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TS Gene Polymorphisms Correlate with Susceptibility to Acute Lymphocytic Leukemia in Children

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Background: Acute lymphocytic leukemia (ALL) in children is a clonal disease of bone marrow hematopoietic stem cells. This study aimed to explore the associations between MTHFR or TS genetic polymorphisms and susceptibility to acute lymphocytic leukemia (ALL) in children.

Material/Methods: This case-control study included 79 ALL patients (case group) and 102 non-ALL patients (control group). Post-PCR genomic DNA sequencing revealed MTHFR C677T and MTHFR A1298C genotypes and TS polymorphisms. The χ^2 test was used to compare differences in MTHFR and TS polymorphisms (including genotypic and allelic distributions) between groups. Logistic regression analysis was used to determine genetic polymorphisms and ALL risk associations.

Results: The results indicated that TS 3R allele frequency was significantly higher in the case group than in the control group (χ^2=7.45, P<0.05). The MTHFR C677T and MTHFR A1298C polymorphisms were not associated with ALL risk. Compared to the TS 2R/2R genotype, subjects carrying TS 2R/3R were twice as likely to develop ALL, and the TS 3R/3R+3R/4R genotype carried a 4-fold higher risk of developing ALL (*OR*=1.96, *CI*: 1.14–3.36).

Conclusions: The TS genetic polymorphisms increase the ALL risk. The TS 3R allele was a risk factor for ALL. There were no associations between MTHFR C677T or MTHFR A1298C polymorphisms and ALL susceptibility.

MeSH Keywords: Disease Susceptibility • Polymorphism, Genetic • Precursor Cell Lymphoblastic Leukemia-Lymphoma

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Background

Acute lymphocytic leukemia (ALL) in children is a clonal disease of bone marrow hematopoietic stem cells. Environmental factors stimulate disease development, and the combination of genetic predisposition and multi-stage responses of environmental response genes determine the risk of developing leukemia. Previous studies have shown that MTHFR and TS gene polymorphisms are associated with acute lymphocytic leukemia susceptibility. However, the results in different regions and populations are also varied. Amigou et al. [1] and Semsei [2] et al. found that the MTHFR gene polymorphism was not associated with the risk of childhood acute lymphoblastic leukemia. Conversely, Silva et al. [3] found that the MTHFR A1298C polymorphism reduced ALL susceptibility in children, and the C677T polymorphism increased susceptibility. However, Jiang et al. [4] reported that the C677T polymorphism reduced ALL risk. Rahimi et al. [5] found that the TS 5'-UTR polymorphism did not increase ALL susceptibility in people from western Iran, while Canalle et al. [6] found that carrying the TS 3R allele reduced ALL risk.

Folate metabolism provides 1-carbon units for normal DNA synthesis and methylation in the body, which is essential for cells to exert their functions. Therefore, gene polymorphisms of folate metabolism-associated enzymes can down-regulate folate levels or alter folate distribution in cells and thus affect folate metabolism, disturb DNA synthesis and methylation, and increase the risk of developing malignancies. Additionally, DNA methylation disturbance can also reduce transmethylation in the methionine cycle, inducing hypomethylation or transmethylation absence in proto-oncogenes and tumor suppressor genes. Previous studies have already shown that folate metabolism-associated genes [7–9] play important roles in ALL development and progression.

In the present study, we used direct sequencing following PCR amplification to determine the MTHFR C677T, MTHFR A1298C, and TS genotypes in people from Hunan Province to investigate the distribution of these 3 genotypes, explore the association between MTHFR and TS gene polymorphisms and ALL susceptibility in children, and evaluate the value of those polymorphisms in predicting ALL susceptibility in children.

Material and Methods

Subjects

Case group: We included 79 treatment-naïve ALL patients hospitalized and treated in the Pediatric Medical Center and Department of Hematology of our hospital, as well as at Hunan Children's Hospital, between February 2010 and February 2013. Among these patients, there were 36 males and 43 females, and their ages ranged from 10 months to 15 years. ALL was diagnosed according to diagnosis and treatment recommendations [10].

Control group: We included 102 non-ALL patients hospitalized in the same period as the controls. Among these patients, there were 63 males and 39 females, and their ages ranged from 2 months to 13.5 years.

All of the case and control group subjects were of Han ethnicity and were born in Hunan Province, China. The Ethics Committee of Hunan Province People's Hospital approved the study (Certification No. 201022).

Apparatus and reagents

We used an electronic analytical balance, Milli-Qlabo superpure water system, ultraviolet spectrophotometer, PCR 9700, gel imaging and analysis system, blood genomic DNA extraction kits (spin column type, DP318), EB substitute, and agarose in the present study.

Sample collection

We received written informed consent from the patients or their guardians. The present study was also approved by the relevant Ethics Committee. We obtained 2 ml of fasting venous blood from all case group and control group subjects and kept it in an anticoagulant tube at 4°C. DNA was extracted within 48–72 h.

