# Interaction between Maternal Serum Folate and the Methylenetetrahydrofolate Reductase (MTHFR) Polymorphisms on Infant Birthweight

Hyesook Park[1,5], Young Ju Kim[2,5], Eun Hee Ha[1,5], Bo Eun Lee[1,5], Bo Hyun Park[1,5], Hwa Young Lee[3,5], Eun Ae Park[4,5], Namsoo Chang[6,7] & Yun-Chul Hong[8]

[1]Department of Preventive Medicine, Ewha Womans University, Seoul, Korea
[2]Department of Obstetrics and Gynecology, Ewha Womans University, Seoul, Korea
[3]Department of Anatomy, Ewha Womans University, Seoul, Korea
[4]Department of Pediatrics, Ewha Womans University, Seoul, Korea
[5]Medical Research Center, Ewha Womans University, Seoul, Korea
[6]Department of Food and Nutritional Sciences, Ewha Womans University, Seoul, Korea
[7]Asia Food and Nutritional Research Center, Ewha Womans University, Seoul, Korea
[8]Departments of Preventive Medicine, Seoul University, Seoul, Korea
Correspondence and requests for materials should be addressed to H.-S. Park (hpark@ewhain.net)

## Abstract

The purpose of this study was to evaluate whether the interactions between maternal folate deficiency and methylenetetrahydrofolate reductase (MTHFR) polymorphism increase the risk of elevated maternal serum homocysteine, short gestation and reduced infant birthweight. Healthy pregnant (n = 170; 24-28 gestational weeks; 20-40 years old) women were analyzed for the MTHFR genotype and serum levels of folate and homocysteine, and were then followed for gestational age and infant birthweight. The mean infant birthweight was highest in mothers carrying MTHFR CC and with a normal folate range, and they were followed by mothers carrying MTHFR CT or TT and a normal range of folate or a folate deficiency. Birthweight was the lowest in mothers whose carrying MTHFR CC with folate deficiency. Using two way ANOVA, we found that folate level and the MTHFR polymorphism interacted to affect birthweight of infants (p=0.05). Among those mothers carrying MTHFR CC, those with folate deficiency showed a 543 g reduction in infant birthweight compared with those with normal folate levels. How-ever, infant birthweight was no different for mothers, those who with folate deficiency compared to those with normal range of folate among mothers carrying the MTHFR CT or TT genotypes. This study suggests an interaction between maternal serum folate and the MTHFR polymorphisms of the mother on the risk of delivering reduced birthweight offspring. Folate supplementation of folate deficient pregnant women with the MTHFR wild type is suggested to reduce the risk of low birthweight.

Keywords: birthweight, folate, methylenetetrahydrofolate reductase, polymorphism, interaction

Pregnancy is associated with an increase in cellular proliferation as a result of uterine enlargement, an expansion of blood volume, placental development and fetal growth[1,2]. These reactions are the basis for the substantial increased vitamin requirement during pregnancy.

Folate is critically important for fetal development. It acts as a cofactor for many essential cellular reactions, including the transfer of single-carbon units[3]. As a result of its role in nucleic acid synthesis, folate-dependent involvements during pregnancy include an increase in red cell mass, enlargement of the uterus, and growth of the placenta and fetus. Moreover, the role of folate in DNA synthesis and cell replication suggests that folate can influence fetal growth and gestational age. Many studies have suggested that poor dietary folate intake and low circulating concentrations of folate are associated with an increased risk of adverse birth outcomes, such as low birthweight and preterm birth[4-6]. However, other studies have failed to find significant correlations between folate levels in maternal serum and gestational age at delivery or infant birthweight[7].

Methylenetetrahydrofolate reductase [MTHFR] is an important enzyme in folate metabolism. This enzyme catabolizes the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulatory form of folate and the methyl donor for the remethylation of homocysteine to methionine. Homozygosity for the C677T variant MTHFR allele causes higher fasting plasma homo-

cysteine concentrations and a lower folate status than those with wild type MTHFR[8]. Moreover, the inverse relation between plasma homocysteine and folate is reported to be more acute in subjects with the TT genotype versus those with CC genotype[9,10]. Therefore, it is possible that risks associated with prenatal maternal serum folate levels are modified by the MTHFR genotypes.

