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Methylenetetrahydrofolate reductase polymorphisms, serum methylenetetrahydrofolate reductase levels, and risk of childhood acute lymphoblastic leukemia in a Chinese population

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Methylenetetrahydrofolate reductase (MTHFR), involved in DNA methylation and nucleotide synthesis, is thought to be associated with a decreased risk of adult and childhood acute lymphoblastic leukemia (ALL). Accumulating evidence has indicated that two common genetic variants, C677T and A1298C, are associated with cancer risk. We hypothesized that these two variants were associated with childhood ALL susceptibility and influence serum MTHFR levels. We genotyped these two polymorphisms and detected MTHFR levels in a case–control study of 361 cases and 508 controls. Compared with the 677CC and 677CC/CT genotypes, the 677TT genotype was associated with a statistically significantly decreased risk of childhood ALL (odds ratio = 0.53, 95% confidence interval = 0.32–0.88, and odds ratio = 0.55, 95% confidence interval = 0.35–0.88, respectively). In addition, a pronounced reduced risk of ALL was observed among low-risk ALL and B-phenotype ALL. Moreover, the mean serum MTHFR level was 8.01 ng/mL (±4.38) in cases and 9.27 ng/mL (±4.80) in controls ($P < 0.001$). MTHFR levels in subjects with 677TT genotype was significantly higher than those with 677CC genotype ($P = 0.010$) or 677CT genotype ($P = 0.043$) in controls. In conclusion, our results provide evidence that the *MTHFR* polymorphisms might contribute to reduced childhood ALL risk in this population. (*Cancer Sci* 2010; 101: 782–786)

Acute lymphoblastic leukemia (ALL) is the most common acute leukemia in children,[1] representing approximately one-third of all pediatric cancers. Approximately 30 new cases per one million people are diagnosed every year, with a peak incidence in patients aged 2–5 years in the USA.[2] To date, the mechanisms that underlie the occurrence of ALL remain largely unknown. Mutations in several genes, together with dietary effects, environmental exposure to carcinogens, and individual immune systems are suggested to be the risk factors that predispose individuals to develop ALL.[3,4]

Folate is one of the most important coenzymes in DNA synthesis, and methylenetetrahydrofolate reductase (MTHFR) is the crucial enzyme in folate metabolism. MTHFR can cause methylation of homocysteine into methionine, leading to methylation of DNA.[5] *MTHFR* C677T (rs1801133) and A1298C (rs1801131) are the two common polymorphisms in the *MTHFR* gene. Studies have revealed that *MTHFR* C677T is associated with reduced enzyme activity, thermolability, and mild to moderate hyperhomocysteinemia, especially during times of folate insufficiency.[6,7] *MTHFR* A1298C is associated with lower enzymatic activity but does not seem to influence homocysteine plasma levels, except when accompanied by C677T variation.[8,9] Previous studies have shown that individuals with homozygous 677TT would have a reduced incidence of colorectal cancer[10,11] and leukemia.[12] However, the associations between the *MTHFR* polymorphisms and risk of ALL remained conflicting.[13,14] In the present study, we explored the role of these two *MTHFR* polymorphisms in childhood ALL patients in a Chinese population and further detected the serum levels of MTHFR expression in our ongoing case–control study.

Materials and Methods

Study group. This study included 361 ALL cases and 508 controls. All subjects were genetically unrelated to ethnic Han Chinese. The cases, aged from 1 to 18 years, were newly diagnosed from January 2007 to June 2009 in The Affiliated Nanjing Children's Hospital of Nanjing Medical University (Nanjing, China) and The Affiliated Children's Hospital of Soochow University (Suzhou, China). All cases were immunologic and pathologic proven by bone marrow aspirate. The control subjects were recruited from the same geographic area and were frequency-matched to the cases by age (±5 years) and gender. The controls were randomly selected following health examination and were without malignant neoplastic or thrombotic disease. Potential study subjects were first surveyed at the clinics using a short questionnaire to determine their willingness to participate in research studies and to obtain demographic information through face-to-face interviews. Some studies indicated that parental smoking, drinking, and house painting status might play a role in the development of childhood ALL.[15–22] To explore the association, we collected information for analysis. It was considered as ''never parental smoking'' if neither the father nor mother was a smoker during pregnancy or after birth, otherwise it was considered as ''ever parental smoking''. It was considered as ''never parental drinking'' if neither the father nor mother drank alcohol, and the rest were considered as ''ever parental drinking''. It was considered as ''ever house painting'' when the house was painted during pregnancy or after birth, else it was considered as ''never house painting''. The classification of childhood ALL was based on the Suggested Diagnosis and Treatment of Children with ALL, published by the Society of Pediatrics, Chinese Medical Association in 2006 (Table S1). The ALL patients were defined into two groups by immunophenotype, T cell ALL (T-ALL) and B-phenotype ALL. Standard