Extracting genomic DNA with kits

Genomic DNA was extracted using DNA extraction kits (Tiangen, spin column type) according to the manufacturer's instructions.

Identification of the DNA purity

We obtained 10 μl DNA and added 990 μl of sterilized superpure water, then added the mixture to a quartz cuvette. We used a spectrophotometer to evaluate DNA concentration. We calculated the A_{260}/A_{280} (namely $OD_{260/280}$) and A_{260}/A_{230} ($OD_{260/230}$) ratios, and the results showed that $OD_{260/280}$ was between 1.7 and 1.9, while $OD_{260/230}$ was higher than 2.0.

Genotyping

Primer synthesis: 1) The nucleotide sequences of the MTHFR and TS genes were obtained from GenBank. The MTHFR C677T and MTHFR A1298C primers were designed and synthesized, while the TS gene primers were obtained from previous studies [11]. 2) The primer sequences are shown in Table 1. All primers were synthesized by the Beijing Genomics Institute (BGI).

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Table 1. Primers for the PCR amplification of MTHFR and TS genes.

Gene	Gene ID	Sequences	Size of target gene (bp)
MTHFR C677T	4524	F: CTGTGCTGTGCTGTTGGAAG R: ACGATGGGGCAAGTGATG	310
MTHFR A1298C	4524	F: TCCTCTTCCCCTGCCTTTG R: CCACTCCAGCATCACTCACTTT	193
TS	7298	F: GTGGCTCCTGCGTTTCCCCC R: CCAAGCTTGGCTCCGAGCCGGCCACAGGCATGGCGCGG	251

PCR amplification: For MTHFR C677T and MTHFR A1298C amplification, the reaction systems were both 50 µl, which contained 25 µl Mix (2×), 2 µl forward primer (10 µM), 2 µl backward primer (10 µM), 4 µl DNA template, and 17 µl deionized water. The reaction conditions were: initial denaturation at 95°C for 2 min, followed by 94°C for 30 s, 54°C for 30 s, and 72°C for 30 s. After 36 cycles of reactions, the samples were extended at 72°C for 7 min. For TS gene amplification, the reaction system was 30 µl, which contained 15 µl Mix, 1.5 µl forward primer, 1.5 µl backward primer, 10 µl DNA template, and 2 µl deionized water. The reaction conditions were: initial denaturation at 95°C for 2 min, followed by 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s. After 36 cycles of reactions, the samples were extended at 72°C for 7 min.

Electrophoresis of the PCR products: We obtained 5 µl PCR products for each amplification along with 7.5 µl Marker, and they were sequentially added into the holes in agarose gel (3%). The electrophoresis was then performed at 100 mv voltage for 45 min, then we used the gel imaging system to analyze and photograph the images.

Sequencing: The remaining PCR products were transferred to the BGI for direct sequencing after purification.

Statistical analysis

We used the chi-square goodness of fit test and the Hardy-Weinberg equilibrium test for the MTHFR C677T, MTHFR A1298C, and TS genotypes, and $P<0.05$ was considered statistically significant. We used the chi-square test to compare the distribution of MTHFR C677T, MTHFR A1298C, and TS genotypes in the case and control groups, and $P<0.05$ was considered statistically significant. Unconditional logistic regression was used to obtain the odds ratios (ORs) and 95% confidence intervals (CIs) of each polymorphism, and $P<0.05$ was considered statistically significant.

Results

PCR amplification results

After agarose gel electrophoresis of the PCR products, the results showed that the product sizes were similar to those anticipated (Figure 1A–1C) and thus could be used in the following experiments.

DNA sequencing results

A C→T mutation at site 677 in the 4th MTHFR gene exon resulted in a mutation of Ala (GCC) to Val (GTC) at site 222 of the peptide chain, resulting in a homozygous Val/Val genotype. If a C/T heterozygote was found at site 677, an Ala/Val heterozygous genotype was also seen; if no mutation was found, a wide type Ala/Ala genotype was seen (Figure 2A).

An A→C mutation at site 1298 in the 7th MTHFR gene exon resulted in a Glu (GAA) mutation to Ala (GCA) at site 429 of the peptide chain, resulting in a homozygous Ala/Ala genotype. If an A/C heterozygote was found at site 1298, a Glu/Ala heterozygous genotype was seen. If no mutation was found, a wild-type Glu/Glu genotype was seen (Figure 2B).

A 28 bp tandem repeat polymorphism was observed in the TS gene enhancer sequences. According to the variable number of tandem repeats (VNTRS), the polymorphism was classified as 2R containing 2 repeats, 3R containing 3 repeats, or 4R containing 4 repeats (Table 2). Additionally, a G→C mutation was observed at the 12th nucleotide of the second repeat in the 3R polymorphism, thus the genotypes included 2R/2R, 2R/3Rg, 2R/3Rc, 3Rg/3Rc, 3Rg/3Rg, and 3Rc/3Rc. In the present study, we found a 3Rg/4R genotype (Figure 3A–3G).

