotype	CC	78 (70.3)	92 (64.3)		Reference	
		AC	32 (28.8)	43 (30.1)	0.641	0.88 (0.507–1.518)	16.2
		AA	1 (0.9)	8 (5.6)	0.041*	0.15 (0.018–1.205)	76.7
	HWE		0.241	0.328			
	Allele	C	188 (84.7)	227 (79.4)		Reference	
		A	34 (15.3)	59 (20.6)	0.124	0.70 (0.437–1.107)	91.3
	Dominant	CC	78 (70.3)	92 (64.3)		Reference	
		CA + AA	33 (29.7)	51 (35.7)	0.319	0.76 (0.448–1.299)	55.3
	Recessive	CC + CA	110 (99.1)	135 (94.4)		Reference	
		AA	1 (0.9)	8 (5.6)	0.045*	0.15 (0.019–1.245)	76.1
	Overdominant	CC + AA	79 (71.2)	100 (69.9)		Reference	
		A/C	32 (28.8)	43 (30.1)	0.83	0.94 (0.547–1.624)	7.4
MTHFR rs1537514 (4869 C>G)	Genotype	CC	92 (82.9)	124 (86.7)		Reference	
		CG	19 (17.1)	17 (11.9)	0.254	1.51 (0.742–3.057)	55.9
		GG	0 (0.0)	2 (1.4)	0.128	NA	0.0
	HWE		0.324	0.131			
	Allele	C	203 (91.4)	265 (92.7)		Reference	
		G	19 (8.6)	21 (7.3)	0.614	1.18 (0.618–2.256)	18.1
	Dominant	CC	92 (82.9)	124 (86.7)		Reference	
		CG + GG	19 (17.1)	19 (13.3)	0.396	1.35 (0.676–2.689)	36.3
	Recessive	CC + CG	111 (100.0)	141 (98.6)		Reference	
		GG	0 (0.0)	2 (1.4)	0.118	NA	0.0
	Overdominant	CC + GG	92 (82.9)	126 (88.1)		Reference	
		CG	19 (17.1)	17 (11.9)	0.236	1.53 (0.755–3.105)	59.5
MTR rs1805087 (2576 A>G)	Genotype	AA	82 (73.9)	109 (76.2)		Reference	
		AG	29 (26.1)	30 (21.0)	0.401	1.28 (0.716–2.307)	36.7
		GG	0 (0.0)	4 (2.8)	0.031*	NA	2.5
	HWE		0.113	0.284			
	Allele	A	248 (61.6)	193 (59.9)		Reference	
		G	38 (38.4)	29 (40.1)	1.000	0.98 (0.584–1.647)	12.4
	Dominant	AA	82 (73.9)	109 (76.2)		Reference	
		AG + GG	29 (26.1)	34 (23.8)	0.667	1.13 (0.640–2.009)	13.3
	Recessive	AA + AG	111 (100.0)	139 (97.2)		Reference	
		GG	0 (0.0)	4 (2.8)	0.027*	NA	2.6
	Overdominant	AA + GG	82 (73.9)	113 (79.0)		Reference	
		AG	29 (26.1)	30 (21.0)	0.335	1.33 (0.743–2.389)	45.7
MTRR rs1801394 (66 A>G)	Genotype	AA	45 (40.5)	63 (44.0)		Reference	
		AG	50 (45.0)	70 (49.0)	1.000	1.00 (0.59–1.694)	5.0
		GG	16 (14.4)	10 (7.0)	0.068	2.24 (0.931–5.389)	38.0
	HWE		0.727	0.107			
	Allele	A	140 (63.1)	196 (68.5)		Reference	
		G	82 (36.9)	90 (31.5)	0.196	1.28 (0.881–1.846)	71.3
	Dominant	AA	45 (40.5)	63 (44.0)		Reference	
		AG + GG	66 (59.5)	80 (56.0)	0.574	1.15 (0.699–1.909)	20.6
	Recessive	AA + AG	95 (85.6)	133 (93.0)		Reference	
		GG	16 (14.4)	10 (7.0)	0.053	2.24 (0.974–5.151)	42.0
	Overdominant	AA + GG	61 (55.0)	73 (51.0)		Reference	

Table 3 (continued)

