

Letter to the Editor

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Determinants of homocysteine concentrations in mother and neonatal girl pairs

Keywords: C677T/MTHFR genotype; folate; homocysteine; vitamin B12.

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To the Editor,

Elevated plasma homocysteine (Hcy) concentration is considered to have associations with common pregnancy complications and adverse pregnancy outcomes such as preeclampsia, prematurity, low birth weight, stillbirth, placental abruption, neural tube defects, and clubfoot [1]. Folate and vitamin B12 intake and their blood level status, impaired renal function, and genetic factors in enzymes are involved in the Hcy metabolism and are therefore indicated as primary determinants of elevated Hcy

concentration [2]. A common gene variant of methylenetetrahydrofolate reductase (MTHFR), which synthesizes N⁵-methyltetrahydrofolate, the methyl donor for methylation of Hcy to methionine, is the most frequent genetic cause of elevated Hcy concentration [3]. An identified C to T substitution at 677 in the *MTHFR* gene [C677T/*MTHFR* (A222V)] relatively increases Hcy concentration in subjects with the TT genotype. In this present study, we aim to evaluate the effects of nutritional and genetic factors on plasma Hcy concentration among neonatal girls and their mothers in Japan.

Prior to this study, ethical approval was obtained from a special committee at Nagasaki University (project registration number 100614189). Blood samples were obtained the day after delivery from 200 healthy mothers with uncomplicated pregnancies at Nagasaki University Hospital and four clinics/hospitals in Nagasaki City. Dried blood spots from their 200 neonatal girls were obtained from a routine nationwide screening program conducted in Japan around 5 days after birth [4]. Informed consent was obtained from all participants. The exclusion criteria for mothers included diabetes mellitus, metabolic disease, renal insufficiency, and recurrent abortion (three times or more), and those for neonatal girls were congenital malformation, premature birth (before 37 weeks), and post-term birth (after 42 weeks).

After fasting blood samples were obtained, serum and plasma were collected and kept at –20°C until assaying. Plasma Hcy, cysteine, and methionine concentrations were measured using high performance liquid chromatography with fluorescence detection. The assay procedures used to measure the Hcy concentration from dried blood spots were modifications of the methods previously reported [5]. Serum folate and vitamin B12 concentrations were measured by a chemiluminescent immunoassay (ADVIA Centaur®, Bayer, Leverkusen, Germany) and serum creatinine concentration was measured by an enzyme method (HITACHI 7450®, Hitachi High-Technologies Corporation, Tokyo, Japan).

Genomic DNA from mothers was extracted from blood cells using a MagExtractor MFX[®] kit (Toyobo, Osaka, Japan) and a prepGEM[™] DNA extraction kit (ZyGEM, Hamilton, New Zealand) was used to extract genomic DNA from neonatal dried blood spots. For the determination of C677T/*MTHFR* genotype, we used the TaqMan[®] polymerase chain reaction method (Applied Biosystems Japan, Tokyo, Japan).

Data are expressed as mean±standard deviation or as a median (25th–75th percentiles). Differences between the C677T/*MTHFR* genotypes (CC+CT vs. TT) were evaluated using the Mann-Whitney's U-test. Differences in the ratio of folate and vitamin B12 supplement intake among mothers were evaluated by the χ^2 -test. All statistical analyses were performed using SPSS Statistics 18.0[®] software for Windows (SPSS Japan, Tokyo, Japan). Probability values <0.05 were considered indicative of statistical significance.

Table 1 shows the characteristics of the study participants. In neonates, there were no physical differences between CC+CT and TT genotypes. Also, plasma Hcy, cysteine, and methionine concentrations were not statistically different ($p=0.40$, $p=0.087$, and $p=0.66$, respectively) (Table 1A). Characteristics among mothers are shown in Table 1B. The mean ages were 30.3±4.6 years in mothers and the range was 19–41 years. Plasma Hcy, cysteine, and methionine concentrations were not statistically different ($p=0.20$, $p=0.30$, and $p=0.44$, respectively). There were 28 mothers who took folate supplements during pregnancy, although the Hcy concentration did not differ statistically between those who took supplements and those who did not ($p=0.76$). Also, vitamin B12 supplementation did not decrease Hcy concentration ($p=0.96$).

Table 2 shows the univariate linear regression analysis on neonatal and maternal Hcy concentrations and other variables. In neonates, Hcy concentration was associated with maternal folate concentration ($r=0.17$, $p=0.016$) and maternal vitamin B12 concentration ($r=0.14$, $p=0.047$), but not with maternal Hcy concentration ($r=0.091$, $p=0.20$), maternal C677T/*MTHFR* genotypes ($r=0.044$, $p=0.54$),

and neonatal C677T/*MTHFR* genotypes ($r=0.12$, $p=0.095$) (Table 2A). However, maternal Hcy concentration was significantly associated with maternal folate concentration ($r=0.23$, $p=0.001$), but not with maternal vitamin B12 concentration ($r=0.073$, $p=0.31$) and maternal C677T/*MTHFR* genotypes ($r=0.14$, $p=0.057$) (Table 2B).

