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Genetic variations of MTHFR gene and their association with preterm birth in Korean women

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ABSTRACT

Background and objective: The MTHFR gene encodes the methylenetetrahydrofolate reductase known to be involved in the homocysteine–methionine pathway. It has been reported that the deficiency of MTHFR activity may cause hyperhomocysteinemia which results in adverse pregnancy outcomes. Previous studies reported a correlation between the MTHFR gene polymorphisms (677 T/C and 1298 A/C) and lower MTHFR activity and its association with preterm birth in various populations. Since these results were conflicting, we analyzed the genetic association of MTHFR gene 677 T/C and 1298 A/C polymorphisms with preterm birth in Korean women.

Materials and methods: The subjects for case–control study were collected a total of 226 Korean women (98 preterm-birth patients and 128 controls). Genotype frequency differences between the case and the control were assessed using chi-square tests. Mann–Whitney t-test was used to estimate the effects of 1298 A/C genotype on clinicopathological characteristics (systolic blood pressure, diastolic blood pressure, birth weight, and gestational age at delivery) in preterm-birth patients.

Results: Our results showed that the MTHFR 677 C/T polymorphism was significantly associated with preterm-birth patients in the analysis of genotype frequency ($P = 0.044$) and the over-dominant model (OR = 0.54; 95% CI, 0.320–0.920; $P = 0.023$). The recessive model showed a marginal trend toward significance (OR = 0.47; 95% CI, 0.220–1.010; $P = 0.046$). The 1298 A/C polymorphism was also associated with reduced preterm-birth risk in the recessive model ($P = 0.032$). In the correlation analysis, the 1298 C allele was significantly associated with increasing of gestational age at delivery in preterm-birth patients ($P = 0.034$).

Conclusions: Our findings suggested that the MTHFR gene 677 C/T and 1298 A/C polymorphisms might have protective effects for preterm birth in the Korean women.

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1. Introduction

Adverse pregnancy outcomes are known to be occupied a high proportion among global diseases [1]. Preterm birth (PB) is one of the common adverse pregnancy outcomes and it is related to high neonatal and maternal mortality [2]. According to previous study, approximately 13.0 million infants worldwide were premature babies, and it accounts for 9.6% of all births [3]. Especially, the incidence rate of preterm birth in the Korean population from 2009 to 2011 was estimated as 22.1% (<http://kostat.go.kr/>). Park et al. in 2016 also reported that the incidence of preterm birth increased 1.5 times during 1997–2014 in Korea [4]. Likewise, the global prevalence of preterm birth is increasing, however, the exact etiologies of preterm birth remain unknown [5].

It has been reported that various factors, including gestational hypertension, gestational diabetes mellitus, maternal education, alcohol intake, smoking status, and genetic factors were associated with preterm birth [6]. In recent years, a number of studies found an association between folate metabolizing genes polymorphism and preterm birth [5–9]. The folate metabolizing genes are known to affect the accumulation of metabolic intermediate such as homocysteine [5]. Previous study showed that the accumulation of homocysteine or hyperhomocysteine influences uteroplacental circulation that is closely related to the preterm birth [10].

The *MTHFR* gene encodes the methylenetetrahydrofolate reductase (*MTHFR*) enzyme involved in folate metabolism. The levels of homocysteine in body fluid are regulated by *MTHFR* activity [7]. It has been reported that a deficiency of *MTHFR* activity significantly increases homocysteine plasma levels [11]. There are common polymorphisms in the *MTHFR* gene as 677 C/T, 1298 A/C, 1317 T/C, and 1793 G/A, of these polymorphisms, the 677 C/T and 1298 A/C polymorphisms are functional [12,13]. Many studies have shown a significant association between preterm birth and *MTHFR* 677 C/T or 1298 A/C polymorphisms in the Asian population [8,9]. However, results from some studies were not consistent [14,15]. Nurk et al. in 2004 found no significant association between *MTHFR* gene polymorphisms (677 C/T and 1298 A/C) and preterm birth in the European population [15]. Gargano et al. in 2009 also reported that genotypes of *MTHFR* gene polymorphisms (677 C/T and 1298 A/C) were not associated with preterm birth in the Caucasian and African American population [14]. Therefore, we investigated the effects of the *MTHFR* gene polymorphisms (677 C/T and 1298 A/C) on the occurrence of preterm birth in Korean women. We also analyzed a possible correlation between *MTHFR* gene polymorphisms (677 C/T and 1298 A/C) and gestational age.