Marker			Patients with PTB n (%) (n = 111)	Control n (%) (n = 143)	*p value*	OR (95% CI)	Statistical power (%)
		AG	50 (45.0)	70 (49.0)	0.536	0.85 (0.520–1.406)	24.9
TCN2 rs1801198 (776 C>G)	Genotype	CC	34 (30.6)	28 (19.6)		Reference	
		CG	51 (46.0)	79 (55.2)	0.042*	0.53 (0.288–0.980)	97.4
		GG	26 (23.4)	36 (25.2)	0.151	0.59 (0.292–1.210)	82.6
	HWE		0.422	0.195			
	Allele	C	119 (53.6)	135 (47.2)		Reference	
		G	103 (46.4)	151 (52.8)	0.152	0.77 (0.545–1.100)	82.4
	Dominant	CC	34 (30.6)	28 (19.6)		Reference	
		CG + GG	77 (69.4)	115 (80.4)	0.042*	0.55 (0.310–0.982)	96.7
	Recessive	CC + CG	85 (76.6)	107 (74.8)		Reference	
		GG	26 (23.4)	36 (25.2)	0.747	0.91 (0.509–1.623)	10.4
	Overdominant	CC + GG	60 (54.0)	64 (44.8)		Reference	
		CG	51 (46.0)	79 (55.2)	0.141	0.69 (0.419–1.133)	83.7

*$p < 0.05$

[a]HWE The p value for Hardy–Weinberg equilibrium

[b]The p value for Chi-square test

of PTB (Micle et al. 2012; Roy et al. 2008). Mohammadpour-Gharehbagh et al. (2018) showed that the *MTHFR* rs1537514 G allele and the rs4846049 C allele protect against the abnormal development of the placenta. However, the results of the present study indicate no association between the *MTHFR* rs1537514 and PTB ($p > 0.05$) (Table 2).

This study also demonstrates the gene–gene interaction among folate-metabolizing gene polymorphisms (Table 4). We obtained conflicting results regarding PTB in the combination analysis for the *MTHFR* rs1537514 CC genotype. The combination with the *MTRR* rs1801394 GG genotype is associated with the development of PTB, whereas the combination of the *MTHFR* rs1537514 CC genotype and the *TCN2* rs1801198 CG genotype has a protective effect against PTB. One potential explanation for this inconsistency is the various etiologies of PTB. Previous studies have demonstrated that there are several causes associated with PTB, including infection, inflammation, uteroplacental ischemia, hemorrhage, uterine overdistension, stress, and other immunologically mediated processes (Goldenberg et al. 2008). Furthermore, various gene polymorphisms are associated with PTB and interact differently with each other (Crider et al. 2005). According to previous studies, the result of our combination analysis of folate metabolic gene polymorphisms supports this explanation (Table 4).

The present study has its limitations. Firstly, the sample size is relatively small (Hattersley and McCarthy 2005). However, the statistical power of the sample size in this study is over 90%, which is higher than the standard of 80% (Hong and Park 2012). Secondly, as PTB is affected by the maternal and fetal genotype, a study that investigates the genetic association of both genotypes is needed (Wilcox et al. 2008). Unfortunately, we analyzed only the genotype data from mothers. In addition, many additional factors have been associated with PTB (Hong and Park 2012), but we did not consider gene-environment interactions, such as maternal smoking or alcohol consumption, low maternal body mass index, and advanced maternal age, which may have a significant effect on the association between folate metabolism and preterm birth (Muglia and Katz 2010).

Despite these limitations, this attempt is the first study to analyze the association between folate metabolic gene polymorphisms and PTB in Korean women. Moreover, we identified the gene–gene interaction in folate metabolic gene polymorphisms for PTB. Thus, our case–control study may provide pathological evidence for further studies.

In conclusion, our results suggest that folate metabolism gene polymorphisms, including *MTHFR* rs4846049, rs1537514, *MTR* rs1805087, *MTRR* rs1801394, and *TCN2* rs1801198, have a significant association with the pathogenesis of PTB. In addition, we identified that these polymorphisms have gene–gene interaction in PTB. However, as PTB is multifactorial, a larger sample set and further functional studies are required.

