Original Article
Gene polymorphisms in the folate metabolic pathway and risk of pediatric acute lymphoblastic leukemia: a case-control study in a Chinese population

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Abstract: Polymorphisms in folate pathway genes may influence susceptibility to pediatric acute lymphoblastic leukemia (ALL). This case-control study was undertaken to analyze the association of genetic polymorphisms (677C>T and 1298A>C) of methylenetetrahydrofolate reductase (MTHFR) and reduced folate carrier (RFC1) (80G>A) with the risk of pediatric ALL in China. A total of 176 pediatric ALL patients and 170 matched healthy subjects (as controls) were included and DNA was extracted from the peripheral blood. SNaPshot single nucleotide polymorphism typing was used to determine the genotypes of MTHFR 677C>T, MTHFR 1298A>C, and RFC1 80G>A. All statistical analyses were conducted with SAS software (version 9.2; SAS Institute). There were no significant differences in the genotype and allele frequencies of MTHFR 677C>T, MTHFR 1298A>C, or RFC1 80G>A between patients and controls. No significant correlation was found between the combined genotypes of these polymorphisms and the risk of developing ALL in this study. Furthermore, no significant differences were observed for 677C>T and 1298A>C frequencies between the control and case groups. There was no association between MTHFR 677C>T, MTHFR 1298A>C, or RFC1 80G>A gene polymorphisms and risk of pediatric ALL in the Han Chinese population.

Keywords: Methylenetetrahydrofolate reductase (MTHFR), reduced folate carrier (RFC1), acute lymphoblastic leukemia (ALL), polymorphisms

Introduction
Folate metabolism plays an essential role in the processes of DNA synthesis and methylation. Folate deficiency or its aberrant metabolism has been associated with a number of malignancies including acute lymphoblastic leukemia (ALL) [1-5]. Folate metabolism may become disrupted by inadequate nutrition, altered cellular transport, and polymorphisms in folate-related genes.

The most widely studied gene variants in folate metabolism in relation to the risk of ALL are methylenetetrahydrofolate reductase (MTHFR) 677C>T and MTHFR 1298A>C [6-10]. MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the major circulatory form of folate in the body that acts as a carbon donor for the conversion of homocysteine to methionine. Methionine is the universal methyl donor for DNA methylation. MTHFR is also involved in DNA synthesis through dTMP production. C677T and A1298C are two common polymorphisms of the MTHFR gene that affect enzyme activity [11, 12]. MTHFR 677TT genotype carriers show approximately 30% of the enzyme activity in vitro as compared to the MTHFR 677CC genotype and carriers of the MTHFR 677CT genotype show nearly 65% of normal enzyme activity [11]. The MTHFR A1298C variant results in a decrease in MTHFR enzymatic activity that is more pronounced in homozygotes (CC) than heterozygotes (AC), although it does not result in a thermo-labile protein [12]. However, previous reports of the association between polymorphisms of the MTHFR gene and risk of ALL are conflicting [6-10].
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Reduced folate carrier 1 (RFC1) facilitates the transport of 5-methyltetrahydrofolate from the circulation to peripheral cells. Cellular transport abnormality and polymorphisms in folate-related genes may disturb the metabolism of folate [13]. From the National Center for Biotechnology Information database, seven potential single nucleotide polymorphisms have been tentatively identified in RFC1. However, only the alteration at position 80 results in an amino acid substitution. The G-to-A transition at nucleotide 80 of RFC1 replaces an arginine with a histidine in the protein and this 80AA variant is associated with higher plasma folate levels [14]. De Jonge reported that the RFC1 80A allele increased the risk of pediatric ALL [15] but others did not [16].

To gain further insight into the association between pediatric ALL and folate metabolism, we conducted a hospital-based case-control genetic studies from 170 Han healthy donors who visited the hospital for a routine health examination.

Material and methods

Patients and samples

The patients (n = 176) included in this study were children (<15 years) of Chinese Han origin with newly diagnosed ALL according to the World Health Organization classification. The patients were treated at the Children’s Hospital of Soochow University in Jiangsu Province, China between January 2007 and January 2009. Patient samples were obtained in complete remission after informed consent was provided from their parents according to the Declaration of Helsinki. The Ethical Committee of the hospital approved the research protocol. Control DNA was obtained with informed consent for this and other studies of polymorphisms of MTHFR and RFC1 genes in children younger than 15 years in the Han Chinese population.

