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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 thermolabile protein [12]. However, previous reports of the association between polymorphisms of the *MTHFR* gene and risk of ALL are conflicting [6-10].

Table 1. Genotype and allele frequencies of MTHFR and RFC1 polymorphisms among the cases-controls and the associations with risk of pediatric ALL

Variant Genotype	ALL cases (%)	Controls (%)	OR (95% CI)	*P* value
RFC 80G>A				
GG	53 (30.1)	48 (28.2)	1.00[a]	
GA	80 (45.5)	89 (52.4)	0.81 (0.50-1.33)	0.41
AA	43 (24.4)	33 (19.4)	1.18 (0.65-2.15)	0.59
GA+AA	123 (69.9)	122 (71.8)	0.91 (0.57-1.45)	0.70
Alleles				
G	186 (52.8)	185 (54.4)	1.00[a]	
A	166 (47.2)	155 (45.6)	1.07 (0.79-1.44)	0.68
MTHFR 677C>T				
CC	62 (35.2)	49 (28.8)	1.00[a]	
CT	70 (39.8)	87 (51.2)	0.64 (0.39-1.04)	0.07
TT	44 (25.0)	34 (20.0)	1.02 (0.57-1.83)	0.94
CT+TT	114 (64.8)	121 (71.2)	0.75 (0.47-1.17)	0.20
Alleles				
C	194 (55.1)	185 (54.4)	1.00[a]	
T	158 (44.9)	155 (45.6)	0.97 (0.72-1.31)	0.85
MTHFR 1298A>C				
AA	121 (68.7)	121 (71.2)	1.00[a]	
AC	50 (28.5)	47 (27.6)	1.06 (0.66-1.7)	0.80
CC	5 (2.8)	2 (1.2)	2.50 (0.48-13.14)	0.45
AC+CC	55 (31.3)	49 (28.8)	1.12 (0.71-1.78)	0.62
Alleles				
A	292 (83.0)	289 (85.0)	1.00[a]	
C	60 (17.0)	51 (15.0)	1.16 (0.77-1.75)	0.46

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

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 study of polymorphisms of *MTHFR* and *RFC1* genes in children younger than 15 years in the Han Chinese population.

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 genetic studies from 170 Han healthy donors who visited the hospital for a routine health examination.

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 2. Frequencies of MTHFR haplotypes among the cases-controls and the associations between the MTHFR haplotypes and risk of pediatric ALL

Haplotypes	Cases (n = 352 alleles) n (%)	Controls (n = 340 alleles) n (%)	OR (95% CI)	P
677C-1298A	149 (42.33)	147 (43.24)	1.00[a]	
677C-1298C	46 (13.07)	38 (11.18)	1.19 (0.73-1.94)	0.47
677T-1298A	143 (40.63)	142 (41.76)	0.99 (0.72-1.38)	0.97
677T-1298C	14 (3.98)	13 (3.82)	1.06 (0.48-2.34)	0.88

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

ong the subjects with the expected genotype frequencies. Chi-square test or Fisher's exact test was performed to obtain odds ratios (ORs) and 95% confidence intervals (CIs) when appropriate to assess the relative risk conferred by a particular allele and genotype, independently. Potential linkage disequilibrium between the *MTHFR* C677T and A1298C polymorphisms was also explored using multiplicative interaction terms in a logistic regression model. All statistical analyses were conducted with SAS software (version 9.2; SAS Institute). A *P* value of <0.05 was considered to be statistically significant.

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 *MTHFR* C677T 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) polymorphis-

ms 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

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 methyltetrahydrofolate 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 methyltetrahydrofolate. 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.

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Disclosure of conflict of interest

None.

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References

[1] Naushad SM, Pavani A, Digumarti RR, Gottumukkala SR, Kutala VK. Epistatic interactions between loci of one-carbon metabolism modulate susceptibility to breast cancer. Mol Biol Rep 2011; 38: 4893-901.

[2] Pu D, Jiang SW, Wu J. Association between MTHFR gene polymorphism and the risk of ovarian cancer: a meta-analysis of the literature. Curr Pharm Des 2014; 20: 1632-8.

[3] Botezatu A, Socolov D, Iancu IV, Huica I, Plesa A, Ungureanu C, Anton G. Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and promoter methylation in cervical oncogenic lesions and cancer. J Cell Mol Med 2013; 17: 543-9.

