Cellular and Molecular Biology

Bahari *et al. Cell. Mol. Biol.*2016, 62 (2): 45-51
ISSN: 1165-158X
doi: 10.14715/cmb/2016.62.2.8

Original Research

Association of SHMT1 gene polymorphisms with the risk of childhood acute lymphoblastic leukemia in a sample of Iranian population

G. Bahari[1,2], M. Hashemi[1,2*], M. Naderi[3], S. Sadeghi-Bojd[3], M. Taheri[4]

[1] Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan, Iran
[2] Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
[3] Department of Pediatrics, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
[4] Genetics of Non Communicable Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

Abstract: The enzymes serine hydroxymethyltransferase 1 (SHMT1) regulate key reaction in folate-mediated one-carbon metabolism. In the current study we aimed to examine the possible association between *SHMT1* gene polymorphisms and childhood acute lymphoblastic leukemia (ALL) in a sample of Iranian population. The rs9901160, rs2273027, rs9909104, rs1979277, and rs11868708 gene polymorphisms of *SHMT1* were genotyped in 120 children diagnosed with ALL and 120 healthy children by the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). The results showed that rs9901160, rs2273027 as well as rs1979277 polymorphism significantly increased the risk of childhood ALL (P<0.05). While, rs9909104 polymorphism significantly decreased the ALL risk (P<0.05). The rs11868708 variant was not associated with risk/protection of childhood ALL (P>0.05). In conclusion, our results suggest that the polymorphisms of *SHMT1* gene are associated with childhood ALL risk in a sample of Iranian population. Further studies with larger sample sizes and different ethnicities are necessary to verify our findings.

Key words: SHMT1, polymorphism, acute lymphocytic leukemia.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy diagnosed in children, accounting for about 25% of cancers in children younger than 15 years of age, and the peak onset arises at 2–5 years of age worldwide (1-3). Although the etiology of ALL is generally unidentified, it is known that genetic and environmental factors play a critical role in disease development (4-6).

The *de novo* pathway of thymidylate biosynthesis requires three enzymes: thymidylate synthase (TYMS), dihydrofolate reductase (DHFR), and SHMT1. *SHMT1*, located on short arm of chromosome 17 (17p11.2), encodes a vitamin B6-dependent enzyme that catalyzes the reversible transfer of the hydroxymethyl group of serine to tetrahydrofolate to form 5,10-methylene tetrahydrofolate and glycine and the irreversible conversion of 5,10-methylene tetrahydrofolate to 5-formyl tetrahydrofolate (7, 8). Formation of 5-formyl tetrahydrofolate helps in maintaining one-carbon homeostasis during the rapidly proliferative stages of development (8, 9). Guerreiro et al (10) showed that *SHMT1* rs1979277 (C1420T) variant increased the risk of colorectal cancer (CRC). However, Hishida et al (11) found that this variant significantly reduced the risk of malignant lymphoma. On the other hand, no significant association was found between *SHMT1* C1420T polymorphism and breast cancer (BC) risk (12). Recently, a meta-analysis performed by Zhao et al (13) showed that rs1979277 (C1420T) variant was associated with decreased risk of BC among Asian populations, but not in Caucasian groups. A meta-analysis achieved by Zhong et al (14) suggest that *SHMT1* C1420T polymorphism is not associated with overall cancer development, but might decrease cancer susceptibility of Asians as well as reduce leukemia risk. Skibola et al (15) reported that Polymorphisms within *SHMT1* in combination with polymorphisms within *TYS* and *MS* were associated with decreased risk of adult ALL.

To date, a number of studies investigated the impact of *SHMT1* polymorphisms on risk of childhood ALL (16-20). However, the results of these studies remain inconclusive. So, we performed case-control study to find out the impact of *SHMT1* rs9901160, rs2273027, rs9909104, rs1979277, and rs11868708 polymorphisms on ALL risk in a sample of Iranian population.

Materials and Methods

Patients

One hundred twenty children diagnosed with ALL and 120 cancer-free age and sex matched healthy children in Zahedan, southeast Iran were enrolled in this case control study.

The study design and enrolment procedure have been defined previously (21-24). Local ethics committee of Zahedan University of Medical sciences approved the project, and informed consent was obtained from parents of cases and controls. Genomic DNA was extracted from peripheral whole blood by salting out method (25).