2. Materials and methods

2.1. Subjects

We analyzed a total of 226 women. The samples were recruited from the Obstetrics and Gynecology Department at Dankook University Hospital in Korea. Of these samples, 98 preterm birth patients were selected as follow: the gestational ages (GA)

were 24–37 weeks [16]. A control group consisted of 128 normal pregnant women with no history of preterm birth or pregnancy loss and GA > 38 weeks. The preterm birth patients and control group had no 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 obstetrician, 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.

2.2. DNA extraction and genotyping

DNA was extracted from leukocytes or buccal cells using the GeneAll Exgene Clinic SV mini kit (GeneALL, Seoul, Korea). *MTHFR* 677 C/T and 1298 A/C SNPs were genotyped using a PCR-RFLP. Primer sets were designed for the determination of the 677 C/T and 1298 A/C polymorphisms in the *MTHFR* gene reported by the Chao et al.: forward, 5'-TGAAGGA-GAAGGTGTCTGCGGGA-3' and reverse 5'-AGGACGGTGC GGTTGAGAGTG-3' for 677 C/T polymorphism; forward, 5'-CTTTGGGGAGCTGAAGGACTACTAC-3' and reverse 5'-CACTTTGTGACCATTCCGGTTTG-3' for 1298 A/C polymorphism [17]. Each PCR reaction was performed in a total volume of 20 μ L containing 10 ng of genomic DNA, 10 pM each primer, 0.2 mM dNTPs, 2.0 mM MgCl₂, 10 \times PCR buffer and 1.0 U NV DNA polymerase (NAVI BioTech, Cheonan, Korea). The PCR amplification was conducted C1000 Touch thermal cycler (Bio Rad, California, USA) under the following conditions: 95 $^{\circ}$ C for 5 min, followed by 38 cycles of 94 $^{\circ}$ C for 1 min, 62 $^{\circ}$ C for 1 min, and 72 $^{\circ}$ C for 1 min, then a final extension at 72 $^{\circ}$ C for 10 min. Each of the PCR products was digested with 1.0 U *Hinf* I (677 C/T) and *Mbo* II (1298 A/C) restriction enzymes (Enzynomics, Daejeon, Korea) for 6 h at 37 $^{\circ}$ C (*Hinf* I) and 37 $^{\circ}$ C (*Mbo* II), respectively, and electrophoresed in 3% agarose gel (Lonza, Morristown, USA). The polymorphic *Hinf* I and *Mbo* II sites were detected by restriction fragment length polymorphism in producing fragments of 198 bp (C allele) or 175, 20 bp (T allele) and 56, 31, 30, 28, 18 bp (A allele) or 84, 31, 30, 18 bp (C allele), respectively (Fig. 1).

2.3. Statistical analysis

The independent (unpaired) samples t test was performed to compare the characteristics data (i.e., age, height, weight, pre-gestation weight, SBP, DBP, birth weight, and GA), and the Mann–Whitney test was used to test the significance of differences between the clinicopathological characteristics (SBP, DBP, birth weight, and GA) and *MTHFR* genotypes in the preterm birth patients using the SPSS 21 Statistics (IBM Korea, Korea). Chi-squared tests were used to assess Hardy–Weinberg equilibrium (HWE). In addition, a test of cross tabulation analyses, and odds ratio (OR) with 95% confidence intervals (CI) were calculated in 2 \times 2 table using the genotype and allele frequencies of cases and controls in web-based statistics tools (SISA, <http://www.quantitativeskills.com/sisa/> and SNPstats, <http://bioinfo.iconcologia.net/SNPstats>). A P value of <0.05 was considered statistically significant. Bonferroni correction was used to adjust the α level according to the number of SNPs.