Genotype analyses

DNA was isolated from peripheral whole blood using a DNA extraction kit (TIANGEN BIOTECH, Holland) according to the manufacturer’s protocol. Genotyping for MTHFR 677C>T, MTHFR 1298A>C, and RFC1 80G>A polymorphisms was performed using SNAPSHOT Multiplex Kit (ABI Prism). The genotyping protocols were performed according to the methods of Tobler et al. [17]. For added quality assurance, 5% of the control samples were selected at random for repeat analysis.

Statistical analysis

Hardy-Weinberg equilibrium was tested to compare the observed genotype frequencies am-

<table>
<thead>
<tr>
<th>Table 1. Genotype and allele frequencies of MTHFR and RFC1 polymorphisms among the cases-controls and the associations with risk of pediatric ALL</th>
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</thead>
<tbody>
<tr>
<td>Variant Genotype</td>
</tr>
<tr>
<td>RFC 80G&gt;A</td>
</tr>
<tr>
<td>GG</td>
</tr>
<tr>
<td>GA</td>
</tr>
<tr>
<td>AA</td>
</tr>
<tr>
<td>GA+AA</td>
</tr>
<tr>
<td>Alleles</td>
</tr>
<tr>
<td>G</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>MTHFR 677C&gt;T</td>
</tr>
<tr>
<td>CC</td>
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<tr>
<td>CT</td>
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<td>TT</td>
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<td>CT+TT</td>
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<td>Alleles</td>
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<tr>
<td>C</td>
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<tr>
<td>T</td>
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<tr>
<td>MTHFR 1298A&gt;C</td>
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<td>AA</td>
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<td>AC+CC</td>
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<td>C</td>
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</tbody>
</table>

*a reference category; OR indicates odds ratio; CI confidence interval; *Significant value.
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Results

Cases and controls were matched for age, sex, and ethnicity. Of the 176 patients, 108 were males and 68 were females (mean age: 5.6 ± 3.3 years). The phenotype distribution was 160 (91.0%) B-lineage and 16 (9.0%) T-lineage in origin. Stratification of the cases according to immunophenotype was not performed because smaller numbers provided insufficient power to detect significant differences. Of the 170 controls, 101 were males and 69 females (mean age: 4.5 ± 3.8 years). There were no significant differences in the frequency distributions of age between cases and controls. All genotype distributions in the controls and the patients were in Hardy-Weinberg equilibrium (data not shown).

The frequencies of MTHFR 677CC, 677CT, and 677TT were 28.8%, 51.2%, and 20.0% in the control group and were 35.2%, 39.8%, and 25.0% in patients, respectively. The frequency of the T allele of MTHFR 677C>T was 0.449 for the cases and 0.456 for the controls. The adjusted ORs and 95% CIs for 677CT vs. 677CC were 0.64 (0.39-1.04) for 677CT vs. 677CC and 1.02 (0.57-1.83) for 677TT vs. 677CC. Indeed, there was no significant correlation between the T allele and the occurrence of ALL (Table 1).

The frequencies of MTHFR 1298AA, 1298AC, and 1298CC were 71.2%, 27.6%, and 1.2% in the control group and were 68.7%, 28.5%, and 2.8% in patients, respectively. The frequency of the C allele of MTHFR 1298A>C was 0.17 for cases and 0.15 for controls. The adjusted ORs and 95% CIs for MTHFR A1298C were 1.06 (0.66-1.7) for 1298AC vs. 1298AA and 2.5 (0.48-13.14) for 1298CC vs. 1298AA, which showed no evidence of a protective effect of MTHFR A1298C against ALL, similar to the results for the C677T polymorphism (Table 1).

Furthermore, regarding the joint effect of these two polymorphisms (Table 2), we found no evidence between the protective effect of MTHFR polymorphisms and ALL in our study population. Similar to previous reports, our results showed strong linkage disequilibrium between these two polymorphisms because there was no 1298CC/677TT genotype in either the control or the patient groups.