Received December 22, 2015; **Accepted** February 26, 2016; **Published** February 29, 2016

*** Corresponding author:** Mohammad Hashemi, PhD, Dept. of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran. Email: mhd.hashemi@gmail.com; hashemim@zdmu.ac.ir

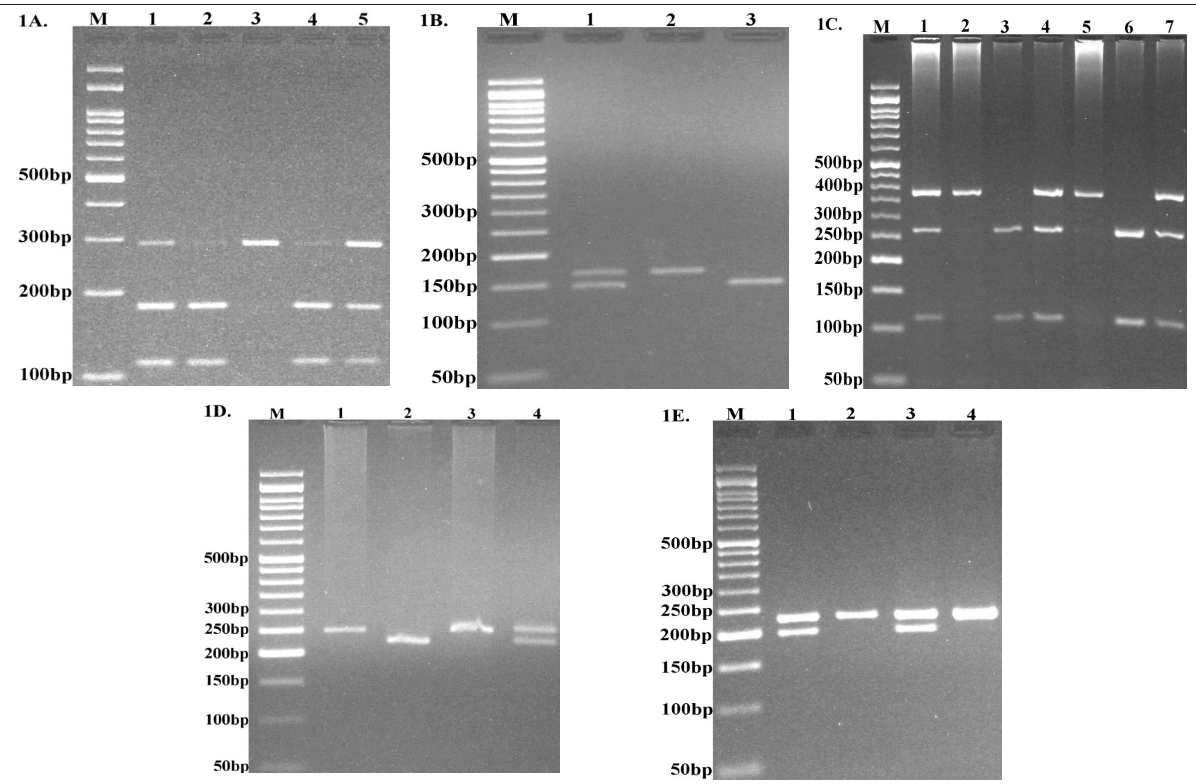

Figure 1. Electrophoresis pattern of polymerase chain reaction restriction fragments length polymorphism (PCR-RFLP) for detection of *SHMT1* rs1979277 (1A), rs2273027 (1B), rs9901160 (1C), rs9909104 (1D) and rs11868708 (1E) polymorphisms. *Figure 1A.* M: DNA marker; Lanes 1 and 5: rs1979277 TC; Lanes 2 and 4: rs1979277 CC; Lane 3: rs1979277 TT.
Figure 1B. M: DNA marker; Lane 1: rs2273027 AG; Lane 2: rs2273027 AA; Lane 3: rs2273027 GG. *Figure 1C.* M: DNA marker; Lanes 1, 4, 7: rs9901160 AG; Lanes 2 and 5: rs9901160 AA; Lanes 3 and 6: GG. *Figure1D.* M: DNA Marker; Lanes 1 and 3: rs9909104 AA; Lane 2: rs9909104 GG; Lane 4: rs9909104 AG. *Figure 1E.* M: DNA Marker; Lanes 1 and 3: rs11868708 AG; Lanes 2 and 4: rs11868708 AA.