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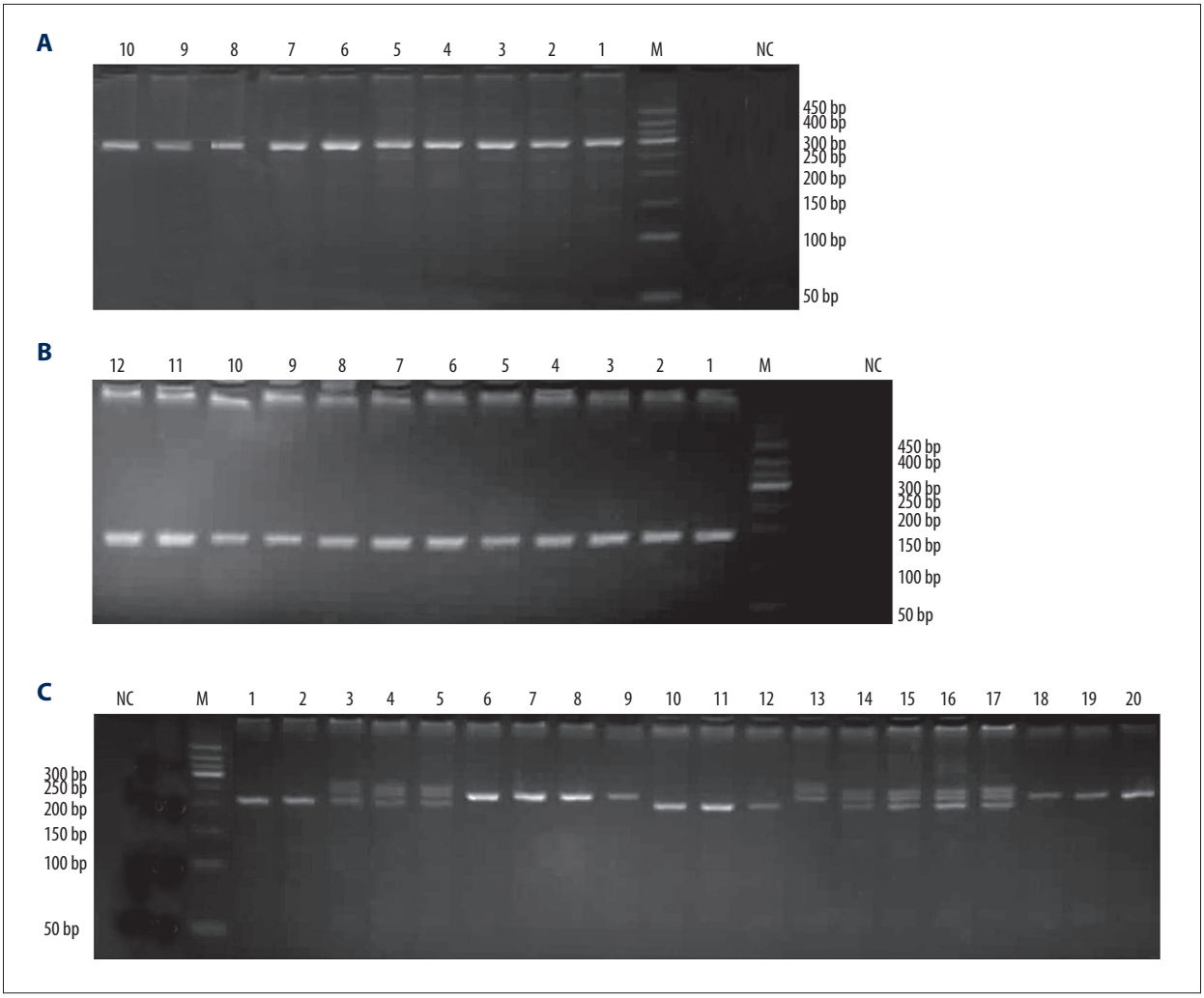

Figure 1. Electrophoresis of the MTHFR C677T, MTHFR A1298C, and TS 5'-UTR PCR products. (**A**) Electrophoresis of the MTHFR C677T PCR products. M: 50 bp Marker; NC: blank control; 1–5: case group; 6–10: control group; (**B**) Electrophoresis of the MTHFR A1298C PCR products. M: 50 bp Marker; NC: blank control; 1–6: case group; 7–12: control group; (**C**) Electrophoresis of the TS 5'-UTR PCR products. The homozygous 2R/2R genotype was 223 bp; the heterozygous 2R/3R genotype had 3 bands, the first 223 bp, the second between 223 and 251 bp, and the third about 251 bp; and the homozygous 3R/3R genotype was about 251 bp. M: 50 bp Marker; NC: blank control; 1–9: case group, among which 1 and 2 were 2R/2R genotypes, 3–5 were 2R/3R genotypes, and 6–9 were 3R/3R genotypes; 10–20: control group, among which 10–12 were 2R/2R genotypes, 13 was a 3R/4R genotype, 14–17 were 2R/3R genotypes, and 18–20 were 3R/3R genotypes.