[4] Jokic M, Brcic-Kostic K, Stefulj J, Catela IT, Bozo L, Gamulin M, Kapitanovic S. Association of MTHFR, MTR, MTRR, RFC1, and DHFR gene

polymorphisms with susceptibility to sporadic colon cancer. DNA Cell Biol 2011; 30: 771-6.
[5] Yiu TT, Li W. Pediatric cancer epigenome and the influence of folate. Epigenomics-UK 2015; 7: 961-73.
[6] Urayama KY, Chokkalingam AP, Manabe A, Mizutani S. Current evidence for an inherited genetic basis of childhood acute lymphoblastic leukemia. Int J Hematol 2013; 97: 3-19.
[7] McNeer JL. The complex interplay between folate metabolism and risk of acute lymphoblastic leukemia. Leuk Lymphoma 2011; 52: 1621-2.
[8] Tong N, Sheng X, Wang M, Fang Y, Shi D, Zhang Z, Zhang Z. Methylenetetrahydrofolate reductase gene polymorphisms and acute lymphoblastic leukemia risk: a meta-analysis based on 28 case-control studies. Leuk Lymphoma 2011; 52: 1949-60.
[9] Wang H, Wang J, Zhao L, Liu X, Mi W. Methylenetetrahydrofolate reductase polymorphisms and risk of acute lymphoblastic leukemia-evidence from an updated meta-analysis including 35 studies. BMC Med Genet 2012; 13: 77.
[10] Xiao Y, Deng TR, Su CL, Shang Z. Methylenetetrahydrofolate reductase polymorphisms and susceptibility to acute lymphoblastic leukemia in a Chinese population: a meta-analysis. Oncol Res Treat 2014; 37: 576-82.
[11] Bueno O, Molloy AM, Fernandez-Ballart JD, Garcia-Minguillan CJ, Ceruelo S, Rios L, Ueland PM, Meyer K, Murphy MM. Common polymorphisms that affect folate transport or metabolism modify the effect of the MTHFR 677C>T polymorphism on folate status. J Nutr 2016; 146: 1-8.
[12] Cabo R, Hernes S, Slettan A, Haugen M, Ye S, Blomhoff R, Mansoor MA. Effect of genetic polymorphisms involved in folate metabolism on the concentration of serum folate and plasma total homocysteine (p-tHcy) in healthy subjects after short-term folic acid supplementation: a randomized, double blind, crossover study. Genes Nutr 2015; 10: 456.
[13] Yee SW, Gong L, Badagnani I, Giacomini KM, Klein TE, Altman RB. SLC19A1 pharmacogenomics summary. Pharmacogenet Genomics 2010; 20: 708-15.
[14] Hou Z, Matherly LH. Biology of the major facilitative folate transporters SLC19A1 and SLC46A1. Curr Top Membr 2014; 73: 175-204.
[15] de Jonge R, Tissing WJ, Hooijberg JH, Jansen G, Kaspers GJ, Lindemans J, Peters GJ, Pieters R. Polymorphisms in folate-related genes and risk of pediatric acute lymphoblastic leukemia. Blood 2009; 113: 2284-9.
[16] Koppen IJ, Hermans FJ, Kaspers GJ. Folate related gene polymorphisms and susceptibility to develop childhood acute lymphoblastic leukaemia. Br J Haematol 2010; 148: 3-14.
[17] Tobler AR, Short S, Andersen MR, Paner TM, Briggs JC, Lambert SM, Wu PP, Wang Y, Spoonde AY, Koehler RT, Peyret N, Chen C, Broomer AJ, Ridzon DA, Zhou H, Hoo BS, Hayashibara KC, Leong LN, Ma CN, Rosenblum BB, Day JP, Ziegle JS, De La Vega FM, Rhodes MD, Hennessy KM, Wenz HM. The SNPlex genotyping system: a flexible and scalable platform for SNP genotyping. J Biomol Tech 2005; 16: 398-406.
[18] Xia LZ, Liu Y, Xu XZ, Jiang PC, Ma G, Bu XF, Zhang YJ, Yu F, Xu KS, Li H. Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and gastric cancer susceptibility. World J Gastroenterol 2014; 20: 11429-38.
[19] Zhang XD, Li YT, Yang SY, Li W. Meta-analysis on MTHFR polymorphism and lung cancer susceptibility in East Asian populations. Biomed Rep 2013; 1: 440-6.
[20] Kennedy DA, Stern SJ, Matok I, Moretti ME, Sarkar M, Adams-Webber T, Koren G. Folate intake, MTHFR polymorphisms, and the risk of colorectal cancer: a systematic review and meta-analysis. J Cancer Epidemiol 2012; 2012: 952508.
[21] Yan J, Yin M, Dreyer ZE, Scheurer ME, Kamdar K, Wei Q, Okcu MF. A meta-analysis of MTHFR C677T and A1298C polymorphisms and risk of acute lymphoblastic leukemia in children. Pediatr Blood Cancer 2012; 58: 513-8.
[22] Damnjanovic T, Milicevic R, Novkovic T, Jovicic O, Bunjevacki V, Jekic B, Lukovic L, Novakovic I, Redzic D, Milasin J. Association between the methylenetetrahydrofolate reductase polymorphisms and risk of acute lymphoblastic leukemia in Serbian children. J Pediatr Hematol Oncol 2010; 32: e148-50.
[23] Goricar K, Erculj N, Faganel KB, Debeljak M, Hovnik T, Jazbec J, Dolzan V. The association of folate pathway and DNA repair polymorphisms with susceptibility to childhood acute lymphoblastic leukemia. Gene 2015; 562: 203-9.
[24] Silva RM, Fontes AC, Silva KA, Sant'Ana TA, Ramos FJ, Marques-Salles TJ, Pombo-de-Oliveira MS, Muniz MT. Polymorphisms involved in folate metabolism pathways and the risk of the development of childhood acute leukemia. Genet Test Mol Biomarkers 2013; 17: 147-52.
[25] Tong N, Fang Y, Li J, Wang M, Lu Q, Wang S, Tian Y, Rong L, Sun J, Xu J, Zhang Z. Methylenetetrahydrofolate reductase polymorphisms, serum methylenetetrahydrofolate reductase levels, and risk of childhood acute lymphoblastic leukemia in a Chinese population. Cancer Sci 2010; 101: 782-6.
[26] Li X, Liao Q, Zhang S, Chen M. Association of methylenetetrahytrofolate reductase (MTHFR)