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risk and medium risk was treated with a similar protocol so the subjects with standard risk and medium risk were classified in together as low risk. The institutional review board approved the research protocol, and informed consent was obtained from all individuals and their parents.

Genotyping. Genomic DNA was extracted from peripheral blood lymphocytes using standard methods. Detection of the *MTHFR* C677T and A1298C polymorphisms was done using PCR-RFLP assay. The primers, lengths, and restriction enzymes have been described previously.[23] All PCR and restriction products were visualized on 3% agarose gel stained with ethidium bromide. Genotyping was randomly repeated in 20% of samples to check for typing reliability.

ELISA. Blood was collected in standard cubes without anticoagulant, and was immediately centrifuged for 20 min at 3000 *g*. Serums were stored at −80°C. Serum levels of MTHFR expression were measured using mouse antihuman 5, 10-methylenetetrahydrofolate reductase, MTHFR monoclonal antibody (Abcam, Cambridge, UK), following the indirect ELISA protocol of the manufacturer. The optical density was determined by measuring the absorbance at 450 nm. The absorbance was correlated against a standard curve.

Statistical analysis. Differences in the distributions of demographic characteristics, selected variables, and frequencies of genotypes of *MTHFR* variants between the cases and controls were evaluated using Student's *t*-test (for continuous variables) or the χ^2-test (for categorical variables). The odds ratios (OR) and 95% confidence internals (CI) were calculated by logistic regression analysis with adjustment for diagnosis age, gender, parental alcohol use and tobacco smoking, as well as house painting status to quantify the association between the *MTHFR* polymorphisms and risk of ALL. The correlation between the serum MTHFR levels and genotypes of the *MTHFR* C677T and A1298C polymorphisms were evaluated by one-way ANOVA. The statistical power was calculated using PS software (available online at http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize). A *P*-value <0.05 was considered statistically significant, and all statistical tests were two-sided. All statistical analyses were carried out using Statistical Analysis System software (version 9.1.3; SAS Institute, Cary, NC, USA).

Results

Characteristic of study group. The characteristics of 361 ALL cases and 508 cancer-free controls are summarized in Table 1. There were no statistically significant differences between the cases and controls in age or gender ($P = 0.353$ for age; $P = 0.409$ for gender). However, there were more smokers and drinkers among the parents of the cases than those of the controls (64.5% *vs* 57.9%, $P = 0.047$, OR = 1.53, 95% CI = 1.00–1.75; 38.5% *vs* 15.5%, $P < 0.001$, OR = 3.40, 95% CI = 2.47–4.68, respectively). There were also more families who had their houses painted among cases than among controls (32.4% *vs* 11.6%, $P < 0.001$, OR = 3.65, 95% CI = 2.57–5.18). Of the 361 ALL patients, 15.8% patients were T-ALL, and the others were B-phenotype ALL. Furthermore, 192 (53.2%) patients were in the low risk category and the remaining 169 (46.8%) were high risk.