Genotyping

Genotyping of *SHMT1* rs9901160 (non-genic), rs2273027 (intronic variant), rs9909104 (intronic variant), rs1979277 (Leu474Phe), and rs11868708 (intronic variant), polymorphisms were determined by PCR-RFLP method. The primers are listed in table 1. In each 0.20 ml PCR reaction tube, 1 µl of genomic DNA (~100 ng/ml), 1 µl of each primers and 10 µl of 2X Prime Taq Premix (Genet Bio, Korea) and 7 µl ddH_2O were added. The PCR conditions for all SNPs were set as follows: 95 ˚C for 5 min, 30 cycles of 95 ˚C for 30 s, 60 ˚C for rs11868708, 61˚C rs9901160, 64˚C rs2273027, 62 ˚C rs9909104 and 62 ˚C for rs1979277, for 30 s, and 72 ˚C for 30 s and a final extension step of 72 ˚C for 5 min. Then, 10 µl of PCR product is digested by appropriate restriction enzyme (Table 1) and electrophoresed onto 2.5 % agarose gels containing 0.5 µg/ml ethidium bromide and visualized under UV light (Figure 1).

Statistical analysis

Statistical analysis was performed using statistical package, SPSS 20 software. Continuous and categorical data were analyzed by independent sample t-test and $\chi 2$ test, respectively. The association between genotypes and ALL was estimated by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses. Haplotype analysis was done using SNPStats software (26). The statistical level of significance was considered as $p< 0.05$.

Results

The study group consisted of 120 ALL patients (70 male, 50 female; age: 5.9 ± 3.8 years) and 120 healthy children (57 male, 63 female; age: 5.6 ± 2.1 years). No statistically significant differences were found between the groups regarding sex and age (p=0.121 and 0.522, respectively).

The genotype and allelic frequencies of *SHMT1* polymorphisms are shown in table 2. The results revealed that rs9901160 A>G variant increased the risk of ALL in the codominant (OR=4.07, 95% CI=2.25-7.35, p<0.0001, AG vs AA; and OR=6.64, 95% CI=2.71-16.31, p<0.0001, GG vs AA), dominant (OR=4.49, 95% CI=2.54-7.93, p<0.0001, AG+GG vs AA), and recessive (OR=2.92, 95% CI=1.29-6.62, p=0.013, GG vs AA+AG) inheritance models. In addition, the rs4132601 G allele increased the risk of ALL (OR=1.82, 95% CI=1.82–3.89; p < 0.0001) compared with T allele.

Regarding the rs2273027 A>G variant, our result proposed that this variant increased the risk of ALL in codominant (OR=6.87, 95% CI=3.59-13.16, p<0.0001, AG vs AA; and OR=7.98, 95% CI=3.51-18.12, p<0.0001, GG vs AA), dominant (OR=7.16, 95% CI=3.86-13.28, p<0.0001, AG+GG vs AA), as well as recessive (OR=2.52, 95% CI=1.26-5.05, p=0.012, GG vs AA+AG) inheritance models. The rs11980379 G allele significantly increased the risk of ALL (OR=3.16, 95% CI=2.16–4.61, p< 0.0001) in comparison with T allele.

Concerning rs9909104 A>G polymorphism, the result indicated that this polymorphism significantly decreased the risk of ALL in codominant (OR=0.56, 95% CI=0.32-0.96, p=0.035, AG vs AA) inheritance model. While, the rs9909104 G allele was not associated with the risk of ALL (OR=0.77, 95% CI=0.53–1.13,

Table 1. The primers used for detection of *SHMT1* polymorphisms using PCR-RFLP methods.

SHMT1 polymorphism	Primer sequence (5`->3`)	Restriction Enzyme	Fragment (bp)
rs9901160 A>G		TaqI	A allele, 379;
Forward	TCAGACATGGGAGTGGTCTTCA		G allele, 266+113
Reverse	TTGGGAGATGATCTTGAGAAACAG		
rs2273027 A>G		FspBI	A allele, 164;
Forward	GCAGTGGCAACTGCTTGGACTA		G allele, 143+21
Reverse	CTGTAGCTACTGCATCCTTGTGCT		
rs9909104 A>G		TaaI	A allele, 251;
Forward	CCCATCTGCAGTTTATTATAAATGACT		G allele, 225+26
Reverse	TAGTGAGATCTTGTTCTCCACAAAA		
rs1979277 T>C		EarI	T allele 292, C allele
Forward	GTGTGGGGTGACTCATTTGTG		180+112
Reverse	GGAGCAGCTCATCCATCTCTC		
rs11868708 A>G		AciI	A allele, 230;
Forward	GTGACCTTGCTAAATTTGCTTATTCTAGC		G allele, 202+28
Reverse	ACGGGACCACACAATGGAAAC		

Table 2. Genotypic and allelic frequencies of *SHMT1* variants in ALL patients and cancer-free subjects.