Some studies suggested a gene-environment interaction between maternal periconceptual folate supplementation use and/or dietary folate intake, also the MTHFR genotypes of the mother on the risk of delivering cleft lip of offspring[11-14], and on unexplained recurrent pregnancy loss[14].

However, the possible effect of an interaction between maternal folate and the MTHFR polymorphism on infant birthweight has not been investigated. The purpose of this study is to evaluate whether an interaction between maternal folate deficiency and the MTHFR CT and TT variants increases the risk of a reduced infant birthweight.

The average maternal age was 32.1 years (range: 22-40 years). Average value for serum folate was 8.7 ±6.2 ng/ml (range: 0.2-43.3 ng/ml), and 12.3% of pregnant women had a folate deficiency during the 24-28th gestational weeks. The average value for serum homocysteine was 7.0±3.1 μmol/l (range : 1.4-18.3 μmol/l). The percentages of the individual MTHFR genotypes were 34.1% for the 677CC homozygote, 48.8% for the 677CT heterozygote, and 17.1% for the 677TT homozygote. The average gestational age was 38.7 weeks (range: 27.4-41.6 weeks) and the average infant birthweight 3152.8 g (range 810.0-4550.0 g).

Table 1 shows the average values of serum homocysteine, gestational age and infant birthweight by

maternal serum folate group and MTHFR polymorphism group. No differences in serum homocysteine concentration or gestational age were observed by folate group. Average infant birthweight was slightly lower in the folate deficient group, but without statistical difference. When the distributions of serum homocysteine, gestational age and infant birthweight were analyzed versus the MTHFR genotypes, the level of homocysteine concentration was found to be significantly higher for 677CT and 677TT. Pregnant women with 677CT or 677TT were more likely to have a shorter gestational age (p = 0.06) and a lower infant birthweight than those with 677CC (p = 0.10).

Table 2 shows the combined effect of folate and MTHFR genotype on serum homocysteine, gestational age and infant birthweight by two-way ANOVA. Those with MTHFR variants had a higher level of homocysteine than those with 677CC. Among subjects with MTHFR variants, the mean homocysteine concentrations were similar in those deficient in folate and in those with normal folate levels. Compared to women with normal folate levels and the 677CC genotype, women with a folate deficiency and the 677CT or the 677TT genotype showed slightly shorter gestational periods, although this association did not reach statistical significance (p > 0.05). Infant birthweight was marginally higher for those with normal folate and 677CC (p = 0.09). An interaction was found between folate status and MTHFR polymorphism type on infant birthweight (p = 0.05).

To determine the effect of the interaction between folate and the MTHFR polymorphism, we figured the effect of folate deficiency on maternal serum homocystein level, gestational age and infant birthweight by MTHFR type (Fig. 1). For all subject, those

Table 1. Serum homocysteine, gestational age, and infant birthweight by maternal serum folate level and MTHFR* polymorphism type

| | N | Homocysteine, μmol/l | Gestational age, weeks | Infant birthweight, g |
|---|---|---|---|---|
| Folate† | | | | |
| ≤3 ng/ml | 21 | 7.0 [4.2] | 38.6 [2.4] | 3025.2 [509.7] |
| >3 ng/ml | 149 | 7.1 [3.0] | 38.7 [2.1] | 3180.7 [576.8] |
| MTHFR‡ | | | | |
| 677CC | 58 | 6.2 [2.8] | 39.2 [1.4] | 3287.1 [454.8] |
| 677CT | 83 | 7.1 [2.7] | 38.4 [2.6] | 3079.6 [675.3] |
| 677TT | 29 | 8.8 [4.2] | 38.7 [1.4] | 3144.8 [388.1] |

*MTHFR, methylenetetrahydrofolate reductase, †Determined by student t-test, p = 0.93 for homocysteine, p = 0.24 for gestational age, and p = 0.84 for infant birthweight, ‡Determined by ANOVA test, p = 0.002 for homocysteine, p = 0.06 for gestational age, and p = 0.10 for infant birthweight