Associations between MTHFR polymorphisms and risk of childhood ALL. The observed genotypes and allele frequencies for ALL cases and controls are shown in Table 2. The genotype frequencies of these two polymorphisms among controls were all in agreement with the Hardy–Weinberg equilibrium ($P = 0.275$ for C677T; $P = 0.069$ for A1298C). The 677T allele was observed in 36.0% of ALL cases compared with 40.6% among controls ($P = 0.050$). The frequency of 1298C allele did not show any difference between the cases and controls ($P = 0.264$). Both frequencies in the controls were similar to our previous study.[24] In single locus analysis, genotype distribution of the C677T polymorphism showed a significant difference between the cases and controls ($P = 0.035$), whereas there was no significant difference in the A1298C polymorphism ($P = 0.530$). The results of multiple logistic regression analysis

Table 1. Frequency distribution of selected variables between cases with childhood acute lymphoblastic leukemia (ALL) and cancer-free controls

Variables	Cases (*n* = 361)		Controls (*n* = 508)		OR (95% CI)†	*P*‡
	n	%	*n*	%		
Age (years)						
<6	164	45.4	247	48.6	1.00	0.353
≥6	197	54.6	261	51.4	1.14 (0.87–1.49)	
Gender						
Male	216	59.8	318	62.6	1.00	0.409
Female	145	40.2	190	37.4	1.12 (0.85–1.48)	
Parental smoking status						
Never	128	35.5	214	42.1	1.00	0.047
Ever	233	64.5	294	57.9	1.53 (1.00–1.75)	
Parental drinking status						
Never	222	61.5	429	84.5	1.00	<0.001
Ever	139	38.5	79	15.5	3.40 (2.47–4.68)	
House painting status						
Never	244	67.6	449	88.4	1.00	<0.001
Ever	117	32.4	59	11.6	3.65 (2.57–5.18)	
Immunophenotype						
T-ALL	57	15.8	–	–	–	–
B-phenotype ALL	304	84.2	–	–	–	–
Treatment branch						
Low risk	192	53.2	–	–	–	–
High risk	169	46.8	–	–	–	–

†Adjusted for age, gender, parental drinking status, parental smoking status, and house painting status except the study factor. ‡Two-sided χ^2-test for either genotype distributions or allele frequencies between cases and controls. CI, confidence interval; OR, odds ratio; T-ALL, T cell ALL; Low risk, standard risk and medium risk; –, not applicable.

Table 2. Logistic regression analysis of associations between methylenetetrahydrofolate reductase polymorphisms and acute lymphoblastic leukemia risk

Genotypes	Cases (*n* = 361)		Controls (*n* = 508)		Adjusted OR (95% CI)†	*P*‡
	n	%	*n*	%		
C677T						
CC	135	37.4	173	34.1	1.00	0.035
CT	192	53.2	257	50.6	0.95 (0.69–1.30)	
TT	34	9.4	78	15.3	0.53 (0.32–0.88)	
Trend test					$P = 0.040$	
CC/CT	327	90.6	430	84.7	1.00	0.010
TT	34	9.4	78	15.3	0.55 (0.35–0.88)	
T allele	260	36.0	413	40.6		0.050
A1298C						
AA	256	70.9	342	67.3	1.00	0.530
AC	90	24.9	142	28.0	0.83 (0.59–1.16)	
CC	15	4.2	24	4.7	0.71 (0.34–1.45)	
Trend test					$P = 0.285$	
AC/CC	105	29.1	166	32.7	0.81 (0.59–1.11)	0.260
C allele	120	16.6	190	18.7		0.264

†Adjusted for age, gender, parental drinking status, parental smoking status, and house painting status. ‡Two-sided χ^2-test for either genotype distributions or allele frequencies between the cases and controls. –, not applicable. CI, confidence interval; OR, odds ratio.

indicated that the risk of ALL associated with the C677T polymorphism was decreased in a dose–response manner as the number of the 677T allele increased (OR = 0.95, 95% CI = 0.69–1.30 for 677CT, and OR = 0.53, 95% CI = 0.32–0.88 for 677TT; P_{trend} = 0.040). There was no significant association between the A1298C polymorphism and ALL risk (Table 2).

In further analysis, as shown in Table 3, we observed that the 677TT genotype was associated with a significantly decreased risk of low-risk ALL compared with the 677CC/CT genotypes (OR = 0.53, 95% CI = 0.29–0.97). Also, when we used the 677CC/CT genotypes as the reference, we found that the 677TT genotype was associated with a significantly decreased risk of B-phenotype ALL (OR = 0.56, 95% CI = 0.35–0.91). However, we did not find any significant association between the A1298C polymorphism and risk of treatment branch or immunophenotype of ALL (Table 3).

