Associations of MTHFR C677T, A1298C and MTRR A66G Polymorphisms With the Risk of Low Birth Weight Infants

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Abstract

Background: To explore the relationship between maternal methylenetetrahydrofolate reductase (MTHFR) gene C677T and A1298C, Methionine Synthase Reductase (MTRR) gene A66G and the recurrence of low birth weight(LBW) in offspring.

Methods: Our case-control study enrolled 106 maternal blood samples of premature infants and 168 maternal blood samples of normal births.Allele-specific polymerase chain reaction (ASPCR) assay combined with TaqMan probe technique were used to detect the mother's MTHFR and MTRR genotypes respectively. And unconditional logistic regression analysis was used to evaluate the associations of MTHFR and MTRR polymorphisms, and gene-gene interaction with low birth weight.

Results: MTHFR 677TT and 1298CC were independently associated with a higher risk of LBW (OR:2.22, 95%CI:1.14-4.34 and OR:2.82, 95% CI:1.15-6.87,respectively). The MTRR A66G polymorphism was associated with an significant association of LBW when combined with the MTHFR 677TT genotype, although there was no association found between LBW and MTRR A66G alone.Moreover, two or more risk genotypes carriers showed higher odds of LBW than null risk genotype one.

Conclusion: Maternal MTHFR gene 677TT, 1298CC can increase the risk of LBW in the offspring.The MTRR A66G polymorphism was not associated with LBW alone. But it may exacerbate the effect of the MTHFR C677T variant.

Background

Low birth weight (LBW) is an adverse pregnancy outcome which refers to a baby with a birth weight less than 2 500 g [1]. The incidence of low birth weight infants in China is 5.87% of live births[2]. LBW can lead to a higher mortality rate, and prone to mental retardation and growth retardation[3–4].Most of the LBW is caused by the combination of environmental and genetic factors.Methylenetetrahydrofolate reductase (MTHFR) and Methionine Synthase Reductase (MTRR) are homocysteine (Hcy) key enzymes of the metabolic pathway, the decreased activity of both may lead to hyperHcyemia[5]. Hyperhomocyticemia is considered to have a toxic effect on the development of the embryo[6]. The level of Hcy in maternal blood is affected by the most common mutation sites of its key enzymes including MTHFR C677T,A1298C and MTRR A66G polymorphism.

MTHFR C677T polymorphism associated with thermolability and reduced enzyme activity, leading to accumulation of Hcy especially under conditions of low dietary folate [7].Most studies have shown that the plasma total homocysteine concentration of homozygous (TT) mutant subjects is significantly increased[8–10]. MTRR A66G polymorphism decreases the enzyme activity and the rate of Hcy remethylation, and further affects plasma homocysteine levels[11].MTRR A66G may also induce DNA hypomethylation by regulation of Hcy levels [12]. Moreover, Vaughn JD et al. indicated additive or synergistic effects of the MTHFR C677T and MTRR A66G polymorphisms on plasma Hcy levels [13].Several studies have shown that MTHFR A1298C was a risk factor for neural tube defects and

combined with C677T resulted in elevated Hcy and decreased plasma folate levels similar to that of 677TT homozygosity[14–16].Gideon Friedman et al. demonstrated that the A1298C mutation affected homocysteine metabolism because the total homocysteine concentration decreased significantly in subjects with 677cc / 1298cc genotype[17].Many epidemiological studies have indicated that the MTHFR C677T, A1298C and MTRR A66G polymorphisms with various diseases, including birth defects, pregnancy complications[18–20].However,whether gene mutations of key enzymes are related to LBW is unclear.Studies by Diptika Tiwari et al. found that MTHFR C677T is a risk factor associated with the susceptibility of LBW in northeast India based correlation analysis[21].So far, no large-scale, well-designed epidemiological studies have clearly demonstrated that MTHFR and MTRR variants are related to LBW in northeast China.We therefore conducted a case-control study to investigate the associations of MTHFR C677T, A1298C MTRR A66G on LBW risk.