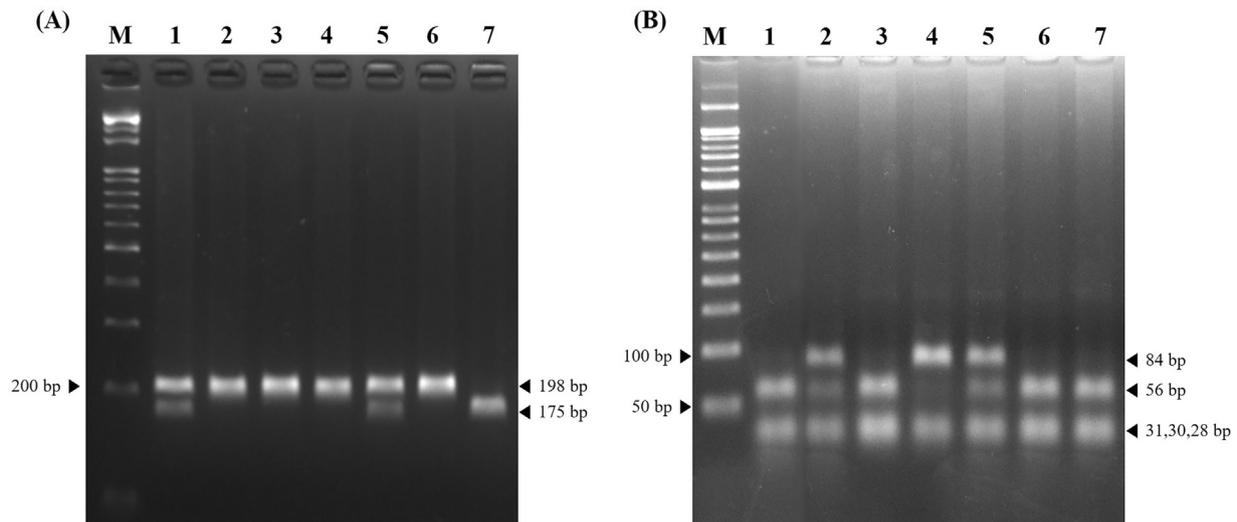


Fig. 1 – Detection of genotypes and allelic variations at the 677 C/T and 1298 A/C polymorphisms in *MTHFR* gene. **(A)** Result of PCR-RFLP electrophoresis of *MTHFR* 677 C/T polymorphism. Lane M: 1-kb + DNA ladder; Lanes 1, 5: CT genotype; Lanes 2–4, 6: CC genotype; Lane 7: TT genotype. **(B)** Result of PCR-RFLP electrophoresis of *MTHFR* 1298 A/C polymorphism. Lane M: 50-bp DNA ladder; Lanes 1, 3, 6, and 7: AA genotype; Lanes 2, 5: AC genotype; Lane 4: CC genotype.

3. Results

We analyzed a total of 226 pregnant women. For the mean values of age, height, weight, pre-gestation weight, SBP, and DBP, no meaningful differences were observed between the preterm-birth patients and controls ($P > 0.05$). In contrast, the birth weight and GA showed significant difference between the preterm-birth patients and controls ($P < 0.05$) (Table 1).

Genotyping data of *MTHFR* 677 C/T and 1298 A/C polymorphisms for the 98 preterm-birth patients and 128 controls are presented in Table 2. The genotype frequencies of the 2 SNPs have no deviation from the Hardy-Weinberg equilibrium except the 677 C/T polymorphism in preterm-birth patients (Table 2). In the comparison of *MTHFR* 677 C/T genotype, OR (95% CI) for each genotype was not significant; however, the difference of genotype frequencies between preterm-birth patients and controls was significant ($P = 0.044$) (Table 2). In the

over-dominant genetic model, we found a significant association (OR = 0.54; 95% CI, 0.320–0.920; $P = 0.023$) and observed a marginal trend toward significance in the recessive genetic model (OR = 0.47; 95% CI, 0.220–1.010; $P = 0.046$) (Table 2). Among these values, the over-dominant P value remained significant even after Bonferroni correction ($< 0.05/2 = 0.025$). The differences in genotype and allele frequencies of *MTHFR* 1298 A/C polymorphism between preterm-birth patients and control group were not significant ($P > 0.05$) (Table 2). Meanwhile, the recessive genetic model revealed a significant difference between preterm-birth and control group ($P = 0.032$) (Table 2). In addition, we performed the correlation analysis to identify a relationship between the clinicopathological characteristics (SBP, DBP, birth weight, and GA) and the *MTHFR* gene polymorphisms (677 C/T and 1298 A/C). In this analysis, we observed that the 1298 A/C polymorphism was significantly associated with GA (1298 A/A 32.82 ± 3.39 vs. 1298 A/C 34.20 ± 2.56 , $P = 0.034$) (Table 3).

Table 1 – Characteristics data of preterm-birth patients ($n = 98$) and control ($n = 128$) group.