However, no statistical evidence was observed for interactions between the *MTHFR* C677T genotypes and the variables (i.e., age, gender, parental smoking status, parental drinking status, and house painting status) (data not shown).

Associations among serum MTHFR levels and risk of childhood ALL. Serum samples were available for 293 ALL cases and 376 controls in the present study, with the mean serum MTHFR level at 8.01 ng/mL (±4.38) in cases and 9.27 ng/mL (±4.80) in controls ($P < 0.001$) (Fig. 1). The correlations between serum MTHFR levels and individual locus were also evaluated. Because MTHFR levels could be affected by disease status and therapeutic effects, we only carried out this analysis in controls. As shown in Figure 2, serum MTHFR levels in subjects with 677TT genotype were significantly higher than those with 677CC genotype ($P = 0.010$) and 677CT genotype ($P = 0.043$). However, no significant difference was observed in serum MTHFR levels among individuals with different genotypes of A1298C ($P = 0.413$). Interestingly, in the subgroup with high serum MTHFR levels (>8.69 ng/mL), the 677TT genotype was associated with a 42% reduced risk of ALL compared with 677CC/CT genotypes (adjusted OR = 0.58, 95% CI = 0.35–0.96) (Table 4).

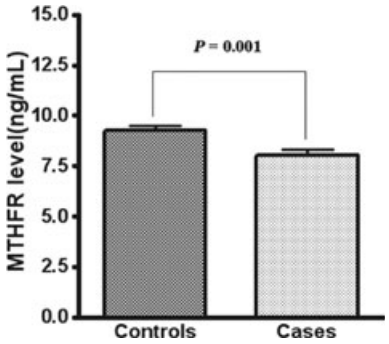

Fig. 1. Mean levels of serum methylenetetrahydrofolate reductase (MTHFR) in 293 cases of childhood acute lymphoblastic leukemia and 376 cancer-free controls. Mean levels of serum MTHFR were significantly different between cases and controls ($P < 0.001$).

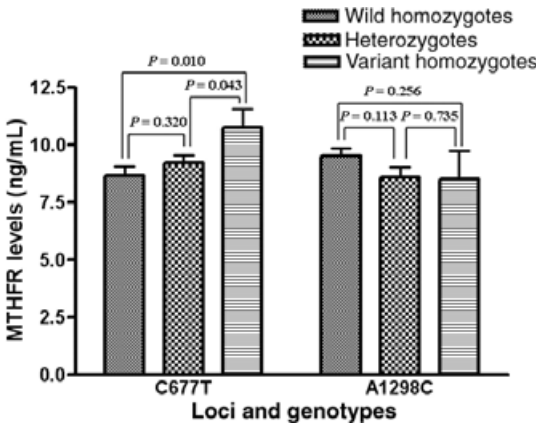

Fig. 2. Mean levels of serum methylenetetrahydrofolate reductase (MTHFR) in cancer-free controls with different genotypes of *MTHFR* C677T and A1298C polymorphisms.

Discussion

In the present case–control study, we explored the associations between *MTHFR* polymorphisms and risk of childhood ALL and serum expression levels of MTHFR in a Chinese population. We found that there were more tobacco smokers and alcohol drinkers among the parents, and more families who had their houses painted, in the cases group than in the controls group. Previous studies[15,16] found that paternal smoking at home was

Table 3. Association between methylenetetrahydrofolate reductase polymorphisms and treatment branch and immunophenotype of acute lymphoblastic leukemia (ALL)