Table 2. Serum homocysteine, gestational age, and infant birthweight by a combination of maternal serum folate level and MTHFR* polymorphism type

| | MTHFR CC type* | | MTHFR CT, TT types* | |
|---|---|---|---|---|
| | FN* (n = 50) | FD* (n = 8) | FN* (n = 99) | FD* (n = 13) |
| Homocysteine (μmol/l) | 6.2 [2.7] | 6.2 [3.6] | 7.5 [3.1] | 7.4 [4.7] |
| Gestational age (weeks) | 39.3 [1.1] | 38.6 [2.6] | 38.4 [2.4] | 38.7 [2.3] |
| Infant birthweight* (g) | 3354.4 [396.8] | 2866.3 [589.4] | 3093.0 [632.9] | 3123.1 [450.7] |

*MTHFR, methylenetetrahydrofolate reductase; FN, normal range of folate; FD, folate deficiency; CC, 677CC; CT, 677CT; TT, 677TT, †Determined by two-way ANOVA test, p = 0.09 for folate group, p = 0. 98 for MTHFR types, and p = 0.05 for the interaction term of folate level and MTHFR types
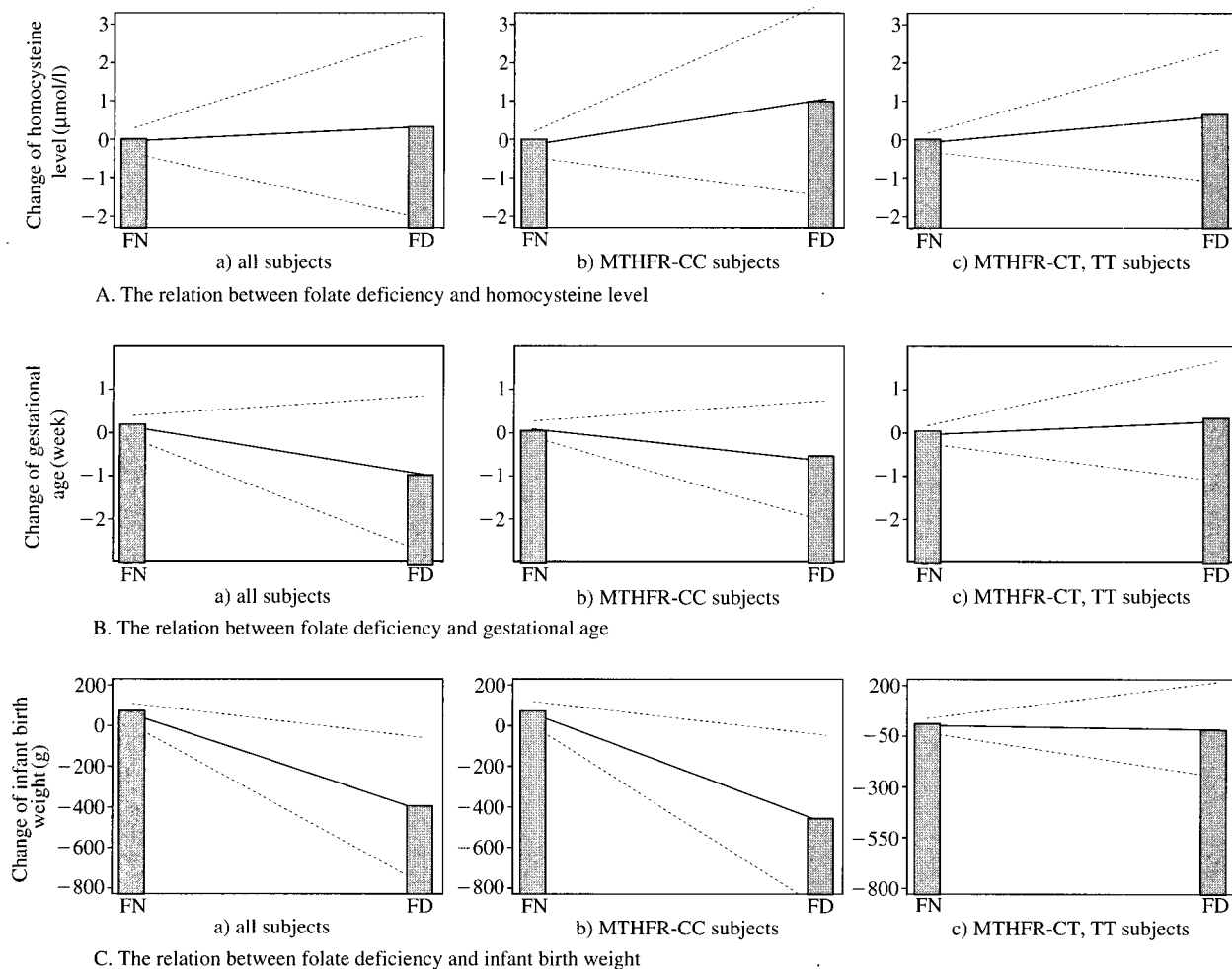
**Fig. 1.** The relation between folate deficiency status and homocysteine level, gestational age, and infant birthweight by MTHFR* types. Fitted: gam (homocysteine/gestational age/infant birthweight-folate group + MTHFR group + (folate group*MTHFR group) + prepregnancy BMI + weight gain + height + smoke exposure status + gestational age†) for all subjects, Fitted: gam (homocysteine/gestational age/infant birthweight ~ folate group + age + prepregnancy BMI + weight gain + height + smoke exposure status + gestational age†) for each MTHFR subtypes, †Additionally added gestational age for infant birthweight, *MTHFR, methylenetetrahydrofolate reductase; FN, normal range of folate; FD, folate deficiency; CC, 677CC; CT, 677CT; TT, 677TT.