SHMT1 polymorphism	Case n (%)	Control n (%)	OR (95%CI)	p
rs9901160 A>G				
Codominant				
AA	25 (20.8)	65 (54.2)	1.00	-
AG	72 (60.0)	46 (38.3)	4.07 (2.25-7.35)	<0.0001
GG	23 (19.2)	9 (7.5)	6.64 (2.71-16.31)	<0.0001
Dominant				
AA	25 (20.8)	65 (54.2)	1.00	
AG+GG	95 (79.2)	55 (45.8)	4.49 (2.54-7.93)	<0.0001
Recessive				
AA+AG	97 (80.8)	111(92.5)	1.00	
GG	23 (19.2)	9 (7.5)	2.92 (1.29-6.62)	0.013
Allele				
A	122 (50.8)	176 (73.3)	1.00	
G	118 (49.2)	64 (26.7)	2.66 (1.82-3.89)	<0.0001
rs2273027 A>G				
Codominant				
AA	18 (15.0)	67 (55.8)	1.00	
AG	72 (60.0)	39 (32.5)	6.87 (3.59-13.16)	<0.0001
GG	30 (25.0)	14 (11.7)	7.98 (3.51-18.12)	<0.0001
Dominant				
AA	18 (15.0)	67 (55.8)	1.00	
AG+GG	102 (85.0)	53 (44.2)	7.16 (3.86-13.28)	<0.0001
Recessive				
AA+AG	90 (75.0)	106 (88.3)	1.00	
GG	30 (25.0)	14 (11.7)	2.52 (1.26-5.05)	0.012
Allele				
A	108 (45.0)	173 (72.1)	1.00	
G	132 (55.0)	67 (27.9)	3.16 (2.16-4.61)	<0.0001
rs9909104 A>G				
Codominant				
AA	52 (43.3)	37 (30.8)	1.00	-
AG	58 (48.3)	74 (61.7)	0.56 (0.32-0.96)	0.035
GG	10 (8.4)	9 (7.5)	0.79 (0.29-2.14)	0.643
Dominant				
AA	52 (43.3)	37 (30.8)	1.00	-
AG+GG	68 (56.7)	83 (69.2)	0.58 (0.34-1.00)	0.061
Recessive				
AA+AG	110 (91.7)	111(92.5)	1.00	-
GG	10 (8.3)	9 (7.5)	1.09 (0.42-2.80)	0.860
Allele				
A	162 (67.5)	148 (61.7)	1.00	-
G	78 (32.5)	92 (38.3)	0.77 (0.53-1.13)	0.215
rs1979277 T>C				
Codominant				
TT	17 (14.2)	63 (52.5)	1.00	-
TC	94 (78.3)	54 (45.0)	6.45 (3.43-12.13)	<0.0001
CC	9 (7.5)	3 (2.5)	11.12 (2.71-45.64)	<0.0001
Dominant				
TT	17 (14.2)	63 (52.5)	1.00	
TC+CC	103 (85.8)	57 (47.5)	6.70 (3.58-12.52)	<0.0001
Recessive				
TT+TC	111(92.5)	117 (97.5)	1.00	
CC	9 (7.5)	3 (2.5)	3.29 (0.85-12.69)	0.135
Allele				
T	128 (53.3)	180 (75.0)	1.00	
C	112 (46.7)	60 (25.0)	2.62 (1.78-3.86)	<0.0001
rs11868708 A>G				
AA	75 (62.5)	78 (65.0)	1.00	
AG	45 (37.5)	42 (35.0)	1.11(0.66-1.89)	0.687
GG	0.0 (0.0)	0.0 (0.0)	-	-
Allele				
A	195 (81.3)	198 (82.5)	1.00	
G	45 (18.7)	42 (17.5)	1.09 (0.68-1.73)	0.813

p=0.215) compared with A allele.

We found that the rs1979277 T>C polymorphism significantly increased the risk of ALL in codominant (OR=6.54, 95% CI=3.43-12.13, p<0.0001, TC vs TT; and OR=11.12, 95% CI=2.71-45.64, p<0.0001, CC vs TT), and dominant (OR=6.70, 95% CI=3.58-12.52, p<0.0001, TC+CC vs TT) inheritance models. The rs1979277 C allele significantly increased the risk of ALL (OR=2.62, 95% CI=1.78-3.86, p<0.0001) compared with T allele.