Distribution of MTHFR C677T, MTHFR A1298C, and TS genotypes and Hardy-Weinberg equilibrium in the case and control groups

We used the chi-square goodness of fit test for the Hardy-Weinberg equilibrium test in the case and control groups (Tables 3–5) to evaluate whether the subjects in the 2 groups had genetic equilibrium. The results showed that the subjects in both the case and control groups had Hardy-Weinberg equilibrium (df=2, $P>0.05$), suggesting that the subjects in these 2 groups were from a large population and had no distinct natural selection or migration effects on genetic equilibrium.

Associations between the polymorphisms and ALL susceptibility in children

For the MTHFR 677 polymorphism, CC, CT, and TT genotype frequencies were 44.30%, 45.57%, and 10.13% in the case group, and 44.12%, 44.12%, and 11.76% in the control group, respectively; the difference between these 2 groups was not statistically significant ($\chi^2=0.129$, $P=0.937$), suggesting that the genotype distribution was not significantly different between the case and control groups. The T allele frequency was 32.91% and 33.82% in the case and control groups, respectively; although the T allele frequency was lower in the case group than

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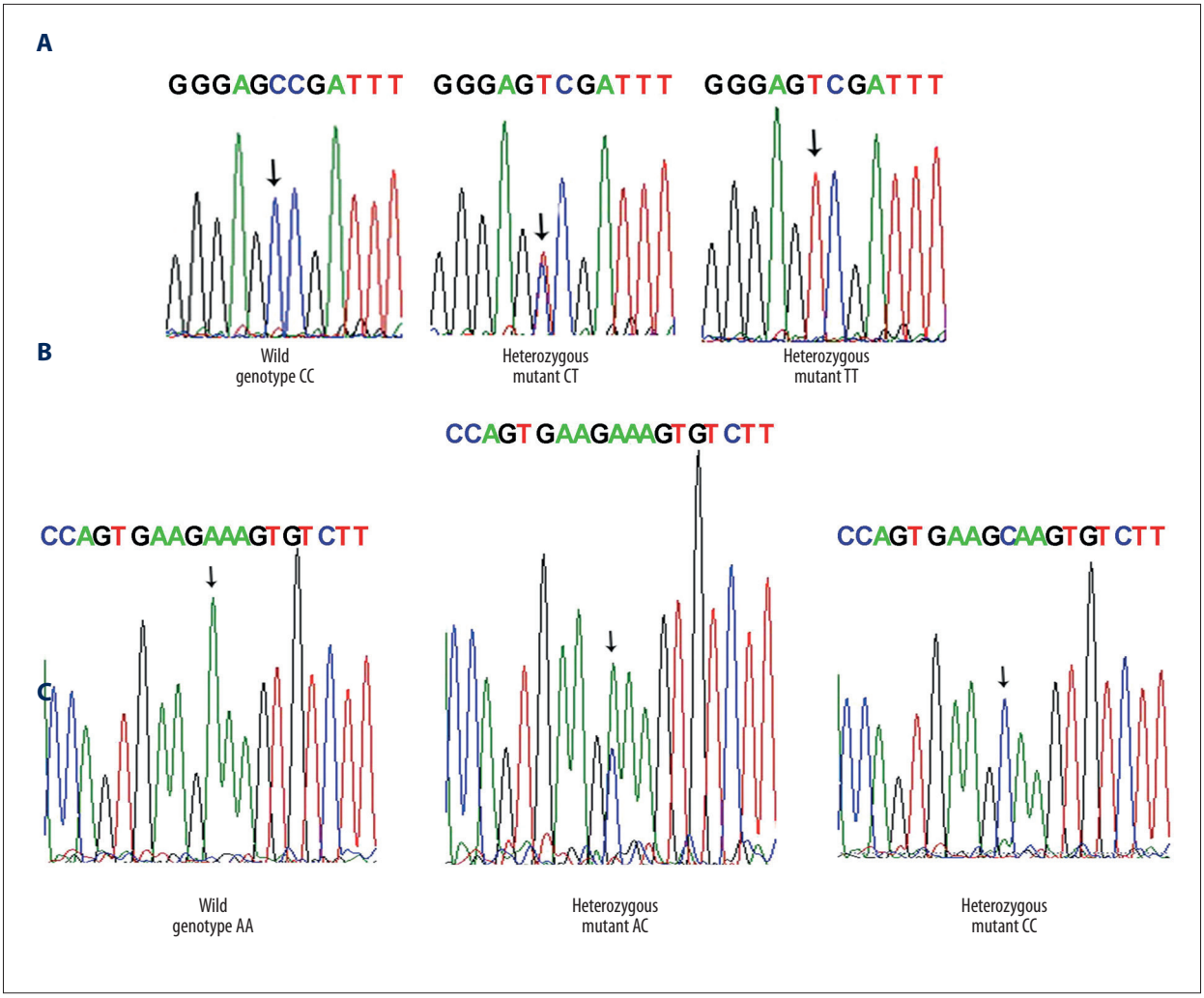