deficient in folate had higher levels of homocysteine even without significance, a shorter gestation and a lower infant birthweight versus those with normal folate levels. This difference was more apparent when subject were further classified in terms of *MTHFR* polymorphisms. The magnitude of homocysteine level, gestational age and infant birthweight changes between those deficient in folate and those with normal folate levels were significantly greater for those with *MTHFR 677CC*. On the other hand, the homocysteine levels, gestational ages and infant birthweights were not different between folate deficient and normal folate groups among the *MTHFR CT* or *TT* types.

In the Table 3, we assessed the risk of folate deficiency status on maternal serum homocysteine level, gestational age and infant birthweight for each of the *MTHFR* subtypes. We found that folate deficiency significantly reduced infant birthweight by 543g ($p < 0.05$) among *MTHFR 677CC*.

Birthweight is an important determinant of adult disease. Barker and colleagues found a strong association between infant birthweight and the risk of later chronic diseases[17,18]. Infant birthweight is influenced by maternal genetic and environmental factors during pregnancy. In particular, nutritional factors are thought to important environmental factors for the normal fetus. Folate is a critical nutrient for

**Table 3.** The risk of folate deficiency status on maternal serum homocysteine concentration, gestational age, and infant birthweight by MTHFR* type using multiple linear regression

| Dependent variables | CC* | | CT, TT* | |
|---|---|---|---|---|
| | Unadjusted β | Adjusted †β | Unadjusted β | Adjusted †β |
| Homocysteine | −0.01 (p = 0.99) | 1.19 (p = 0.41) | −0.11 (p = 0.92) | 0.71 (p = 0.47) |
| Gestational age | −0.76 (p = 0.16) | −0.75 (p = 0.35) | 0.23 (p = 0.74) | 0.32 (p = 0.69) |
| Infant birthweight | −432.67 (p = 0.01) | −543.45 (p = 0.03) | −13.06 (p = 0.92) | −20.93 (p = 0.88) |

Independent variable: folate deficiency vs. normal folate range, †Adjusted for age, prepregnancy BMI, weight gain, height, and smoking status. For risk on infant birthweight, additionally adjusted for gestational age, *MTHFR, methylenetetrahydrofolate reductase; CC, 677CC; CT, 677CT; TT, 677TT