With respect to rs11868708 polymorphism, our result failed to find any significant difference in genotype and allelic distribution between ALL and controls (P>0.05).

As shown in table 3, haplotypes analysis suggested that haplotypes GAATA, GGACA, GGATA, AAACA, AGACA, GGGCG, as well as AAGCG significantly increased the risk of ALL (P<0.05) in comparison with AAATA (rs9901160A/rs2273027A/ rs9909104A/ rs1979277T/rs11868708A).

The association of *SHMT1* polymorphisms with patients' clinical characteristics is presented in table 4. The findings showed that rs9901160 A>G variant was significantly associated with WBC count only. Regarding rs2273027 A>G polymorphism, the results showed that this variant is significantly associated with Lymphadenopathy. The rs9909104 A>G variant was associated with platelet count and the rs1979277 T>C variant was associated with WBC and platelet counts. We found no significant association between rs11868708 variant and clinicopathological characteristics.

Discussion

Acute lymphoblastic leukemia (ALL) is a multifactorial disease influenced by genetic and environmental factors. *SHMT* gene also plays a pivotal role in folate metabolism pathway. It encodes a vitamin B6 dependent enzyme that catalyzes the reversible conversion of tetrahydrofolate to 5,10-methylene tetrahydrofolate and serine to glycine, providing one-carbon units for purine and pyrimidine synthesis. Two different isoforms of SHMT protein are known: one is present in the cytosol (cSHMT or SHMT1) and other in the mitochondrion (mSHMT or SHMT2) (8, 11). Variant of the *SHMT1* gene with C1420T polymorphism has been known to encode enzyme with the amino acid substitution of leucine to phenylalanine and the functional significance is still unknown (7, 10). Considering the important functional role of *SHMT1* in the production of methyl group for multiple metabolic pathways, polymorphism in this gene could mimic a folate deficiency, making this an ideal gene candidate to study of the cancer (15).

In the current study we evaluated the impact of *SHMT1* polymorphisms on childhood ALL risk in a sample of Iranian population. Our findings showed that rs9901160, rs2273027 as well as rs1979277 polymorphism significantly increased the risk of childhood ALL (P<0.05). Whereas, rs9909104 variant significantly decreased the ALL risk (P<0.05) and the rs11868708 polymorphism was not associated with risk/protection of childhood ALL (P>0.05).

In contrast to our findings, Lightfoot et al (16) have found no association between *SHMT1* rs1979277 (1420 C>T) and risk of childhood leukemia.

SHMT1 1420 C>T (Leu474Phe), which reduces circulating folate levels, thus shunting 5,10-MeTHF toward DNA synthesis (27).

Lautner-Csorba et al (17) investigated rs1979277, rs643333 and rs9909104 polymorphisms of *SHMT1* gene in children with ALL and controls. Their findings proposed that the rs9909104 variant was weakly associated with a reduced risk to ALL and the TC genotype was associated with a lower survival rate compared to TT genotype (80.2% vs. 88.8%; p=0.01).

In a population-based case–control study, Metayer et al (18) evaluated rs11868708, rs9909104, rs2273027 and rs9901160 polymorphisms of *SHMT1* in childhood ALL and controls. No single polymorphism or haplotype in the *SHMT1* gene was associated with childhood ALL risk in their study. Gast et al (19), de Jonge et al (20), Yang et al (28) and Lightfoot et al (16) have found no significant association between *SHMT1* rs1979277 variant and risk of childhood ALL. A meta-analysis performed by Vijayakrishnan et al (29) showed that TC genotype of rs1979277 variant decreased the risk of pediatric ALL compared to CC genotype. This finding is agreement with our results. We showed that CT and CC genotypes significantly increased the risk of ALL compared to TT genotype. Furthermore, the C allele increased the risk of ALL compared to T allele. Skibola et al (15) reported that CT as well as TT geno-

Table 3. Haplotype frequencies of SHMT1 polymorphisms in childhood ALL and control subjects.