Table 4 Genotype Combination Analysis for two polymorphisms of *MTHFR, MTR, MTRR, TCN2* genes in the patients with PTB (preterm-birth) and control group

Gene	Combination	Patients with PTB n (%) (n = 111)	Control n (%) (n = 143)	*p* value	OR (95% CI)
MTHFR rs4846049	CC/CC	78 (70.3)	92 (64.3)		Reference
MTHFR rs1537514	CA/CC	14 (12.6)	27 (18.9)	0.174	0.61 (0.300–1.247)
	CA/CG	18 (16.2)	16 (11.2)	0.452	1.33 (0.634–2.775)
	AA/CC	0 (0.0)	5 (3.5)	0.012*	NA
	AA/CG	1 (0.9)	1 (0.7)	1.000	1.18 (0.073–19.168)
	AA/GG	0 (0.0)	2 (1.4)	0.108	NA
MTHFR rs4846049	CC/AA	57 (51.4)	70 (49.0)		Reference
MTR rs1805087	CC/AG	21 (18.9)	19 (13.2)	0.400	1.36 (0.666–2.767)
	CC/GG	0 (0.0)	3 (2.1)	0.054	NA
	CA/AA	24 (21.6)	34 (23.8)	0.656	0.87 (0.462–1.626)
	CA/AG	8 (7.2)	8 (5.6)	0.698	1.23 (0.434–3.476)
	CA/GG	0 (0.0)	1 (0.7)	0.263	NA
	AA/AA	1 (0.9)	5 (3.5)	0.173	0.25 (0.028–2.163)
	AA/AG	0 (0.0)	3 (2.1)	0.054	NA
MTHFR rs4846049	CC/AA	31 (27.9)	36 (25.2)		Reference
MTRR rs1801394	CC/AG	33 (29.7)	50 (35.0)	0.423	0.77 (0.400–1.470)
	CC/GG	14 (12.6)	6 (4.2)	0.062	2.71 (0.929–7.901)
	CA/AA	13 (11.7)	22 (15.4)	0.377	0.69 (0.297–1.585)
	CA/AG	17 (15.3)	17 (11.9)	0.723	1.16 (0.508–2.653)
	CA/GG	2 (1.8)	4 (2.8)	0.542	0.58 (0.099–3.389)
	AA/AA	1 (0.9)	5 (3.5)	0.162	0.23 (0.026–2.096)
	AA/AG	0 (0.0)	3 (2.1)	0.051	NA
MTHFR rs4846049	CC/CC	22 (19.8)	21 (14.7)		Reference
*TCN*2 rs1801198	CC/CG	37 (33.3)	48 (33.6)	0.413	0.74 (0.353–1.536)
	CC/GG	19 (17.1)	23 (16.1)	0.585	0.79 (0.336–1.850)
	CA/CC	11 (9.9)	6 (4.2)	0.342	1.75 (0.548–5.586)
	CA/CG	14 (12.6)	26 (18.2)	0.138	0.51 (0.213–1.243)
	CA/GG	7 (6.3)	11 (7.7)	0.381	0.61 (0.198–1.863)
	AA/CC	1 (0.9)	1 (0.7)	1.000	0.96 (0.056–16.266)
	AA/CG	0 (0.0)	5 (3.5)	0.008*	NA
	AA/GG	0 (0.0)	2 (1.4)	0.084	NA
MTHFR rs1537514	CC/AA	67 (60.4)	94 (65.7)		Reference
MTR rs1805087	CC/AG	25 (22.5)	26 (18.2)	0.352	1.35 (0.717–2.538)
	CC/GG	0 (0.0)	4 (2.8)	0.035*	NA
	CG/AA	15 (13.5)	13 (9.1)	0.239	1.62 (0.723–3.625)
	CG/AG	4 (3.6)	4 (2.8)	0.639	1.40 (0.339–5.810)
	GG/AA	0 (0.0)	2 (1.4)	0.135	NA
MTHFR rs1537514	CC/AA	35 (31.5)	55 (38.5)		Reference
MTRR rs1801394	CC/AG	43 (38.7)	62 (43.4)	0.769	1.09 (0.613–1.937)
	CC/GG	14 (12.6)	7 (4.9)	0.021*	3.14 (1.155–8.555)
	CG/AA	10 (9.0)	7 (4.9)	0.127	2.25 (0.782–6.446)
	CG/AG	7 (6.3)	7 (4.95)	0.431	1.57 (0.508–4.865)
	CG/GG	2 (1.8)	3 (2.1)	1.000	1.05 (0.167–6.588)
	GG/AA	0 (0.0)	1 (0.7)	0.313	NA
	GG/AG	0 (0.0)	1 (0.7)	0.313	NA
MTHFR rs1537514	CC/CC	30 (27.0)	26 (18.2)		Reference
*TCN*2 rs1801198	CC/CG	40 (36.0)	67 (46.9)	0.047*	0.52 (0.269–0.996)
	CC/GG	22 (19.8)	31 (21.7)	0.208	0.62 (0.288–1.312)
	CG/CC	4 (3.6)	1 (0.7)	0.254	3.47 (0.364–33.002)
	CG/CG	11 (9.9)	11 (7.7)	0.776	0.87 (0.323–2.326)