maintaining normal cell growth and division and is thus important in pregnancy. Mothers of growth retarded fetuses generally have a lower folate intake, lower blood folate concentrations and higher homocysteinemia rates than mothers of normal fetuses, were reported[5]. Moreover, studies have shown an inverse correlation between maternal homocysteine levels and infant birthweight[19,20]. A direct association has also been found between maternal serum folate and birthweight[6,21]. In addition, a specific folate gene coding for the heat labile MTHFR enzyme has been found to be common, and to have a harmful effect during pregnancy. The common 677C→ T-MTHFR polymorphisms may be clinically relevant under these conditions. A missense mutation 677C→ T in the MTHFR gene was found to reduce MTHFR enzyme activity and suggested to lead to an elevated homocysteine level[22]. Moreover, it has been hypothesized that if a maternal MTHFR genetic polymorphism affects the folate metabolism it may influence common outcomes such as birthweight and gestational age. Some nonrandomized studies have shown that those receiving folate supplementation had significantly higher infant birthweights (by ca. 200 g-400 g)[23]. Moreover, serum folate values > 15.4 nM appears to neutralize the effects of 677C→T mutations[24].

In this study, we could not find a significant association between maternal serum folate deficiency and the subsequent risk of a reduced birthweight. However, we found an interaction between maternal serum folate deficiency and the MTHFR polymorphism on infant birthweight. Among MTHFR CC genotype, maternal serum folate deficiency significantly effected on reduced birthweight. Thus, among those subjects with 677CC, maternal folate deficiency during

gestation suggested to impair cellular growth and replication in the fetus or placenta, which could increase the risk of intrauterine growth retardation.

Some studies suggested that a more abundant supply of folate to the mother and fetus could support growth, gestation and improve infant birthweight. However, this association between folate deficiency and infant birthweight, was not observed in those with the CT or TT genotypes. These results mean that folate has an important effect on infant birthweight in pregnant women with the MTHFR wild genotype, however; folate in pregnant women with the MTHFR variant genotypes do not seem to affect infant birthweight. This finding does not mean that MTHFR variant genotypes are unrelated to birthweight. As shown in this study, infant birthweights in pregnant women with the MTHFR variant genotypes were low compared to those with the MTHFR wild type. This result suggests that the MTHFR variant genotype itself or MTHFR variant gene-related factors, other than folate, influence infant birthweight.

Several previous studies have reported an association between folate and homocysteine[14,25-27], the latter of which increases the risk of preeclampsia and subsequent preterm birth[28-31]. Ronnenberg suggested that elevated homocysteine links to reduced nitric oxide concentration and glutathione peroxidase activity, and such disruption could affect the length of gestation[32]. MTHFR polymorphism plays a central role in folate metabolism. 677TT homozygotes, and to a lesser extent, 677CT heterozygotes, show decreased plasma folate and increased homocysteine levels.

In this study, we found that folate deficiency alone is not associated with the risk of an increased serum level of homocysteine. However, the MTHFR variant was significantly associated with increased homocysteine levels in the presence of folate deficiency. However, it is unclear whether the link between elevated homocysteine levels in mothers and low birthweight is valid, because we were unable to find a statistically significant association between the two. Even though this relation is unclear, our study shows that folate status combined with specific common polymorphisms of a gene coding for folate-dependent enzymes may be an important determinant of maternal serum homocysteine and infant birthweight. These results suggest that use of prenatal folate supplements have the potential to reduce the risk of low birthweight infants.

This study has some limitations that we could not obtained information about infant MTHFR polymorphism, could not consider the potential relevance of fetal genotype, and could not obtained information

about prenatal vitamins or folic acid supplementation after folate measurement to delivery. However, prenatal vitamins or folic acid is not routine recommendation for pregnancy in South Korea. More importantly, this study is limited by its sample size and the applicability of its results to pregnant women elsewhere. We interpreted our results for gene-environment interaction based on a small number of folate deficiency women, and need to consider bias.

In spite of limitation, we suggest that these results are important avenues for future research. We believe that our study is the first to consider the *MTHFR* gene-folate interaction on infant birthweight, and importantly the study suggests the possibility of reducing the incidence of low birthweight by folate supplementation.

In conclusion, the present study suggests a gene-environment interaction between maternal serum folate during pregnancy and the *MTHFR* polymorphisms of the mother on the risk of delivering low birthweight offspring. Folate supplementation during pregnancy in the *MTHFR* wild type woman with folate deficiency is considered to reduce the likelihood of low birthweight.