Characteristic	Control ($n = 128$)	Preterm-birth ($n = 98$)	P^a
Age, years	30.22 (4.76)	30.64 (4.44)	0.495
Height, cm	160.67 (5.56)	160.48 (4.78)	0.785
Weight, kg	69.71 (10.68)	68.29 (10.43)	0.318
Pre-gestation weight, kg	55.89 (10.38)	57.01 (10.11)	0.419
Systolic blood pressure	122.23 (13.86)	121.19 (14.98)	0.590
Diastolic blood pressure	76.70 (11.60)	75.26 (12.86)	0.376
Birth weight, g	3142.27 (446.56)	2388.89 (670.81)	<0.000*
Gestational age at delivery, weeks	38.73 (1.05)	33.23 (3.21)	<0.000*

Values are mean (standard deviation).

^a Independent t test.

* $P < 0.05$.

Table 2 – Genotypes and allele frequencies of MTHFR 677 C/T and 1298 A/C polymorphisms in the preterm-birth patients and control group.

Marker			Preterm-birth n (%) (n = 98)	Control n (%) (n = 128)	P ^a	OR (95% CI)
MTHFR 677 C/T	Genotype	C/C	30 (30.6)	46 (35.9)	0.044 [*]	Reference
		C/T	57 (58.2)	55 (43.0)		1.59 (0.880–2.868)
		T/T	11 (11.2)	27 (21.1)		0.63 (0.270–1.445)
	Allele	C	117 (59.7)	147 (57.4)	0.628	0.91 (0.624–1.329)
		T	79 (40.3)	109 (42.6)		
	p ^b		0.039 [*]	0.170		
	Dominant	C/C	30 (30.6)	46 (35.9)	0.400	0.79 (0.450–1.380)
		C/T + T/T	68 (69.4)	82 (64.1)		
	Recessive	C/C + C/T	87 (88.8)	101 (78.9)	0.046 [*]	0.47 (0.220–1.010)
		T/T	11 (11.2)	27 (21.1)		
Overdominant	C/C + T/T	41 (41.8)	73 (57.0)	0.023 [*]	0.54 (0.320–0.920)	
	C/T	57 (58.2)	55 (43.0)			
MTHFR 1298 A/C	Genotype	A/A	69 (70.4)	84 (65.6)	0.229	Reference
		A/C	29 (29.6)	40 (31.3)		0.88 (0.497–1.568)
		C/C	0 (0.0)	4 (3.1)		–
	Allele	A	167 (85.2)	208 (81.3)	0.268	0.75 (0.455–1.246)
		C	29 (14.8)	48 (18.7)		
	p ^b		0.086	0.772		
	Dominant	A/A	69 (70.4)	84 (65.6)	0.450	1.25 (0.710–2.200)
		A/C + C/C	29 (29.6)	44 (34.4)		
	Recessive	A/A + A/C	98 (100.0)	124 (96.9)	0.032 [*]	–
		C/C	0 (0.0)	4 (3.1)		
Overdominant	A/A + C/C	69 (70.4)	88 (68.8)	0.790	1.08 (0.610–1.920)	
	A/C	29 (29.6)	40 (31.2)			

^a P values were estimated by SNPstats web-based tool.

^b Hardy–Weinberg equilibrium P value.

* P < 0.05.

Table 3 – Association between the MTHFR 1298 A/C polymorphism and clinicopathological characteristics of preterm-birth patients.

Clinicopathological characteristics	Preterm-birth patients		P ^a
	MTHFR 1298 A/A (n = 69)	MTHFR 1298 A/C (n = 29)	
Systolic blood pressure	120.03 (15.17)	123.97 (14.39)	0.237
Diastolic blood pressure	74.48 (13.22)	77.10 (11.97)	0.359
Birth weight (g)	2375.91 (690.80)	2419.76 (631.33)	0.769
Gestational age at delivery (week)	32.82 (3.39)	34.20 (2.56)	0.034 [*]

Values are mean (standard deviation).

^a Mann–Whitney test.

* P < 0.05.

4. Discussion

We evaluated the effect of MTHFR gene polymorphisms (677 C/T and 1298 A/C) in preterm birth and compared the clinicopathological characteristics (SBP, DBP, birth weight, and GA) by MTHFR gene polymorphisms within preterm-birth patients.