Table 4 (continued)

Gene	Combination	Patients with PTB n (%) (n = 111)	Control n (%) (n = 143)	*p* value	OR (95% CI)
	CG/GG	4 (3.6)	5 (3.5)	0.611	0.69 (0.168–2.856)
	GG/CC	0 (0.0)	1 (0.7)	0.198	NA
	GG/CG	0 (0.0)	1 (0.7)	0.198	NA
MTR rs1805087	AA/AA	33 (29.7)	48 (33.6)		Reference
MTRR rs1801394	AA/AG	39 (35.1)	53 (37.1)	0.826	1.07 (0.584–1.962)
	AA/GG	10 (9.0)	8 (5.6)	0.251	1.82 (0.649–5.093)
	AG/AA	12 (10.8)	14 (9.8)	0.627	1.25 (0.512–3.034)
	AG/AG	11 (9.9)	14 (9.8)	0.772	1.14 (0.462–2.827)
	AG/GG	6 (5.4)	2 (1.4)	0.062	4.36 (0.829–22.962)
	GG/AA	0 (0.0)	1 (0.7)	0.298	NA
	GG/AG	0 (0.0)	3 (2.1)	0.074	NA
MTR rs1805087	AA/CC	23 (20.7)	21 (14.7)		Reference
*TCN*2 rs1801198	AA/CG	39 (35.1)	57 (39.9)	0.046*	0.49 (0.248–0.992)
	AA/GG	20 (18.0)	31 (21.7)	0.059	0.47 (0.211–1.034)
	AG/CC	11 (9.95)	6 (4.2)	0.626	1.33 (0.424–4.161)
	AG/CG	12 (10.8)	20 (14.0)	0.070	0.43 (0.175–1.079)
	AG/GG	6 (5.4)	4 (2.8)	1.000	1.09 (0.231–4.336)
	GG/CC	0 (0.0)	1 (0.7)	0.167	NA
	GG/CG	0 (0.0)	2 (1.4)	0.053	NA
	GG/GG	0 (0.0)	1 (0.7)	0.167	NA
MTRR rs1801394	AA/CC	14 (12.6)	18 (12.6)		Reference
*TCN*2 rs1801198	AA/CG	22 (19.8)	31 (21.7)	0.839	0.91 (0.376–2.215)
	AA/GG	9 (8.1)	14 (9.8)	0.732	0.83 (0.278–2.459)
	AG/CC	18 (16.2)	8 (5.6)	0.052	2.89 (0.976–8.578)
	AG/CG	18 (16.2)	43 (30.1)	0.170	0.54 (0.221–1.309)
	AG/GG	14 (12.6)	19 (13.3)	1.000	0.95 (0.355–2.529)
	GG/CC	2 (1.8)	2 (1.4)	0.813	1.29 (0.161–10.299)
	GG/CG	11 (9.9)	5 (3.5)	0.102	2.83 (0.797–10.042)
	GG/GG	3 (2.7)	3 (2.1)	0.778	1.29 (0.224–7.370)

*$p < 0.05$

[a]The p value is for t test

Acknowledgements We are grateful to all volunteers for providing DNA samples.

Declarations

Conflict of interest Bit Na Kwon, Noo Ri Lee, Hyung Jun Kim, Yun Dan Kang, Jong Soo Kim, Jin Wan Park, Han Jun Jin declare that they have no conflict of interest.

Ethical approval The study was approved by the Ethics Committee of the Dankook University. Informed consent was obtained from all individual participants included in the study.

References

Bailey LB, Gregory JF (1999) Folate metabolism and requirements. J Nutr 129(4):779–782

Beck S, Wojdyla D, Say L, Betran AP, Merialdi M, Requejo JH (2010) The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull World Health Organ 88(1):31–38

Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R (2012) National, regional, and worldwide estimates of preterm birth rates in the year 2010 with timetrends since 1990 for selected countries: a systematic analysis and implication. Lancet 379(9832):2162–2172

Cai D, Ling L, Pan C, Liu X, Bu R, Chen X, Wang K, Cheng Y, Wu B (2010) Association of polymorphisms in folate metabolic genes and prostate cancer risk: a case-control study in a Chinese population. J Genet 89(2):263–267