Our current study evaluated the confounding factors of Hcy concentration, including C677T/*MTHFR* genotype, among mother and child pairs. In Norway, the Hcy concentration did not differ according to C677T/*MTHFR* genotype among 4992 samples from the newborn screening program [6]. In 201 Irish maternal-fetal pairs using maternal and umbilical cord blood, the maternal Hcy concentration was the primary predictor of Hcy concentration in the fetus ($p<0.0001$) and maternal vitamin B12 concentration played a secondary role ($p=0.0045$), but C677T/*MTHFR* genotype in the fetus played almost none ($p=0.54$) [7]. The maternal plasma folate concentrations also had no significant effect ($p=0.35$), probably because folate supplement is commonly used in Ireland. A previous study conducted to determine the influence of folate fortification showed that the impact of C677T/*MTHFR* genotype on Hcy concentration was substantially smaller in the cohort with folate fortification compared to the cohort without folate fortification after adjusting for other covariates including folate and vitamin B12 concentrations ($r^2=0.021$ vs. $r^2=0.095$) [8]. Another study evaluating relationships between mothers and neonates showed that maternal Hcy, maternal vitamin B12, and maternal folate concentrations predicted the neonatal Hcy concentrations [9]. In addition, the *MTHFR* 677T allele was associated with high Hcy concentrations in mothers, although these effects were not found in neonates carrying the *MTHFR* 677T allele. Our result that neonatal Hcy concentration is not determined by the C677T/*MTHFR* genotype is consistent with these previous studies. However, neonatal Hcy concentration was not associated with maternal Hcy concentration in our study. As the neonatal dried blood spots were collected several days after delivery, maternal breast milk could be another confounding factor. Little is

Neonatal girls	CC+CT (n=173)	TT (n=27)	p-Value
Height, cm	49.0±2.1	49.0±1.6	0.65
Weight, g	3028 (2720–3277)	3008 (2794–3332)	0.47
Head circumference, cm	32.8±1.3	33.0±1.4	0.39
Hcy, $\mu\text{mol/L}$	3.8 (2.5–5.6)	4.4 (2.5–6.6)	0.40
Cysteine, $\mu\text{mol/L}$	35.1 (25.5–45.4)	30.7 (23.9–38.2)	0.087
Methionine, $\mu\text{mol/L}$	7.5 (5.2–10.6)	7.0 (5.7–10.6)	0.66

Table 1A Characteristics of neonatal girls.

Mothers	CC+CT (n=174)	TT (n=26)	p-Value
Age, years	30.1±4.7	31.6±3.6	0.093
Creatinine, mg/L	5.3±1.0	5.3±0.8	0.69
Hcy, µmol/L	5.1 (3.3–7.5)	6.3 (3.6–9.0)	0.20
Cysteine, µmol/L	101.8 (69.6–126.5)	110.2 (82.0–131.5)	0.30
Methionine, µmol/L	25.0 (20.1–31.1)	27.7 (19.7–33.3)	0.44
Folate, µg/L	3.8 (2.8–6.0)	2.9 (2.4–5.8)	0.19
Vitamin B12, ng/L	162.5 (123.8–200.3)	150.0 (129.0–209.0)	0.96
Folate supplement, n (%)	24 (13.8%)	4 (15.4%)	0.83
Vitamin B12 supplement, n (%)	10 (5.7%)	1 (3.8%)	0.69

Table 1B Characteristics of mothers.

Values are mean ± standard deviation or median (25th–75th percentile). Hcy, homocysteine; CC, CT, TT, C677T/MTHFR genotypes.

Neonatal homocysteine	Correlation	p-Value
Maternal folate	r=0.17	0.016
Maternal vitamin B12	r=0.14	0.047
Maternal homocysteine	r=0.091	0.20
Maternal genotypes	r=0.044	0.54
Neonatal genotypes	r=0.12	0.095

Table 2A Univariate linear regression analysis of neonatal homocysteine and other variables.

known about the factors that influence neonatal Hcy concentration and therefore, additional studies are required to clarify the interactions between maternal and neonatal Hcy metabolites. Appropriate nutritional education may be essential to maintain mother and child health.

In our study, the genotypes are in Hardy-Weinberg equilibrium, suggesting that there is not a strong selection against the homozygous state. However, we did not evaluate the folate and vitamin B12 concentrations in the neonates, which are study limitations. As the male sex is associated with a higher Hcy concentration, we conducted this study only in females to remove that confounding factor. Further studies in a larger population and evaluation among different ethnic groups are required because

Maternal homocysteine	Correlation	p-Value
Maternal folate	r=0.23	0.001
Maternal vitamin B12	r=0.073	0.31
Maternal genotypes	r=0.14	0.057

Table 2B Univariate linear regression analysis of maternal homocysteine and other variables.

our study is entirely an epidemiological study among Japanese women.

In conclusion, neonatal Hcy concentration is associated with maternal folate and vitamin B12 concentrations but not with C677T/MTHFR genotype. However, maternal Hcy concentration is associated with folate concentration but not with vitamin B12 concentration and C677T/MTHFR genotype. Elevated Hcy concentration is associated with many health consequences, especially in women of child-bearing age, so women should be aware of the possible pregnancy complications. We need to continue research to identify further determinants of Hcy concentration and implement effective preventive strategies against high Hcy concentrations.

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Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

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