Figure 2. Sequencing of MTHFR C677T (Ala 222 Val) genotypes and MTHFR A1298C (Glu 429 Ala) genotypes. (**A**) MTHFR C677T (Ala 222 Val) genotypes. From left to right, the genotypes are CC, CT, and TT, respectively. Arrows show the mutation sites. (**B**) MTHFR A1298C (Glu 429 Ala) genotypes. From the left to right, the genotypes are AA, AC, and CC, respectively. Arrows show the mutation sites.

Table 2. Tandem repeat polymorphisms in the 5'-UTR of the TS gene.

Genotype	Base sequence
2R	CCGCGCCACTTGGCCTGCCTCCGTCCCG CCGCGCCACTTCGCCTGCCTCCGTCCCCCGCCCG
3Rg	CCGCGCCACTTGGCCTGCCTCCGTCCCG CCGCGCCACTT**G**GCCTGCCTCCGTCCCG CCGCGCCACTTCGCCTGCCTCCGTCCCCCGCCCG
3Rc	CCGCGCCACTTGGCCTGCCTCCGTCCCG CCGCGCCACTTCGCCTGCCTCCGTCCCG CCGCGCCACTT**C**GCCTGCCTCCGTCCCCCGCCCG
4R	CCGCGCCACTTGGCCTGCCTCCGTCCCG CCGCGCCACTTGGCCTGCCTCCGTCCCG CCGCGCCACTTGGCCTGCCTCCGTCCCG CCGCGCCACTTCGCCTGCCTCCGTCCCCCGCCCG

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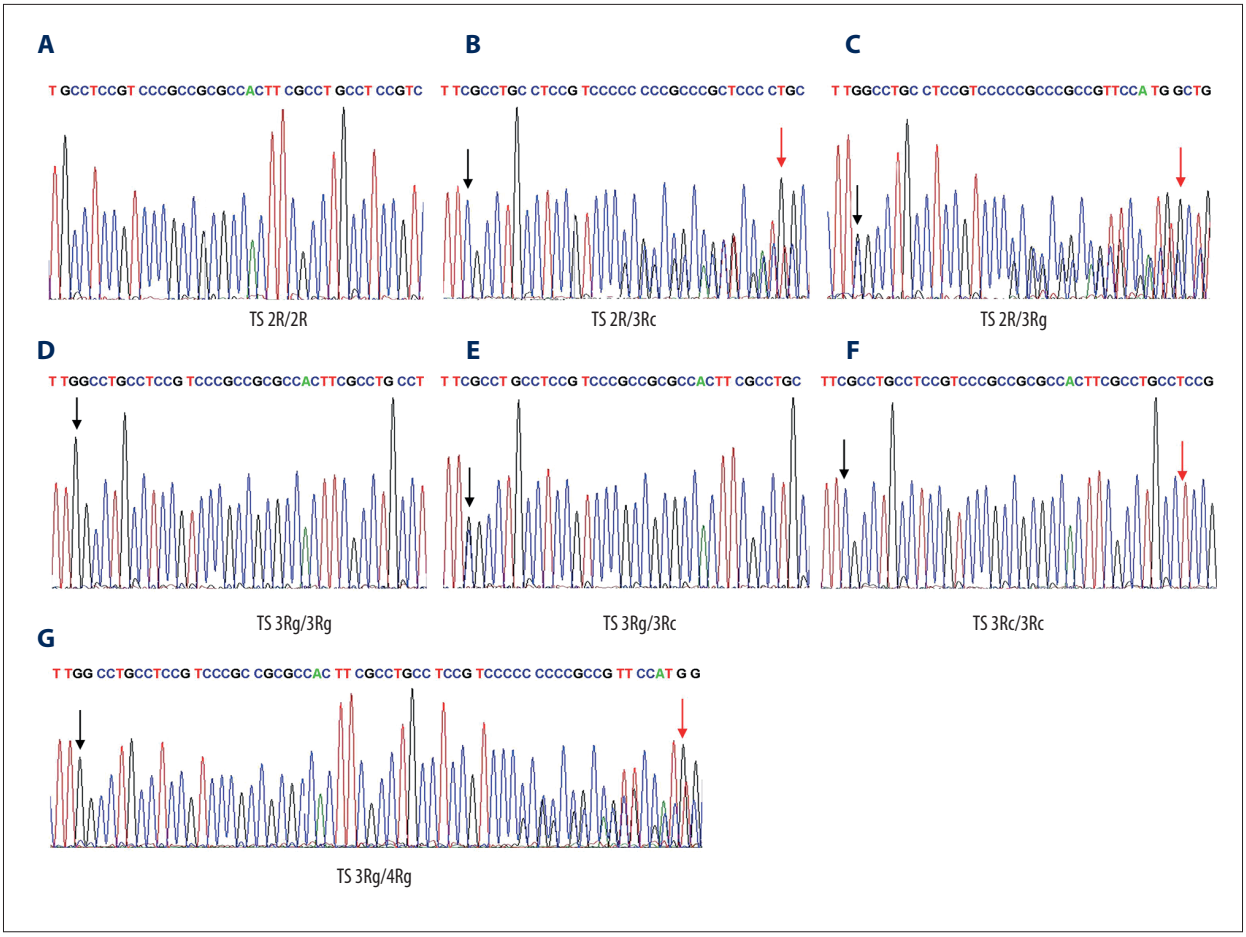