Materials And Methods

Subjects

This study enrolled 106 maternal blood samples of premature infants admitted to the Department of Neonatology, Jilin Province Maternal and Child Health Hospital from April 2019 to April 2021 as the case group.168 maternal blood samples of normal births admitted to obstetrics during the same period were selected as the control group.Maternal inclusion criteria: a.No obstetric complications such as eclampsia, diabetes, placenta previa; b.Take multivitamins instead of conventional folic acid during pregnancy. The criteria for selection of case parturients who gave birth to low birth weight infants: a. Gestational age >28 weeks, body weight < 2 500 g; b. Normal delivery, exclude premature babies caused by accidents; c.Eliminate twins or multiple births and congenital malformations. Selection criteria of control parturients who gave birth to infants with normal weight: a.Choose the gestational week born in the same hospital >28 weeks, body weight >2 500 g, difference in birth time <7 d, singleton normal live birth infants; b.Excluding pregnant women and pregnant women with complications and complications during pregnancy newborn. Comparing the general data of the two groups, the difference was not statistically significant (P 0.05).

Serum folate levels

The fasting venous blood of the subjects was extracted, the serum was separated in time and placed in the refrigerator at - 20 ℃ for examination. The serum folate level was measured by chemiluminescence method with Abbott i2000R(Abbott ,USA) .

DNA Extraction and Genotyping

We extracted DNA from 2 mL whole blood, which was collected in ethylenediaminetetraacetic acid (EDTA) and stored at -20 ℃based on provided instructions(Nucleic Acid Extraction and Purification Kit,

Kuangyuan Bio.,Suzhou,China). Polymorphisms MTHFR C677T, A1298C and MTRR A66G were typed in an allele-specific polymerase chain reaction (ASPCR) assay combined with TaqMan probe technique (Applied Biosystems, Foster City, CA,USA) with the ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA,USA). A sample of the PCR system contained 2 μl of genomic DNA, 22 μl of amplification reagent that included labelled primer pairs (MTHFR gene detection kit; Kuangyuan Bio.,Suzhou,China), and 1 μl of reaction solution A (MTHFR gene detection kit; Kuangyuan Bio.,Suzhou,China). The positive controls (TT genotype) and negative controls (MTHFR gene detection kit; Kuangyuan Bio.,Suzhou,China) were amplified at the same time.The amplification steps were 50℃ for 2 min, 95℃for 3 min, and 45 cycles of the following steps: 95℃ for 10 s, 56℃for 30 s, and 60℃ for 30 s.Fluorescence signal was collected in the last 35 amplification cycles.Finally, the type of MTHFR C677T, A1298C and MTRR A66G genotype was determined according to the number of cycles (CT value) of the fluorescence signal forming the amplification curve at a specific threshold.The main parameters for ASPCR of the three single nucleotide polymorphisms (SNPs) were shown in Table 1.

Table 1
Primer sequences for MTHFR C677T, A1298C and MTRR A66G polymorphisms.

Gene	Primer sequence [1]	Product Size
MTHFR C677T	F: 5'-TGAAGGAGAAGGTGTCTGCGGGA-3'	198bp
	R: 5'-AGGACGGTGCGGTGAGAGTG-3'	
MTHFRA1298C	F: 5'-CTTTGGGGAGCTGAAGGACTACTAC-3'	163bp
	R: 5'-CACTTTGTGACCATTCCGGTTTG-3'	
MTRR A66G	F: 5'-GCAAAGGCCATCGCAGAAGACAT-3'	151bp
	R: 5'-GTGAAGATCTGCAGAAAATCCATGTA-3'	

[1] F: forward primer; R: reverse primer.

Statistical Analysis

The relationship between MTHFR C677T, A1298C and MTRR A66G polymorphisms and LBW used SPSS v22.0 (IBM, Inc., Armonk, NY, USA) for statistical testing in our study.Quantitative data were expressed as mean±standard deviation (x±s) and qualitative data as n (%).Comparisons of difference between the case and control with respect to general characteristics were performed by two-sample t test and Chi-square (χ^2)test, respectively.Analysis of variance was used for multi group comparison. Unconditional logistic regression analysis was used to evaluate the associations of multi-factors and the three polymorphisms by Odds ratios (OR) and 95% confidence interval (CI). The trend test was used to verify the above results.Hardy-Weinberg equilibrium (HWE) was also assessed for the distributions of

genotypes withχ^2 test between cases and controls. A two sided *p* value below 0.05 was considered statistical significance.