The frequency of the A allele of RFC1 80G>A was 47.2% for cases and 45.6% for controls. However, the RFC1 80G>A A allele frequency of the controls (0.456) was higher than that reported in a previous study including 500 healthy America donors (0.38) [15]. There was no evidence for a significant association between the risk effect of RFC1 80G>A and pediatric ALL in our study population.

Overall, no significant differences for the MTHFR 677C>T, MTHFR 1298A>C, and RFC1 80G>A polymorphisms were found between cases and controls.

Discussion

The association between polymorphisms of the MTHFR and RFC1 genes and pediatric ALL risk has been examined in many studies but the results have been inconsistent [6-10, 15, 16]. Here, we conducted a case-control study to investigate the role of MTHFR (677C>T and 1298A>C) and RFC1 (80G>A) polymorphism.

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Cases (n = 352 alleles) n (%)</th>
<th>Controls (n = 340 alleles) n (%)</th>
<th>OR (95% CI) P</th>
</tr>
</thead>
<tbody>
<tr>
<td>677C-1298A</td>
<td>149 (42.33)</td>
<td>147 (43.24)</td>
<td>1.00*</td>
</tr>
<tr>
<td>677C-1298C</td>
<td>46 (13.07)</td>
<td>38 (11.18)</td>
<td>1.19 (0.73-1.94) 0.47</td>
</tr>
<tr>
<td>677T-1298A</td>
<td>143 (40.63)</td>
<td>142 (41.76)</td>
<td>0.99 (0.72-1.38) 0.97</td>
</tr>
<tr>
<td>677T-1298C</td>
<td>14 (3.98)</td>
<td>13 (3.82)</td>
<td>1.06 (0.48-2.34) 0.88</td>
</tr>
</tbody>
</table>

*Reference category; OR indicates odds ratio; CI confidence interval; *Significant value.
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Gene polymorphisms in susceptibility to pediatric ALL in China. Both the cases and controls belonged to the same ethnic background and all shared a common geographic origin in southern China. There were no associations between the MTHFR polymorphisms (677C>T and 1298A>C), RFC1 (80G>A) polymorphisms, and pediatric ALL in our study population. In addition, no significant correlation was found between the combined genotypes of these polymorphisms and the risk of developing ALL. There were no significant differences observed for the 677C>T and 1298A>C frequencies between the control and case groups.

The MTHFR enzyme has been considered a risk factor in leukemogenesis. On one hand, MTHFR polymorphisms are associated with hypomethylation or dysmethylation of protooncogenes or tumor suppressor genes [1, 2]. This may promote malignant processes as observed in breast cancer [1], gastric cancer [18], and lung squamous carcinoma [19]. On the other hand, the reduced activity of MTHFR enhances the availability of methylenetetrahydrofolate in DNA synthesis pathways. Consequently, misincorporation of dUMP instead of dTMP into the DNA structure is reduced. Therefore, double-stranded breaks and chromosomal damage during the uracil excision repair will be decreased. As a result of this process, MTHFR polymorphisms can protect individuals against cancers, as has been shown in colorectal cancers [20].

Many previous studies have reported that MTHFR polymorphisms (677C>T and 1298A>C) could reduce the risks of adult and childhood ALL [21-26], whereas other studies [27-30] have indicated that MTHFR variants have no role in the development of pediatric ALL. A recent meta-analysis concluded that MTHFR 677TT reduces the risk of adult ALL but not childhood ALL and the MTHFR 1298A>C polymorphism did not influence susceptibility to childhood or adult ALL [31]. In line with another study conducted in China [32], no significant differences were observed for the 677C>T and 1298A>C frequencies between the control and case groups in our present study. Lightfoot et al. [33, 34] also could not find any association between MTHFR polymorphisms and the risk of childhood ALL.