Genotypes	Controls *n* (%)	Treatment branch		Adjusted OR (95% CI)†		Immunophenotype		Adjusted OR (95% CI)†	
		High risk ALL *n* (%)	Low risk ALL *n* (%)	High risk ALL	Low risk ALL	T-ALL *n* (%)	B-phenotype ALL *n* (%)	T-ALL	B-phenotype ALL
C677T									
CC	173 (34.1)	66 (39.0)	69 (35.9)	1.00	1.00	23 (40.4)	112 (36.8)	1.00	1.00
CT	257 (50.6)	87 (51.5)	105 (54.7)	0.89 (0.60–1.32)	1.03 (0.69–1.52)	30 (52.6)	162 (53.3)	0.81 (0.44–1.50)	1.00 (0.72–1.40)
TT	78 (15.3)	16 (9.5)	18 (9.4)	0.53 (0.28–1.01)	0.54 (0.28–1.03)	4 (7.0)	30 (9.9)	0.45 (0.14–1.39)	0.56 (0.33–0.95)
CC/CT	430 (84.7)	153 (90.5)	174 (90.6)	1.00	1.00	53 (93.0)	274 (90.1)	1.00	1.00
TT	78 (15.3)	16 (9.5)	18 (9.4)	0.57 (0.31–1.03)	0.53 (0.29–0.97)	4 (7.0)	30 (9.9)	0.50 (0.17–1.49)	0.56 (0.35–0.91)
A1298C									
AA	342 (67.3)	116 (68.6)	140 (72.9)	1.00	1.00	38 (66.7)	218 (71.7)	1.00	1.00
AC	142 (28.0)	46 (27.2)	44 (22.9)	0.89 (0.59–1.35)	0.78 (0.51–1.20)	15 (26.3)	75 (24.7)	0.78 (0.40–1.53)	0.83 (0.59–1.19)
CC	24 (4.7)	7 (4.2)	8 (4.2)	0.77 (0.31–1.91)	0.64 (0.26–1.57)	4 (7.0)	11 (3.6)	1.41 (0.44–4.52)	0.60 (0.27–1.19)
AC/CC	166 (32.7)	53 (31.4)	52 (27.1)	0.87 (0.59–1.29)	0.75 (0.50–1.13)	19 (33.3)	86 (28.3)	0.87 (0.47–1.62)	0.80 (0.57–1.12)

†Adjusted for age, gender, parental drinking status, parental smoking status, and house painting status.CI, confidence interval; OR, odds ratio; T-ALL, T cell ALL.

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Table 4. Stratification analyses between methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism and risk of acute lymphoblastic leukemia (ALL) by serum MTHFR level

Serum MTHFR level cutoff by the control median (ng/mL)†	*n* (cases/controls)	*MTHFR* C677T (cases/controls)		Adjusted OR (95% CI)‡
		CC/CT	TT	
≤8.69	161/189	153/169	8/20	0.40 (0.16–1.01)
>8.69	132/187	118/158	14/29	0.58 (0.35–0.96)

†Serum MTHFR levels were measured in 293 childhood cases of ALL and 376 controls. ‡Adjusted for age, gender, parental drinking status, parental smoking status, and house painting status, except the stratified variable. CI, confidence interval; OR, odds ratio.

associated with all leukemia and ALL. An increasing trend in risk was observed for pack-years smoked after birth (P_{trend} = 0.06 for all leukemia and 0.02 for ALL) and the number of smokers in the home during the child's life (P_{trend} = 0.05 for all leukemia and 0.03 for ALL).[15] Ji *et al.* reported that carcinogens in the cigarette can pass the placental barrier and transfer to the liver of the infant, then induce mutations of oncogenes or anti-oncogenes.[17] To our knowledge, several studies investigated the role of maternal alcohol drinking during pregnancy and childhood leukemia.[18–21] In addition, our results indicated that parental drinking will increase the risk of ALL, but the mechanism was largely unknown. It has been suggested that ethanol might affect the immune function and be involved in precarcinogen metabolism. Nevertheless, it is still unclear how parental alcohol drinking might influence the risk of childhood leukemia. Freedman *et al.*[22] found that childhood ALL was associated with frequent (>4 times/month) exposure to model building and artwork using solvents, and they also found elevated risk (OR = 1.7) among children whose mothers lived in homes painted extensively (>4 rooms) in the year before childbirth.