Figure 3. Genotype sequencing for TS 2R/2R, TS 2R/3Rc, TS 2R/3Rg, TS 3Rg/3Rg, TS 3Rg/3Rc, TS 3Rc/3Rc, and TS 3Rg/4R. (**A**) TS 2R/2R genotype sequencing; (**B**) TS 2R/3Rc genotype sequencing. The black arrow shows the G-C mutation site in the second repetition of the 3R polymorphism, which was a 2R/3Rc genotype. The peaks under the red arrow were caused by the different DNA duplex repetitions. (**C**) TS 2R/3Rg genotype sequencing. The black arrow shows the G-C mutation site in the second repeat of the 3R polymorphism, which was a 2R/3Rg genotype. The peaks under the red arrow were caused by the different DNA duplex repetitions. (**D**) TS 3Rg/3Rg genotype sequencing. The arrow shows the G-C mutation site in the second repetition of the 3R polymorphism, which was the 3Rg/3Rg genotype. (**E**) TS 3Rg/3Rc genotype sequencing. The arrow shows the G-C mutation site in the second repetition of the 3R polymorphism, which was the 3Rg/3Rc genotype. (**F**) TS 3Rc/3Rc genotype sequencing. The arrow shows the G-C mutation site in the second repetition of the 3R polymorphism, which was the 3Rc/3Rc genotype. (**G**) TS 3Rg/4R genotype sequencing. The arrow shows the G-C mutation site in the second repetition of the 3R polymorphism, which was the 3Rg/4R genotype. The peaks under the red arrow were caused by the different DNA duplex repetitions.

in the control group, the difference was not statistically significant (χ^2=0.033, *P*=0.855) (Tables 6, 7).

For the MTHFR1298 polymorphism, the AA, AC, and CC genotype frequencies were 59.49%, 39.24%, and 1.27% in the case group, and 59.80%, 38.24%, and 1.96% in the control group, respectively; the difference between these 2 groups was not statistically significant (χ^2=0.142, *P*=0.931), suggesting that the genotype distribution was not significantly different between the case and control groups. The C allele frequency was 20.89% and 21.08% in the case and control groups, respectively; although the C allele frequency was higher in the case group than in the control group, the difference was not statistically significant (χ^2=0.002, *P*=0.964), suggesting that the MTHFR A1298C polymorphism genotypes were not statistically associated with ALL development risk (Tables 8, 9).

The TS 2R/2R, 2R/3R, and 3R/3R genotype frequencies were 3.80%, 17.72%, and 78.48% in the case group, and 8.82%, 30.39%, and 60.79% in the control group, respectively; the difference between these 2 groups was statistically significant (χ^2=6.606, *P*=0.033), suggesting that the genotype distribution was significantly different between the case and control groups. The frequency of 3R repetitions was 87.34% and

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Table 3. MTHFR C677T genotype distribution and the Hardy-Weinberg equilibrium.

Group	CC		CT		TT		χ^2	*P*
	Observed value	Expected value	Observed value	Expected value	Observed value	Expected value		
Case group	35	35.6	36	34.9	8	8.6	0.081	0.777
Control group	45	44.7	45	45.7	12	11.7	0.021	0.884

Table 4. MTHFR A1298C genotype distribution and the Hardy-Weinberg equilibrium.

Group	AA		AC		CC		χ^2	*P*
	Observed value	Expected value	Observed value	Expected value	Observed value	Expected value		
Case group	47	49.4	31	26.1	1	3.4	2.774	0.096
Control group	61	63.5	39	33.9	2	4.5	2.271	0.132

Table 5. TS5'-UTR genotype distribution and the Hardy-Weinberg equilibrium.