Results

Population characteristics

Table 2 summarizes the clinical characteristics of the cases and controls.A total of 106 low birth weight infants were collected, of which 63 were males and 43 were females. The birth weight was (2234.6±275.3) g.There were 168 normal-weight control children, 98 males and 70 females. The birth weight was (3374.7±523.4)g. Two-sample t test and X^2 test showed that there were statistically significant in infant birth weight, gestational week of delivery, maternal serum folate level, blood pressure, heart rate and alcohol intake during pregnancy between the two groups(P <0.05).

Table 2

Clinical characteristics of case and controls.

Variable	Case(N = 106)	Control(N = 168)	*P*
	N(%) / $\bar{x}$±s	N(%) / $\bar{x}$±s	
Maternal age, year	32.37±10.10	31.38±9.40	>0.05
Maternal Height, cm	160.1±4.8	160.8±4.1	>0.05
Prepregnancy weight, kg	53.9±7.5	55.6±7.6	>0.05
Maternal serum folate, nmol/L	12.5±1.5	16.9±1.0	<0.05
SBP, mmHg	128.3±14.9	117.9±9.5	<0.05
DBP, mmHg	84.3±10.2	79.2±3.0	<0.05
FBG, mmol/L	4.88±1.20	4.92±0.95	>0.05
HR, Times/min	81.3±4.4	75.4±2.8	<0.05
Gestational week of delivery	38.3±4.2	39.9±1.8	<0.05
Infant birth weight ,g	2234.6±275.3	3374.7±523.4	<0.05
Number of pregnancies			>0.05
1	75(70.7)	138(70.8)	
2	29(27.3)	57(29.2)	
Infant sex			>0.05
Male	63(59.4)	98(58.3)	
Female	43(40.6)	70(41.7)	
Alcohol intake during pregnancy			<0.05
No	90(88.8)	159(94.6)	
Yes	16(15.1)	9(5.3)	
Tobacco smoking during pregnancy			>0.05
Nonsmoker	57(53.7)	97(57.7)	
Passive smoker	46(43.3)	65(38.7)	
Smoker	3(3.0)	6(3.6)	
SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HR, heart rate.			

Gene distribution

In this study, the HWE test was used to verify the reliability of the specimen.The results showed that the collected MTHFR C677T, A1298C, and MTRR A66G gene polymorphisms in the population of Northeast China are in line with genetic balance. The data came from the same Mendelian group, and the specimens were regionally representative.

Association between gene polymorphisms and LBW

The genotypic frequencies of MTHFR C677T, A1298C and MTRR A66G were shown in Table 3. MTHFR 677TT and 1298CC genotype were more prevalent in cases than in controls(49.1% vs29.2%;13.2% vs5.3%,repetitively). There was significant difference in the distribution of genotype between cases and controls, with ORs of 2.22(95% CI:1.14-4.34, P = 0.02) and 1.94(95% CI: 1.66-3.21, P =0.01) for 677TT, when compared with CC and CC + CT, respectively.Similarly,a significant difference in 1298CC genotype distribution were also found between the two study groups,with ORs of 2.82(95% CI:1.15-6.87, P = 0.02) when compared with AA genotype.The results suggest that MTHFR gene 677TT, 1298CCgenotype were associated with an increased risk of LBW.However,none significant result was found in MTRR A66G polymorphism alone.