C677T and A1298C polymorphisms with the susceptibility of childhood acute lymphoblastic leukaemia (ALL) in Chinese population. Eur J Med Res 2014; 19: 5.
[27] Kreile M, Rots D, Piekuse L, Cebura E, Grutupa M, Kovalova Z, Lace B. Lack of association between polymorphisms in genes MTHFR and MDR1 with risk of childhood acute lymphoblastic leukemia. Asian Pac J Cancer Prev 2014; 15: 9707-11.
[28] Sadananda AM, Chandy S, Ramachandra N, Appaji L, Aruna KB, Ramaswamy G, Savithri HS, Krishnamoorthy L. Methylenetetrahydrofolate reductase gene polymorphisms and risk of acute lymphoblastic leukemia in children. Indian J Cancer 2010; 47: 40-5.
[29] Nikbakht M, MalekZadeh K, Kumar JA, Askari M, Marwaha R, Kaul D, Kaur J. Polymorphisms of MTHFR and MTR genes are not related to susceptibility to childhood ALL in North India. Exp Oncol 2012; 34: 43-8.
[30] Li SY, Ye JY, Liang EY, Zhou LX, Yang M. Association between MTHFR C677T polymorphism and risk of acute lymphoblastic leukemia: a meta-analysis based on 51 case-control studies. Med Sci Monit 2015; 21: 740-8.
[31] Vijayakrishnan J, Houlston RS. Candidate gene association studies and risk of childhood acute lymphoblastic leukemia: a systematic review and meta-analysis. Haematologica 2010; 95: 1405-14.
[32] Yang L, Liu L, Wang J, Qiu L, Mi Y, Ma X, Xiao Z. Polymorphisms in folate-related genes: impact on risk of adult acute lymphoblastic leukemia rather than pediatric in Han Chinese. Leuk Lymphoma 2011; 52: 1770-6.
[33] Lightfoot TJ, Johnston WT, Painter D, Simpson J, Roman E, Skibola CF, Smith MT, Allan JM, Taylor GM. Genetic variation in the folate metabolic pathway and risk of childhood leukemia. Blood 2010; 115: 3923-9.
[34] Lightfoot TJ, Roman E, Smith MT, Skibola CF. Acute lymphoblastic leukaemia in children-is there a role for MTHFR? Br J Haematol 2010; 149: 797-8, author reply 799-800.
[35] Milne E, Greenop KR, Scott RJ, Haber M, Norris MD, Attia J, Jamieson SE, Miller M, Bower C, Bailey HD, Dawson S, McCowage GB, de Klerk NH, van Bockxmeer FM, Armstrong BK. Folate pathway gene polymorphisms, maternal folic acid use, and risk of childhood acute lymphoblastic leukemia. Cancer Epidemiol Biomarkers Prev 2015; 24: 48-56.
[36] Amigou A, Rudant J, Orsi L, Goujon-Bellec S, Leverger G, Baruchel A, Bertrand Y, Nelken B, Plat G, Michel G, Haouy S, Chastagner P, Ducassou S, Rialland X, Hemon D, Clavel J. Folic acid supplementation, MTHFR and MTRR polymorphisms, and the risk of childhood leukemia: the ESCALE study (SFCE). Cancer Causes Control 2012; 23: 1265-77.
[37] Figueiredo JC, Levine AJ, Crott JW, Baurley J, Haile RW. Folate-genetics and colorectal neoplasia: what we know and need to know next. Mol Nutr Food Res 2013; 57: 607-27.
[38] Lee JE, Wei EK, Fuchs CS, Hunter DJ, Lee IM, Selhub J, Stampfer MJ, Willett WC, Ma J, Giovannucci E. Plasma folate, methylenetetrahydrofolate reductase (MTHFR), and colorectal cancer risk in three large nested case-control studies. Cancer Causes Control 2012; 23: 537-45.
[39] Metayer C, Scelo G, Chokkalingam AP, Barcellos LF, Aldrich MC, Chang JS, Guha N, Urayama KY, Hansen HM, Block G, Kiley V, Wiencke JK, Wiemels JL, Buffler PA. Genetic variants in the folate pathway and risk of childhood acute lymphoblastic leukemia. Cancer Causes Control 2011; 22: 1243-58.
[40] Lupo PJ, Nousome D, Kamdar KY, Okcu MF, Scheurer ME. A case-parent triad assessment of folate metabolic genes and the risk of childhood acute lymphoblastic leukemia. Cancer Causes Control 2012; 23: 1797-803.
[41] Suthandiram S, Gan GG, Mohd ZS, Bee PC, Lian LH, Chang KM, Ong TC, Mohamed Z. Genetic polymorphisms in the one-carbon metabolism pathway genes and susceptibility to non-Hodgkin lymphoma. Tumour Biol 2015; 36: 1819-34.
[42] Collin SM, Metcalfe C, Zuccolo L, Lewis SJ, Chen L, Cox A, Davis M, Lane JA, Donovan J, Smith GD, Neal DE, Hamdy FC, Gudmundsson J, Sulem P, Rafnar T, Benediktsdottir KR, Eeles RA, Guy M, Kote-Jarai Z, Morrison J, Al OA, Stefansson K, Easton DF, Martin RM. Association of folate-pathway gene polymorphisms with the risk of prostate cancer: a population-based nested case-control study, systematic review, and meta-analysis. Cancer Epidemiol Biomarkers Prev 2009; 18: 2528-39.
[43] Figueiredo JC, Levine AJ, Lee WH, Conti DV, Poynter JN, Campbell PT, Duggan D, Lewinger JP, Martinez ME, Ulrich CM, Newcomb P, Potter J, Limburg PJ, Hopper J, Jenkins MA, Le Marchand L, Baron JA, Haile RW. Genes involved with folate uptake and distribution and their association with colorectal cancer risk. Cancer Causes Control 2010; 21: 597-608.
[44] Huang X, Gao Y, He J, Cai J, Ta N, Jiang H, Zhu J, Zheng J. The association between RFC1 G80A polymorphism and cancer susceptibility: Evidence from 33 studies. J Cancer 2016; 7: 144-52.
[45] Yeoh AE, Lu Y, Chan JY, Chan YH, Ariffin H, Kham SK, Quah TC. Genetic susceptibility to