Group	2R/2R		2R/3R		3R/3R+3R/4R		χ^2	*P*
	Observed value	Expected value	Observed value	Expected value	Observed value	Expected value		
Case group	3	1.3	14	17.5	62	60.3	3.114	0.078
Control group	9	5.9	31	37.2	62	58.9	2.857	0.091

Table 6. Comparison of the MTHFR C677T polymorphism genotype distribution.

Group	n	Frequency of the genotypes		
		CC (%)	CT (%)	TT (%)
Case group	79	35 (44.30)	36 (45.57)	8 (10.13)
Control group	102	45 (44.12)	45 (44.12)	12 (11.76)
Total	181	80 (44.20)	81 (44.75)	20 (11.05)

Comparison between the case and control groups: χ^2=0.129, *P*=0.937.

Table 7. Comparison of the MTHFR C677T polymorphism C and T allele distribution.

Group	n	Frequency of the alleles	
		C (%)	T (%)
Case group	79	106 (67.09)	52 (32.91)
Control group	102	135 (66.18)	69 (33.82)

Comparison between the case and control groups: χ^2=0.033, *P*=0.855.

75.98% in the case and control groups, respectively, which was significantly higher in the case group than in the control group (χ^2=7.450, *P*=0.006), suggesting that the frequency of 3R repetitions was distributed differently between the 2 groups, and that 3R repetitions were risk factors that could increase ALL susceptibility (Tables 10, 11).

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Table 8. Comparison of the MTHFR A1298C polymorphism genotype distribution.

Group	n	Frequency of the genotypes		
		AA (%)	AC (%)	CC (%)
Case group	79	47 (59.49)	31 (39.24)	1 (1.27)
Control group	102	61 (59.80)	39 (38.24)	2 (1.96)
Total	181	108 (59.67)	70 (38.67)	3 (1.66)

Comparison between the case and control groups: χ^2=0.142, *P*=0.931.

Table 9. Comparison of the MTHFR A1298C polymorphism A and C allele distribution.

Group	n	Frequency of the alleles	
		A (%)	C (%)
Case group	79	125 (79.11)	33 (20.89)
Control group	102	161 (78.92)	43 (21.08)

Comparison between the case and control groups: χ^2=0.002, *P*=0.964

Table 10. Comparison of the TS polymorphism genotype distribution.

Group	n	Frequency of the genotypes		
		2R/2R (%)	2R/3R (%)	3R/3R+3R/4R (%)
Case group	79	3 (3.80)	14 (17.72)	62 (78.48)
Control group	102	9 (8.82)	31 (30.39)	62 (60.79)
Total	181	12 (6.63)	45 (24.86)	124 (68.51)

Comparison between the case and control groups: χ^2=6.606, *P*=0.033.

Table 11. Comparison of the TS polymorphism 2R and 3R frequencies.

Group	n	Frequency of the alleles	
		2R (%)	3R+4R (%)
Case group	79	20 (12.66)	138 (87.34)
Control group	102	49 (24.02)	155 (75.98)

Comparison between the case and control groups: χ^2=7.45, *P*=0.006.

We used logistic regression analysis to evaluate the associations between 5 potential risk factors (sex, age, MTHFR C677T, MTHFR A1298C, and TS) and ALL susceptibility. We included 2 factors – age, sex, and TS – in the regression model (Table 12). The results showed that compared with females, males had a 1.9-fold lower risk of developing ALL, and the difference was statistically significant (χ^2=4.344, *P*=0.037), suggesting that being a male is a protective factor (*OR*<1). Additionally, the TS polymorphism could increase ALL risk in children. Compared to carrying the TS 2R/2R genotype, those carrying the TS 2R/3R genotype had a 2-fold higher risk of developing ALL, while those carrying TS 3R/3R+3R/3R genotypes had a 4-fold higher risk of developing ALL, and the difference was statistically significant (χ^2=5.959, *P*=0.015). However, we found no significant association between the MTHFR C677T or MTHFR A1298C polymorphisms and ALL susceptibility.

To further clarify the association between the TS gene polymorphism and ALL susceptibility, we used logistic regression to analyze the association between each TS genotype and ALL susceptibility, and the results showed that compared to carrying the TS 3R/3R+3R/4R genotype, patients carrying the 2R/2R+2R/3R genotype had a 2.4-fold lower risk of developing ALL, and the difference was statistically significant (χ^2=6.307, *P*=0.012) (Table 13).

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Table 12. Associations between the 5 included potential factors and ALL susceptibility in children.

Factor	χ^2	OR (95%CI)		P
Age	0.060	0.932	(0.531–1.636)	0.806
Sex	4.344	1.907	(1.039–3.499)	0.037
MTHFR C677T	0.141	0.916	(0.579–1.449)	0.708
MTHFR A1298C	0.005	0.979	(0.531–1.805)	0.947
TS	5.959	1.959	(1.142–3.362)	0.015

Table 13. Association between the TS gene polymorphism and ALL susceptibility in children susceptibility.