Table 3
Effects of gene polymorphisms on LBW

Genotype	Cases(N = 106),n (%)	Controls(N = 168),n (%)	OR (95% CI)[a]	*P*	HWE
MTHFR C677T					0.17
CC	19(12.3)	44(25.0)	Reference		
CT	40(38.6)	75(44.6)	1.24(0.64-2.39)	0.53	
TT	47(49.1)	49(29.2)	2.22(1.14-4.34)	0.02	
CC+CT	59(50.9)	119(70.8)	Reference		
TT	47(49.1)	49(29.2)	1.94(1.66-3.21)	0.01	
MTHFR A1298C					0.12
AA	63(59.4)	114(67.9)	Reference		
AC	29(27.4)	45(26.8)	1.17(0.67-2.04)	0.59	
CC	14(13.2)	9(5.3)	2.82(1.15-6.87)	0.02	
AA	63(59.4)	114(67.9)	Reference		
AC+CC	43(40.6)	54(32.1)	1.44(0.87-2.39)	0.16	
MTRR A66G					0.68
AA	49(46.2)	84(50.0)	Reference		
AG	39(36.8)	68(40.5)	0.98(0.58-1.67)	0.95	
GG	18(17.0)	16(9.5)	1.93(0.90-4.12)	0.09	
AA	49(46.2)	84(50.0)	Reference		
AG+GG	57(71.7)	84(50.0)	1.16(0.72-1.70)	0.54	

[a] Adjusted for Maternal age,Height, weight, serum folate, SBP, DBP, FBG, HR, Gestational week of delivery, Infant birth weight.

Association between genetic interaction and LBW

The joint effects of MTHFR C677T, A1298C and MTRR A66G polymorphisms on LBW were next examined (Table 4). None of the study subjects had the 677TT/1298AC + CC genotypes. When compared with the 677CC + CT/1298AA, the cases with the 677CC + CT/1298AC + CC and 677TT/1298AA genotypes had higher odds of LBW risk (OR = 3.24, 95% CI 1.64-6.37,p = 0.00;OR = 3.90, 95% CI 1.98-7.67,p = 0.00;repetitively).Moreover, the 677CC + CT/66AG + GG and 677TT/66AG + GG carriers both had higher risks of LBW compared with the reference group (OR = 2.08, 95% CI 1.11-3.93, p = 0.02;OR = 7.68, 95% CI 3.43-17.17, p = 0.00,repetitively).The trend test was further performed to verify these results, MTHFR C677T/MTHFR C677T and MTHFR C677T/MTRR A66G combinations dramatically increased the LBW risk.

Table 4

Effects of gene-gene interactions on LBW

Genotype 1	Genotype 2	Case/Control	OR (95%CI)	*P*	P_t
MTHFR C677T	MTHFR A1298C				
CC + CT	AA	16/65	Reference		0.00
CC + CT	AC + CC	43/54	3.24(1.64-6.37)	0.00	
TT	AA	47/49	3.90(1.98-7.67)	0.00	
TT	AC + CC	0/0	-	-	
MTHFR C677T	MTRR A66G				
CC + CT	AA	25/72	Reference		0.00
CC + CT	AG + GG	34/47	2.08(1.11-3.93)	0.02	
TT	AA	15/37	1.17(0.55-2.48)	0.69	
TT	AG + GG	32/12	7.68(3.43-17.17)	0.00	
MTHFR A1298C	MTRR A66G				
AC + CC	AA	22/26	Reference		0.82
AC + CC	AG + GG	21/28	0.89(0.40-1.98)	0.77	
AA	AA	27/58	0.55(0.27-1.14)	0.11	
AA	AG + GG	36/56	0.76(0.38-1.54)	0.45	
[a] Adjusted for Maternal age,Height, weight, serum folate, SBP,DBP,FBG,HR,Gestational week of delivery, Infant birth weight.					
P_t:P value of trend test.					

Discussion

LBW is the result of premature delivery and intrauterine growth retardation. At present, the etiology of LBW mainly focuses on maternal diseases and infections, behavioral risk factors and social demographics.Studies at home and abroad have shown that multiple births, insufficient gestational weeks, poor nutrition during pregnancy, history of maternal diseases, umbilical cord abnormalities, history of dysmenorrhea and mental stimulation during pregnancy are risk factors for the occurrence of LBW[22–24]. In view of the importance of genetic factors, a more comprehensive analysis of the risk factors of LBW should be made in conjunction with environmental factors and genetic factors.