rs 9901160	rs 2273027	rs 9909104	rs 1979277	rs 11868708	ALL (frequency)	Control (frequency)	OR (95%CI)	p
A	A	A	T	A	0.0595	0.2152	1.00	-
G	A	A	T	A	0.155	0.101	14.42 (2.49-83.38)	0.003
G	G	A	C	A	0.1008	0.0243	155.30 (19.47-1238.75)	<0.0001
A	A	G	T	A	0.0469	0.1558	2.03 (0.42-9.97)	0.380
A	G	A	T	A	0.0662	0.0708	4.90 (0.78-3065)	0.091
A	A	G	T	G	0.0331	0.0695	1.28 (0.21-7.72)	0.792
G	G	A	T	A	0.1301	0.0443	20.80 (3.18-136.21)	0.002
A	A	A	C	A	0.0476	0.0713	17.16 (1.61-183.14)	0.021
A	G	A	C	A	0.0751	0.0525	14.26 (1.31-154.78)	0.031
G	G	G	C	G	0.009	0.0167	281.85 (4.83-16443.98)	0.007
A	A	G	C	G	0.0281	0.0278	34.17 (2.36-495.25)	0.010
A	G	G	T	A	NA	0.0455	0.33 (0.01-19.71)	0.591
G	A	G	T	A	0.0337	0.007	5.48 (0.48-62.48)	0.174
G	A	A	C	A	0.0202	0.0209	1.22 (0.04-1541.80)	0.913

Table 4. Association of *SHMT1* gene polymorphisms with demographic and clinical features of patients.

Factors	rs9901160		p	rs2273027		p	rs9909104		p	rs1979277		p	rs11868708		p
	AA	AG+GG		AA	AG+GG		AA	AG+GG		TT	TC+CC		AA	AG+GG	
Sex			0.790			0.795			0.213			0.269			0.987
Male	14	56		11	59		27	43		12	58		44	26	
Female	11	39		7	43		25	25		5	45		31	19	
Age at diagnosis (Year)	5.9±3.9	5.9±3.8	0.363	5.2±3.6	6.0±3.8	0.994	5.6±3.5	6.2±4.0	0.234	5.6±3.4	5.9±3.8	0.585	6.1±4.1	5.6±3.4	0.528
WBC ($\times 10^6$/mL)	26.1±30.8	39.9±53.9	**0.044**	35.0±40.2	37.4±51.9	0.436	37.5±44.8	36.7±54.2	0.659	54.3±65.8	34.2±46.9	0.006	40±51.4	32.1±48.2	0.408
Hemoglobin (g/dL)	6.8±2.4	8.1±7.7	0.640	6.8±2.6	8.1±7.5	0.904	7.4± 2. 1	8.2±9.1	0.302	7.4±2.3	7.9±7.5	0.718	7.3±2.1	8.7±11.1	0.286
Platelet ($\times 10^6$/mL)	66.1±64.6	53.4±44.1	0.074	70.6±48.0	53.5±49.0	0.892	51.9±37.8	59.3±56.2	**0.037**	41.5±23.8	58.5±51.7	0.005	52.7±49.1	61.8±48.9	0.327
Organomegally			0.708			0.061			0.177			0.541			0.363
Positive	23	85		14	94		49	59		16	92		69	39	
Negative	2	10		4	8		3	9		1	11		6	6	
Lymphadenopathy			0.885			**0.035**			0.946			.088			0.985
Positive	18	67		9	76		37	48		15	70		53	32	
Negative	7	28		9	26		15	20		2	33		22	13	
Cerebrospinal fluid involvement			0.337			0.734			0.184			0.471			0.481
Positive	3	6		1	8		2	7		2	7		7	2	
Negative	22	89		17	94		50	61		15	96		68	43	

type decreased the risk of adult ALL compared to CC genotype. Lightfoot et al (30) have found no significant association between *SHMT1* rs1979277 variant and risk of non-Hodgkin lymphoma.

In a meta-analysis performed by Zhao et al (13) proposed that the *SHMT1* rs1979277 (C1420T) polymorphism was associated with decreased risk of breast cancer. While, Wang et al (31) conducted a meta-analysis and found that *SHMT1* C1420T polymorphism do not have a significant association with the risk of cancer overall. Otherwise, *SHMT1* C1420T polymorphism may have a protective effect on colorectal cancer in Asian population.

Genetic variants of one-carbon metabolism can affect DNA synthesis, repair, and methylation. The basic mechanisms of carcinogenesis by the variants are uracil misincorporation into DNA causing DNA damage (32), or aberrant methylation that causes the inactivation of tumor suppressor genes and inactivation of proto-oncogenes (33).