Chen GB, Xu Y, Xu HM, Li MD, Zhu J, Lou XY (2011) Practical and theoretical considerations in study design for detecting gene-gene interactions using MDR and GMDR. PLoS ONE 6(2):e16981

Chen J, Chen L, Zhu LH, Zhang ST, Wu YL (2016) Association of methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism with preterm delivery and placental abruption: a systematic review and meta-analysis. Acta Obstet Gynecol Scand 95(2):157–165

Crider KS, Whitehead N, Buus RM (2005) Genetic variation associated with preterm birth: a HuGE review. Genet Med 7(9):593–604
Engel SM, Olshan AF, Siega-Riz AM, Savitz DA, Chanock SJ (2006) Polymorphisms in folate metabolizing genes and risk for spontaneous preterm and small-for-gestational age birth. Am J Obstet Gynecol 195(5):1231.e1-1231.e11
Faul F, Erdfelder E, Lang AG, Buchner A (2007) G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 39(2):175–191
Ferguson SE, Smith GN, Walker MC (2001) Maternal plasma homocysteine levels in women with preterm premature rupture of membranes. Med Hypotheses 56(1):85–90
Goldenberg RL, Culhane JF, Dlams J, Romero R (2008) Epidemiology and causes of preterm birth. Lancet 371(9606):75–84
Haghiri R, Mashayekhi F, Bidabadi E, Salehi Z (2016) Analysis of methionine synthase (rs1805087) gene polymorphism in autism patients in Northern Iran. Acta Neurobiol Exp (Wars) 76(4):318–323
Han YS, Ha EH, Park HS, Kim YJ (2011) Relationships between pregnancy outcomes, biochemical markers and pre-pregnancy body mass index. Int J Obes (Lond) 35(4):570–577
Hankey GJ, Eikelboom JW (1999) Homocysteine and vascular disease. Lancet 354(9176):407–413
Hashemi M, Mokhtari M, Yazdani-Shahrbabaki V, Danesh H, Bizhani F, Taheri M (2018) Evaluation of transcobalamin II rs1801198 and transcobalamin II receptor rs2336573 gene polymorphisms in recurrent spontaneous abortion. J Obstet Gynaecol 38(6):860–863
Hattersley AT, McCarthy MI (2005) What makes a good genetic association study? Lancet 366(9493):1315–1323
Hong EP, Park JW (2012) Sample size and statistical power calculation in genetic association studies. Genom Inform 10(2):117–122
Hozyasz KK, Mostowska A, Szaflarska-Poplawska A, Lianeri M, Jagodzinski PP (2012) Polymorphic variants of genes involved in homocysteine metabolism in celiac disease. Mol Biol Rep 39(3):3123–3130
Jones P, Lucock M, Veysy M, Jablonski N, Chaplin G, Beckett E (2018) Frequency of folate-related polymorphisms varies by skin pigmentation. Am J Hum Biol 30(2):e23079
Kim JH, Jeon YJ, Lee BE, Kang HJ, Shin JE, Choi DH, Lee WS, Kim NK (2013) Association of methionine synthase and thymidylate synthase genetic polymorphisms with idiopathic recurrent pregnancy loss. Fertil Steril 99(6):1674–1680
Kim HS, Lee BE, Jeon YJ, Rah HC, Lee WS, Shin JE, Choi DH, Kim NK (2014) Transcobalamin II (TCN 2 67A>G and TCN 2 776C>G) and transcobalamin II receptor (TC blR 1104C>T) polymorphisms in Korean patients with idiopathic recurrent spontaneous abortion. Am J Reprod Immunol 72(3):337–346
Kim ES, Kim JO, An HJ, Jung HS, Lee HA, Kim JH, Ahn EH, Kim YR, Lee WS, Kim NK (2017) MTHFR 3′-untranslated region polymorphisms contribute to recurrent pregnancy loss risk and alterations in peripheral natural killer cell proportions. Clin Exp Reprod Med 44(3):152–158
Kurzwelly D, Knop S, Guenther M, Loeffler J, Korfel A, Thiel E, Hebert H, Simon M, Weller M, Linnebank MA et al (2010) Genetic variants of folate and methionine metabolism and PCNSL incidence in German patient population. J Neurooncol 100(2):187–192
Mattson MP, Shea TB (2003) Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. Trends Neurosci 26(3):137–146
Mfady DS, Sadiq MF, Khabour OF, Fararjeh AS, Abu-Awad A, Khader Y (2014) Associations of variants in MTHFR and MTRR genes with male infertility in the Jordanian population. Gene 536(1):40–44