As studies have found that hyperHcyemia can induce certain diseases such as congenital malformations and cardiovascular diseases[25–27].Hcy is a sulfur-containing amino acid whose metabolism is affected by three key enzymes such as MTHFR, as well as folic acid and a variety of vitamins [28].5-Methylenetetrahydrofolate is the methyl donor for Hcy methylation. MTHFR plays an important role in the conversion of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate. Therefore, MTHFR can indirectly regulate the plasma Hcy level [29].The MTHFR gene C677T can cause the originally synthesized alanine to become valine, thereby reducing the enzyme activity, leading to the hindrance of Hcy metabolism, and which finally causing hyperhomocystinemia (Hhcy)[30].The maternal blood circulation is connected to the fetus through the placenta. Hhcy will also be manifested in the fetal blood circulation, which can cause fetal blood vessel development abnormalities, neural tube development abnormalities, and premature delivery. This may be one of the reasons for the LBW of the fetus.

After adjusting the confounding factors,our analysis showed that MTHFR 677TT and 1298CC were independently associated with a higher risk of LBW. The MTRR A66G polymorphism was associated with an significant association with LBW when combined with the MTHFR 677TT genotype, but no association was found between LBW and MTRR A66G alone.Moreover, two or more risk genotypes carriers showed higher odds of LBW than null risk genotype one.In line with the above mechanism our data indicate MTHFR 677TT and 1298CC that affects key enzymes in the Hcy metabolic pathway is an independent risk factor for LBW.Additionally, judging from the results of the interaction analysis, it is suggested that the joint action of multiple gene polymorphisms will increase the risk of LBW.

There were several studies on the correlation between folic acid metabolism gene polymorphisms and LBW, which included low birth weight live birth babies as the case group[31–32].However, in our study we used maternal blood samples of parturients who gave birth to low birth weight infants as the case group, because we wanted to achieve primary prevention of LBW and screen high-risk pregnant women by identifying the maternal MTHFR C677T and MTRR mutations.As far as we know, we are the first to prove that MTHFR C677T, MTHFR A1298C and MTRR A66G gene polymorphisms affect the risk of LBW in Northeast China.The limitation of our study is that the sample size is relatively small. Therefore, we believe that large sample studies can be carried out in the future to verify our results in a wider population

Conclusions

In conclusion,our study found that maternal MTHFR gene 677TT, 1298CC can increase the risk of LBW in the offspring.The MTRR A66G polymorphism was not associated with LBW alone. But it may exacerbate the effect of the MTHFR C677T variant .Further large prospective population-based studies are required to confirm our findings.

Abbreviations

MTHFR, maternal methylenetetrahydrofolate reductase; LBW, low birth weight; MTRR, Methionine Synthase Reductase; ASPCR, Allele-specific polymerase chain reaction; Hcy, homocysteine; EDTA, ethylenediaminetetraacetic acid; SNPs, single nucleotide polymorphisms; HWE, Hardy–Weinberg equilibrium; SPSS, Statistical Package for the Social Science; ORs, odds ratios; CIs, Hhcy, confidence intervals; hyperhomocystinemia.

Declarations

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All procedures conducted in this study involving human participants complied with the ethical standards of the Handbook for Good Clinical Research Practice of the World Health Organization and the Declaration of Helsinki principles (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Institutional Review Board (IRB) ofMaternal and Child Health Hospital of Jilin Province, China. Written informed consent was obtained from all subjects.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author Contribution

Each author's contribution(s) to the manuscript, using the numbered list below:

1. Research concept and design: Shuang Sun

2. Collection and/or assembly of data: Shuang Sun

3. Data analysis and interpretation: Shuang Sun

4. Writing the article: Shuang Sun

5. Critical revision of the article:Ying Liu and Xiaoling Hu

6. Final approval of article:Ying Liu and Xiaoling Hu

7. Table editing: Xun Zhao and Xuehua Guo

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