childhood acute lymphoblastic leukemia shows protection in Malay boys: results from the malaysia-singapore ALL study group. Leuk Res 2010; 34: 276-83.

[46] Chan JY, Ugrasena DG, Lum DW, Lu Y, Yeoh AE. Xenobiotic and folate pathway gene polymorphisms and risk of childhood acute lymphoblastic leukaemia in Javanese children. Hematol Oncol 2011; 29: 116-23.

[47] Lim JY, Bhatia S, Robison LL, Yang JJ. Genomics of racial and ethnic disparities in childhood acute lymphoblastic leukemia. Cancer-Am Cancer Soc 2014; 120: 955-62.

[48] Sherborne AL, Hemminki K, Kumar R, Bartram CR, Stanulla M, Schrappe M, Petridou E, Semsei AF, Szalai C, Sinnett D, Krajinovic M, Healy J, Lanciotti M, Dufour C, Indaco S, El-Ghouroury EA, Sawangpanich R, Hongeng S, Pakakasama S, Gonzalez-Neira A, Ugarte EL, Leal VP, Espinoza JP, Kamel AM, Ebid GT, Radwan ER, Yalin S, Yalin E, Berkoz M, Simpson J, Roman E, Lightfoot T, Hosking FJ, Vijayakrishnan J, Greaves M, Houlston RS. Rationale for an international consortium to study inherited genetic susceptibility to childhood acute lymphoblastic leukemia. Haematologica 2011; 96: 1049-54.