Our results suggested that the 677TT genotype was associated with a decreased risk of childhood ALL, which are supported by several similar studies on *MTHFR* C677T polymorphism and risk of cancers.[4,11,25–28] For example, Franco *et al.*[28] reported that in the Brazilian population *MTHFR* 677TT genotype decreased childhood ALL risk, and Ma *et al.*[11] found that individuals with the 677TT variant have one-half the risk of colorectal cancer compared to those with the 677CC genotype. However, no association between *MTHFR* polymorphisms and risk of childhood ALL was found in other studies.[29–33] The protective effect of 677TT was also observed among the low risk cases and B-phenotype ALL. Other high levels of genetic susceptibility or other unknown risk factors might contribute to this result.[34,35] There may be many factors that can influence the results of genotyping studies, including differences in study groups and sample sizes. Our study group is different from those of previous studies. However, the sample size of these studies was relatively smaller than ours. These reasons might be part of the explanation for this conflicting result.

The association between the *MTHFR* A1298C polymorphism and childhood ALL risk remain conflicting. Kim *et al.*[36] found that *MTHFR* 1298AC genotype was associated with increased risk of childhood ALL. However, Franco *et al.*[28] found that *MTHFR* A1298C polymorphism had no effect on the development of childhood ALL. In our study, we did not find any significant difference in genotype frequencies for the A1298C polymorphism between cases and controls. These apparent inconsistencies between our results and those of previous studies, in different populations, might be due to the diverse number of patients studied, different genotype frequencies in the polymorphisms, different ethnicities, and the absence of information on dietary folate intake.

Schnakenberg *et al.*[31] found no significant association between *MTHFR* C677T and A1298C polymorphisms and risk of ALL either in the total patient group or in stratified groups according to gender, age, DNA index, or immunophenotype. The gender bias of the *MTHFR* polymorphisms in ALL was reported for the first time by Reddy and Jamil in 2006.[37] They suggested that these two polymorphisms were more common in male children with ALL than in females. Furthermore, in our study, the protective effect of 677TT was pronounced in the subgroup of no parental drinking and no house painting families, as well as 1298AC/CC genotype in the subgroup of no parental drinking families, although there were no statistical significant interactions between these variables and the genotypes. These findings might reflect that ALL tumor formation could be subjected to a variety of environmental and genetic factors. In these subgroups, other high levels of genetic susceptibilities, possibly owing to reduced DNA repair capacity or other unknown risk factors, might contribute to this result.

Accumulated evidence has shown that folate metabolism pathway plays an important role in carcinogenesis. As MTHFR is the key enzyme in the folate metabolism pathway, mutations in the *MTHFR* gene might reduce the specific activity of the enzyme and increase plasma homocysteine levels, and then affect the normal function of the human body.[37–39] In the present study, we found that high levels of MTHFR was associated with decreased risk of childhood ALL. In addition, in the control group, we also found that MTHFR levels of subjects with 677TT genotype were higher than that of subjects with 677CT or 677CC genotypes, which supported our hypothesis. However, the possibility also exists that these variants might be influenced by the folate uptake of mothers during pregnancy, thereby influencing MTHFR activity and the ethnicity of cases examined to date.[3,4,37,40]

The limitation of this study is that we failed to obtain data regarding folate intake levels. However, our case–control study also has some advantages. First, there are substantial numbers of cases and controls, which greatly increased the statistical power of the analysis. With our study sample size, we had 80% power at 0.05 or smaller to detect an OR of 1.50 or greater and 0.64 or smaller with an exposure frequency of 30% (data not shown). Second, the availability of the exposure information and immunophenotype information, as well as treatment branch information, was satisfied our study. Finally, the data regarding MTHFR levels can compensate for the absence of folate intake information.

In conclusion, our data suggested that *MTHFR* polymorphisms, especially the C677T variation, might play a role in the development of ALL in Chinese children. However, folate assessment and other potential exposure variables are strongly recommended to verify these findings in future studies, in which the effect of gene–gene and gene–environment interaction on ALL could be further examined, leading to a better, more comprehensive understanding of the association between *MTHFR* polymorphisms and childhood ALL.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Suggested diagnosis and treatment of children with acute lymphoblastic leukemia (Society of Pediatrics, Chinese Medical Association, 2006).

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