There are several possible reasons for these inconsistencies. First, there could be an influence of the type of population studied, given the difference between Asian [32] and European study results [9]. Based on meta-analysis results, it is plausible that polymorphisms in the MTHFR gene, 677C>T and 1298A>C, are associated with decreased susceptibility to childhood ALL in non-Asian populations [8, 9, 16]. Second, nutritional factors, particularly folate, may contribute to these contradictory results. Interestingly, Milne investigated associations between ALL risk and folate pathway gene polymorphisms and their modification by maternal folic acid supplements in a population-based case-control study (2003-2007) in Australia. This study included 392 cases of ALL and 535 controls. There was no evidence of protective effects of MTHFR 677 T allele and MTHFR 1298 C allele by maternal folic acid supplementation [35]. In a nationwide registry-based case-control study, ESCALE, carried out in 2003-2004 including 764 ALL cases and 1,681 controls, Amigou and colleagues reported that childhood leukemia was significantly inversely associated with maternal folic acid supplementation before and during pregnancy (OR = 0.4; 95% CI: 0.3-0.6); MTHFR genetic polymorphisms were not associated with ALL [36]. Analogous findings have been observed for colorectal cancer in which association between polymorphisms in genes involved in the folate pathway and colorectal cancer risk appear to be modified by folate levels [37, 38]. In other words, a suitable diet with proper folate intake could change the protective effect of MTHFR polymorphisms against malignancies. In our present study, we were not able to assess the folate status of our patients. Since pregnant Chinese women take folate supplementation during pregnancy, the serum folate level was expected to be in the normal range in our patients, making our results resemble those reported by Metayer et al. [39] and Lupo et al. [40].

Many studies have examined the relationships between the RFC1 80G>A polymorphism and disease risk, including ALL [41-44]. Earlier studies have not shown a relationship between the RFC1 80G>A polymorphism and risk of non-Hodgkin's lymphoma [41], prostate cancer [42], and colon cancer [43]. However, the RFC1 A allele was associated with an increased risk of
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distal gastric cancer [44]. For the first time, de Jonge et al. reported [15] that the RFC1 80G>A variant was the strongest modulator of leukemia risk and the risk was increased by 1.5 times and 2.1 times in A-allelic carriers and 80AA homozygotes, respectively. However, Yang et al. [32] reported that the RFC1 80AA variant significantly increased susceptibility to adult ALL, while the RFC1 80GA or 80AA polymorphism had no effect on the risk of pediatric ALL in China. This differs from the results of Yeoh et al. [45] and Chan et al. [46].

The RFC1 80G>A polymorphism results in a change of arginine-27 to histidine-27. The functional effect of the 80G>A variant has been investigated and it has been demonstrated that the mean plasma folate level was slightly higher in healthy subjects carrying the RFC1 80AA variant [13, 14]. These observations suggest that the RFC1 80G>A polymorphism leads to reduced efficiency in the cellular uptake of folate and methylenetetrahydrofolate which may cause increased risk of ALL in carriers of the RFC1 80GA and RFC1 80AA variants. However, the cellular uptake of most oxidized folates such as folic acid is predominantly mediated by the folate receptor and not via RFC. Thus, if adequate levels of folate are available, even if the RFC1 80G>A polymorphism causes reduced efficiency, there would still be a sufficient level of folate and methylenetetrahydrofolate. This suggests that differences in folate availability may influence the functional effects of the RFC1 80G>A polymorphism which could possibly account for the different findings among studies. Indeed, studies have suggested that whether or not the RFC1 80A allele is a risk factor depends on dietary folate intake [47, 48].

Conclusions

In conclusion, we found no association between the presence of MTHFR polymorphisms (C677T and A1298C) or RFC1 polymorphism (G80A) and the risk of pediatric ALL among Chinese patients. Results from previous studies that have examined polymorphisms in MTHFR and RFC1 in relation to pediatric ALL etiology have been inconsistent. These contradictory results indicate the possible influence of factors such as race, ethnic background, and nutritional status as well as the dietary intake of folate, which may affect the role of these polymorphisms in developing leukemia. More comprehensive international studies that consider population substructure are needed to identify potentially important gene-environment interactions involving folate fortification in different populations. Furthermore, our study only examined two critical genes that regulate DNA synthesis and methylation, though there are more than 30 different genes involved in the folate metabolic pathway. Thus, the inclusion of additional folate-metabolizing genes in further investigations may help to clarify the role of this pathway in lymphomagenesis.

Acknowledgements

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Disclosure of conflict of interest

None.

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