Genotype	Case group	Control group	χ^2	OR (95% CI)		P
2R/2R+2R/3R	17	40	6.307	2.353	(1.207–4.588)	0.012
3R/3R+3R/4R	62	62				
CT/TT+3R/3R	36	34	5.381	2.21	(1.13–4.33)	0.020
Non (CT/TT+3R/3R)	43	68				
AC/CC+3R/3R	24	25	5.059	2.41	(1.12–5.19)	0.024
Non (AC/CC+3R/3R)	55	77				

Discussion

Acute lymphocytic leukemia (ALL) is one of the most common malignancies in children, and the incidence rate has increased during recent years. Although great improvements have been achieved in ALL treatment methods and basic investigations, its etiologies are still unclear. Previous studies have shown that abnormal expressions of oncogenes and tumor suppressor genes play important roles in the mechanisms involved in tumor development.

In the present study, we found that the MTHFR C677T polymorphism TT genotype frequency was 10.13% and 11.76% in the case and control groups, respectively. Although the frequency was lower in the case group than in the control group, the difference was not statistically significant (P = 0.937), which was in accordance with findings by Yang et al. [12]. The frequency of CC genotype of MTHFR A1298C polymorphism was 1.27% and 1.96% in the case and control groups, respectively; the frequency was lower in the case group than in the control group, but the difference was not statistically significant (P=0.855), which was in accordance with the findings of Yang et al. [12]. By contrast, the TS polymorphism 3R/3R genotype frequency was 78.48% and 60.79% in the case and control groups, respectively. This was slightly higher in the study performed by Hu Q et al. [13], in which study patients with non-small cell lung cancers were included. The 3R/3R genotype frequency was significantly higher in the case group than in the control group in the present study (P=0.033). Males had a 1.9-fold lower risk of developing ALL than females, and the difference was statistically significant, suggesting being male was a protective factor.

No association between MTHFR C677T and MTHFR A1298C polymorphisms and ALL susceptibility was found in the present study, which was in accordance with the findings of Amigou [1] and Semsei et al. [2]. However, several other studies [3] showed that carrying the MTHFR 677CT genotype increased ALL risk in children from Brazil. Some other studies [14] showed that patients carrying MTHFR 677TT genotype had higher overall survival rates than those carrying 677CC/CT genotypes, and those carrying the MHTFR 1298CC genotype had higher overall survival rates than those carrying 1298AA/AC genotypes. In addition, Rafaela Maria Seabra Silva et al. [3] found that carrying the MTHFR1298 C allele reduced ALL risk, while carrying the 677 CT genotype increased ALL susceptibility. Some other studies [15–17] found that the MTHFR gene polymorphism was a protective factor against ALL development, and carrying MTHFR gene polymorphisms could reduce the risk of developing ALL. However, a meta-analysis [18] showed that carrying the MTHFR 677TT genotype increased ALL risk in adults but not children.

Findings of the present study show that compared with patients carrying the TS 2R/2R genotype, those carrying the 2R/3R genotype had a 2-fold higher risk of developing ALL, while those carrying 3R/3R+3R/4R genotypes had a 4-fold higher risk of developing ALL, and the differences were statistically significant.

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However, compared with patients carrying TS 3R/3R+3R/4R genotypes, those carrying 2R/2R+2R/3R genotypes had a 2.4-fold lower risk of developing ALL, and the difference was statistically significant, suggesting that the TS 3R genotype is an ALL risk factor. In accordance with our findings, De-Jonge et al. [15] also showed that the TS 2R genotype was a protective factor against ALL. However, another study, by Skibola et al. [19], found that patients carrying TS 2R/3R had a 2.8-fold lower risk of developing ALL, while those carrying the 3R/3R genotype had a 4.0-fold lower risk. Hishida et al. [20] found that carrying the TS 2R genotype increased the risk of developing lymphoma by 1.6-fold. Canalle et al. [6] found that carrying the TS 3R genotype significantly decreased ALL risk, suggesting it could be a protective factor for children.

Although this study produced some significant findings, there were also a few limitations. This was only a preliminary study exploring the associations between MTHFR or TS genetic polymorphisms and susceptibility to ALL in children. We have not emphasized the comparison between the genetic polymorphisms for ALL and the established prognostic factors (e.g., cytogenetics, flowcytometry, and morphology). Therefore, the clinical use of genetic polymorphisms will probably take a considerable length of time to become a reality. In further studies, we intend to investigate genetic polymorphisms as prognostic and risk factor for ALL, and to compare these with the established prognostic factors. We believe that these genetic polymorphisms may be clinically valuable prognostic factors for ALL and could have significant practical value.

Conclusions

Carrying MTHFR and TS genotypes can predict ALL risk in children. However, other factors, including other folate metabolism-related genes and folate levels in the body, should also be considered to further clarify this prediction, and further studies with larger sample sizes are needed to validate our findings.

Conflict of interests

None.

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