Micle O, Muresan M, Antal L, Bodog F, Bodog A (2012) The influence of homocysteine and oxidative stress on pregnancy outcome. J Med Life 5(1):68–73
Mohammadpour-Gharehbagh A, Teimoori B, Narooei-nejad M, Mehrabani M, Saravani R, Salimi S (2018) The association of the placental MTHFR 3'-UTR polymorphisms, promoter methylation, and MTHFR expression with preeclampsia. J Cell Biochem 119(2):1346–1354
Muglia LJ, Katz M (2010) The Enigma of Spontaneous Preterm Birth. N Engl J Med 362(6):529–535
Oussalah A, Levy J, Filhine-Trésarrieu P, Namour F, Guéant JL (2017) Association of TCN2 rs1801198 c.776G>C polymorphism with markers of one-carbon metabolism and related diseases: a systematic review and meta-analysis of genetic association studies. Am J Clin Nutr 106(4):1142–1156
Park HS, Kim JO, An HJ, Ryu CS, Ko EJ, Kim YR, Ahn EH, Lee WS, Kim JH, Kim NK (2019) Genetic polymorphisms of the cobalamin transport system are associated with idiopathic recurrent implantation failure. J Ass Reprod Genet 36:1513–1522
Regec A, Quadros EV, Platica O, Rothenberg SP (1995) The cloning and characterization of the human transcobalamin II gene. Blood 85(10):2711–2719
Roy M, Leclerc D, Wu Q, Gupta S, Kruger WD, Rozen R (2008) Valproic acid increases expression of methylenetetrahydrofolate reductase (MTHFR) and induces lower teratogenicity in MTHFR deficiency. J Cell Biochem 105(2):467–476
Salehi M, Amin-Beidokhti M, Lima BS, Gholami M, Javadi GR, Mirfakhraie R (2018) The rs4846049 polymorphism in the 3'UTR region of the MTHFR gene increases the migraine susceptibility in an Iranian population. J Pain Res 11:145–149
Sata F, Yamada H, Kishi R, Minakami H (2012) Maternal folate, alcohol and energy metabolism-related gene polymorphisms and the risk of recurrent pregnancy loss. J Dev Orig Health Dis 3(5):327–332
Shi X, Xie X, Jia Y, Li S (2017) Maternal genetic polymorphisms and unexplained recurrent miscarriage: a systematic review and meta-analysis. Clin Genet 91(2):265–284
Smith GCS (2012) Researching new methods of screening for adverse pregnancy outcome: lessons from pre-eclampsia. PLoS Med 9(7):e1001274
Song CS, Song WB, Bao JY, Luo J, Zuo X, An N, Zhang Y (2018) Association between decreased plasma folate levels and MTHFR C677T, and MTRR A66G gene polymorphisms as determinants for elevated total homocysteine concentration in pregnant women. Hered Genet Curr Res 7:e1000193
Stanislawska-Sachadyn A, Woodside JV, Sayers CM, Yarnell JW, Young IS, Evans AE, Mitchell LE, Whitehead AS (2010) The transcobalamin (TCN2) 776C>G polymorphism affects homocysteine concentrations among subjects with low vitamin B12 status. Eur J Clin Nutr 64(11):1338–1343
Tellapragada C, Eshwara VK, Bhat P, Acharya S, Kamath A, Bhat S, Rao C, Nayak S, Mukhopadhyay C (2016) Risk factors for preterm birth and low birth weight among pregnant Indian women: a hospital-based prospective study. J Prev Med Public Health 49(3):165–175
Wang BJ, Liu MJ, Wang Y, Dai JR, Tao JY, Wang SN (2015) Association between SNPs in genes involved in folate metabolism and preterm birth risk. Genet Mol Res 14(1):850–859
Wilcox AJ, Skjaerven R, Lie RT (2008) Familial patterns of preterm delivery: maternal and fetal contributions. Am J Epidemiol 167(4):474–479
Wu C, Gong Y, Sun A, Zhang Y, Zhang C, Zhang W, Zhao G, Zou Y, Ge J (2013) The human MTHFR rs4846049 polymorphism increases coronary heart disease risk through modifying miRNA binding. Nutr Metab Cardiovasc Dis 23(7):693–698
Wu H, Zhu P, Geng X, Liu Z, Cui L, Gao Z (2017) Genetic polymorphism of MTHFR C677T with preterm birth and low birth weight susceptibility: a meta-analysis. Arch Gynecol Obstet 295(5):1105–1118