The genotype data MTHFR gene 677 C/T and 1298 A/C polymorphisms are summarized in Table 2. We observed the slight deviation from the Hardy–Weinberg equilibrium in MTHFR 677 C/T genotype distribution of preterm-birth patients (C/C 30.6%, C/T 58.2%, T/T 11.2%). Previously, Ogus et al. in 2004

suggested that the deviation from the Hardy–Weinberg equilibrium in the case group reflects the real genetic association with disease [18]. Therefore, we expected a significant role of the 677 C/T polymorphism on preterm birth in the Korean women.

The MTHFR gene is located on chromosome 1 (1p36.3), and encodes a key enzyme in the metabolism of folate. It has been known that the MTHFR gene polymorphisms (677 C/T and 1298 A/C) were associated with decreased enzyme activity [19]. The 677 C/T polymorphism is located at the folate binding site and, makes a non-conservative amino acid change from alanine to valine residue (A222V). The 1298 A/C polymorphism is located on a presumptive regulatory domain, and it replaces glutamic

acid with alanine residue (E429A) [20]. In vitro study reported that the 677 C/T, TT genotype and CT genotype accounted for 30% and 60% of the MTHFR enzyme activity, respectively. The 1298 A/C, CC genotype showed about 60% of MTHFR enzyme activity [21]. Previously, many studies showed that preterm birth was associated with the MTHFR gene 677 TT genotype and 1298 CC genotype [22,23]. In contrast, the MTHFR gene 677 TT genotype and 1298 CC genotype frequencies were lower in preterm-birth patients of our study (Table 2). It means that the MTHFR gene 677 TT genotype and 1298 CC genotype are indeed a protective factor for preterm birth in the Korean women. A number of studies already reported that the MTHFR gene 1298 CC genotype and C allele were associated with a reduced incidence of preterm-birth [8,15,24]. However, the effect as protective factor the 677 TT genotype in preterm birth has not reported so far. But, several studies reported a protective effect of the 677 TT genotype in colorectal cancer, acute lymphoblastic leukemia, and Parkinson's disease [25-27]. Interestingly, it has been reported that the roles of MTHFR genotypes can be changed by the different dietary conditions. Deb et al. in 2011 found that the MTHFR 677 TT genotype and lower folate status were related to a higher incidence of neural tube defect [28]. It has also been suggested that many Korean foods have a high folate content and that such food intake can prevent the increase of homocysteine due to decreased MTHFR enzyme activity [5,29]. Thus, a functional study for the role of MTHFR gene 677 C/T polymorphism in preterm birth in the Korean women is a subject for further analysis.

It is well known that the 1298 C allele is associated with a reduced risk of preterm birth [8]. To confirm this, we investigated the association between MTHFR gene 1298 C allele and gestational age at delivery (week) within preterm-birth patients. Here, we found that carriers of the 1298 C allele within preterm-birth patients showed increased gestational age at delivery (Table 3). Similar associations have been reported that MTHFR gene 1298 C allele was associated with a reduced risk of small for gestational age (SGA) and low birth weight infants [15,24]. Therefore, our finding supports the previous studies in which the MTHFR gene 1298 C allele reduced the risk of premature birth.

The present study has limitations including the sample size. In general, standard sample power for clinical research studies is considered to be 80% [30]; however, in the statistical analysis we obtained over a 90% sample power of 226 subjects in the comparison of genotype frequencies using the G*Power program [31]. In addition, some of our significant results did not survive after Bonferroni correction for multiple corrections. However, for avoiding type I error in the multiple comparisons, Bonferroni correction is almost always controversial in association studies. Recently, Vieira et al. in 2017 suggested that Bonferroni correction is very strict and it rather increases type II errors. Therefore, additional analysis should be performed using more samples to validate our results [32].

Despite these limitations, our study has advantages. First, maternal age, gestational hypertension, and pre-pregnancy BMI score are known to affect a risk of preterm birth [33,34]. So, we adjusted these effects by matching these variables in preterm-birth patients and control groups. Second, we reported the MTHFR gene 677 C/T, TT genotype

as a protective factor of preterm birth in Korean women, which has not been shown in previous studies. Thus, our finding could be subject for meta-analyses and further functional studies.

5. Conclusions

Our results imply that the MTHFR gene polymorphisms (677 C/T and 1298 A/C) may provide a significant effect on the occurrence of preterm birth and the 1298 A/C polymorphism is related to increase of GA within preterm-birth patients, although larger sample sizes and functional studies are necessary to further elucidate these findings.

Conflict of interest

All authors disclose no conflict of interest.

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