# Methods

## Subjects and Demographic Characteristics

The study protocol was approved by the Institutional Review Board on human subjects at Ewha Womans University, Seoul, Korea, and informed consent was obtained from participants. The study subjects were 170 healthy pregnant women who visited an obstetric clinic for antenatal care and their singleton live births in Seoul, Korea between August 2001 and March 2003. The criteria for a healthy pregnant woman were as follows: no previous pregnancy complication, no medication, and free of chronic disease (pregnancy induced hypertension, gestational diabetes, and so on). We collected information about demographic and health related characteristics using a questionnaire and a medical chart. The self-administered questionnaires provided information about age, prepregnancy weight, height, current working status and exposure to smoke. Prepregnancy Body Mass Index (BMI) was calculated from height and prepregnancy weight (kg/m²). Current working status was classified as 'yes' or 'no'. For smoke exposure, we asked about both passive exposure to smoke and active smoking status during pregnancy. Answers were dichotomized as yes or no. At the time of delivery, trained nurses recorded the weight gain during pregnancy and the pregnancy

outcomes (birthweight, gestational age, and multiple births) on a medical chart. The gestational age was estimated based on maternal report for last menstrual period (LMP) and ultrasound measurements by gynaecologists.

## Biochemical Analysis

Fasting venous blood was drawn from the antecubital vein. Blood samples for folate and homocysteine were centrifuged for 10 minutes at 3000 × g to obtain the serum. Serum homocysteine analyses were conducted using a modification of a high performance liquid chromatography (HPLC)-fluorescence method, as described by Araki and Sako[15]. Serum folate concentration was determined using a radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, USA).

## *MTHFR* Mutation Analysis

DNA was extracted from whole blood using an Aquapure Genomic DNA blood kit (Biorad Pacific LTD., Kowloon, HongKong) and stored at −80°C for future analysis. DNA fragments were amplified from the genomic DNA by PCR. Amplification was carried out in a total volume of 10 μl containing 50 ng of genomic DNA, 200 mM dNTPs, 0.30 mmol/ml of each primer, i.e., (5′-TGAAGGAGAAGGTGTCTG-CGGGA-3′), (5′-AGGACGGTGGGTGAAGTG-3′) in PCR buffer containing 0.5 units Taq DNA polymerase (Takara Shuzo Co., Shiga, Japan). All reactions were performed for 33 cycles of; 95°C for 60 seconds, the appropriate annealing temperature for 60 seconds, and 72°C for 60 seconds. Four microliters of the PCR products were digested using 16 units of the restriction enzyme *HinfI* (8 U/μL; Takara Shuzo Co., Shiga, Japan). C to T substitution at nucleotide 677 creates an extra *HinFI* restriction site that cleaves the original 198-base pair PCR fragment into 175-and 23-base pair fragments. Size fractionation of the PCR products was performed by electrophoresis on 2.5% agarose gel containing 0.5 mg/ml ethidium bromide. Visualization was performed under ultraviolet light.

## Statistical Analysis

Serum homocysteine levels, gestational age, and infant birthweight by *MTHFR* genotype and serum folate were compared by ANOVA and the student t-test. To determine the risk of folate and the *MTHFR* genotype on serum homocysteine, gestational age, and infant birthweight, we placed those with less than 3.0 ng/ml of folate in the folate deficiency group[16] and those with *MTHFR* 677CT or 677TT in the *MTHFR* variant group. Two-way ANOVA testing was used to determine the interaction between folate and

*MTHFR.*

The relations between folate deficiency and homocysteine level, gestational age, or infant birthweight considering *MTHFR* variants, interaction term of folate deficiency and *MTHFR* variants, prepregnancy BMI, weight gain, height, and smoke exposure status for all subjects were figured using GAM models in SPLUS version 11.0. Gestational age was additionally adjusted for only to the infant birthweight model. We also figured the relation between folate deficiency and homocysteine level, gestational age, or infant birthweight for each *MTHFR* subtype.

Finally, we estimated the risk of folate deficiency versus normal folate levels, on serum homocysteine, gestational age, and infant birthweight by using a linear regression model. We also adjusted for age, prepregnancy BMI, weight gain, height, and smoke exposure status. In addition, to determine the relation between folate deficiency and infant birthweight, we adjusted for gestational age.

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