It has been proposed that *SHMT1* rs1979277 (C1420T) polymorphism is associated with reduce availability of methyl derivates for DNA synthesis as well as remethylation of homocysteine (8, 11). According to Anderson and Stover (34) report, this variant does not affect catalytic activity, however, it results in accumulation of altered protein in the cytoplasm, where it may inhibit cellular methylation reactions by sequestering 5,10-methylenetetrahydrofolate.

The data regarding the impact of *SHMT1* on childhood ALL are inconsistent. There is no perfect reason for the inconsistent results in diverse studies. Ethnic, genetic, and/or environmental may interact in various ways to either decrease or increase the risk of childhood ALL in different areas.

In summary, our finding emphasizes the impact of *SHMT1* polymorphisms on childhood ALL risk in a sample of Iranian population. Larger sample sizes and different ethnicities are necessary to confirm these findings.

Acknowledgements
The authors wish to acknowledge deputy for research of Zahedan University of Medical Sciences for supporting dissertation grant (PhD thesis of GB #6858) from Zahedan University of Medical Sciences.

References

1. Eden, T., Pieters R., Richards S., Childhood Acute Lymphoblastic Leukaemia Collaborative G. Systematic review of the addition of vincristine plus steroid pulses in maintenance treatment for childhood acute lymphoblastic leukaemia - an individual patient data meta-analysis involving 5,659 children. *Br J Haematol*. 2010, **149**:722-33. doi: 10.1111/j.1365-2141.2010.08148.x
2. Kaatsch, P. Epidemiology of childhood cancer. *Cancer treatment reviews*. 2010, **36**:277-85. doi: 10.1016/j.ctrv.2010.02.003
3. Ribera, J. M., Oriol A. Acute lymphoblastic leukemia in adolescents and young adults. *Hematol Oncol Clin North Am*. 2009, **23**:1033-42, vi. doi: 10.1016/j.hoc.2009.07.002
4. Guo, L. M., Xi J. S., Ma Y., Shao L., Nie C. L., Wang G. J. ARID5B gene rs10821936 polymorphism is associated with childhood acute lymphoblastic leukemia: a meta-analysis based on 39,116 subjects. *Tumour Biol*. 2014, **35**:709-13. doi: 10.1007/s13277-013-1097-0
5. Ma, Y., Sui Y., Wang L., Li H. Effect of GSTM1 null genotype on risk of childhood acute leukemia: a meta-analysis. *Tumour Biol*. 2014, **35**:397-402. doi: 10.1007/s13277-013-1055-x
6. Belson, M., Kingsley B., Holmes A. Risk factors for acute leukemia in children: a review. *Environ Health Perspect*. 2007, **115**:138-45. doi:
7. Komlosi, V., Hitre E., Pap E., Adleff V., Reti A., Szekely E., et al. SHMT1 1420 and MTHFR 677 variants are associated with rectal but not colon cancer. *BMC Cancer*. 2010, **10**:525. doi: 10.1186/1471-2407-10-525
8. Niclot, S., Pruvot Q., Besson C., Savoy D., Macintyre E., Salles G., et al. Implication of the folate-methionine metabolism pathways in susceptibility to follicular lymphomas. *Blood*. 2006, **108**:278-85. doi: 10.1182/blood-2005-04-1567
9. Fu, T. F., Hunt S., Schirch V., Safo M. K., Chen B. H. Properties of human and rabbit cytosolic serine hydroxymethyltransferase are changed by single nucleotide polymorphic mutations. *Arch Biochem Biophys*. 2005, **442**:92-101. doi: 10.1016/j.abb.2005.07.018
10. Guerreiro, C. S., Carmona B., Goncalves S., Carolino E., Fidalgo P., Brito M., et al. Risk of colorectal cancer associated with the C677T polymorphism in 5,10-methylenetetrahydrofolate reductase in Portuguese patients depends on the intake of methyl-donor nutrients. *Am J Clin Nutr*. 2008, **88**:1413-8. doi:
11. Hishida, A., Matsuo K., Hamajima N., Ito H., Ogura M., Kagami Y., et al. Associations between polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and susceptibility to malignant lymphoma. *Haematologica*. 2003, **88**:159-66. doi:
12. Bentley, A. R., Raiszadeh F., Stover P. J., Hunter D. J., Hankinson S. E., Cassano P. A. No association between cSHMT genotypes and the risk of breast cancer in the Nurses' Health Study. *Eur J Clin Nutr*. 2010, **64**:108-10. doi: 10.1038/ejcn.2009.104
13. Zhao, T. T., Shen L. L., Zhang X. L., Gu D. Y., Zhang Q., Huo X. Y., et al. Clinical significance of SHMT1 rs1979277 polymorphism in Asian solid tumors: evidence from a meta-analysis. *Genet Mol Res*. 2015, **14**:5602-14. doi: 10.4238/2015.May.25.12
14. Zhong, S. L., Zhang J., Hu Q., Chen W. X., Ma T. F., Zhao J. H. C1420T polymorphism of cytosolic serine hydroxymethyltransferase and risk of cancer: a meta-analysis. *Asian Pac J Cancer Prev*. 2014, **15**:2257-62. doi:
15. Skibola, C. F., Smith M. T., Hubbard A., Shane B., Roberts A. C., Law G. R., et al. Polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and risk of adult acute lymphocytic leukemia. *Blood*. 2002, **99**:3786-91. doi:
16. Lightfoot, T. J., Johnston W. T., Painter D., Simpson J., Roman E., Skibola C. F., et al. Genetic variation in the folate metabolic pathway and risk of childhood leukemia. *Blood*. 2010, **115**:3923-9. doi: 10.1182/blood-2009-10-249722
17. Lautner-Csorba, O., Gezsi A., Erdelyi D. J., Hullam G., Antal P., Semsei A. F., et al. Roles of genetic polymorphisms in the folate pathway in childhood acute lymphoblastic leukemia evaluated by Bayesian relevance and effect size analysis. *PLoS One*. 2013, **8**:e69843. doi: 10.1371/journal.pone.0069843
18. Metayer, C., Scelo G., Chokkalingam A. P., Barcellos L. F., Aldrich M. C., Chang J. S., et al. Genetic variants in the folate pathway and risk of childhood acute lymphoblastic leukemia. *Cancer Causes Control*. 2011, **22**:1243-58. doi: 10.1007/s10552-011-9795-7
19. Gast, A., Bermejo J. L., Flohr T., Stanulla M., Burwinkel B., Schrappe M., et al. Folate metabolic gene polymorphisms and childhood acute lymphoblastic leukemia: a case-control study. *Leukemia*. 2007, **21**:320-5. doi: 10.1038/sj.leu.2404474
20. de Jonge, R., Tissing W. J., Hooijberg J. H., Jansen G., Kaspers G. J., Lindemans J., et al. Polymorphisms in folate-related genes and risk of pediatric acute lymphoblastic leukemia. *Blood*. 2009, **113**:2284-9. doi: 10.1182/blood-2008-07-165928
21. Hashemi, M., Sheybani-Nasab M., Naderi M., Roodbari F., Taheri M. Association of functional polymorphism at the miR-502-binding site in the 3' untranslated region of the SETD8 gene with risk of childhood acute lymphoblastic leukemia, a preliminary report. *Tumour Biol*. 2014, **35**:10375-9. doi: 10.1007/s13277-014-2359-1
22. Hasani, S. S., Hashemi M., Eskandari-Nasab E., Naderi M., Omrani M., Sheybani-Nasab M. A functional polymorphism in the miR-146a gene is associated with the risk of childhood acute lymphoblastic leukemia: a preliminary report. *Tumour Biol*. 2014, **35**:219-25. doi: 10.1007/s13277-013-1027-1
23. Hashemi, M., Ebrahimi M., Amininia S., Naderi M., Eskanadri-Nasab E., Taheri M. Evaluation of osteoprotegerin gene polymorphisms and the risk of childhood acute lymphocytic leukemia in Zahedan Southeast Iran. *International Journal of Hematology-Oncology and Stem Cell Research*. 2014, **8**:39-44. doi:
24. Bahari, G., Hashemi M., Naderi M., Taheri M. IKZF1 gene polymorphisms increased the risk of childhood acute lymphoblastic leukemia in an Iranian population. *Tumour Biol*. 2016, doi: 10.1007/

s13277-016-4853-0
25. Hashemi, M., Hanafi Bojd H., Eskandari Nasab E., Bahari A., Hashemzehi N. A., Shafieipour S., et al. Association of Adiponectin rs1501299 and rs266729 Gene Polymorphisms With Nonalcoholic Fatty Liver Disease. *Hepatitis monthly*. 2013, **13**:e9527. doi: 10.5812/hepatmon.9527
26. Sole, X., Guino E., Valls J., Iniesta R., Moreno V. SNPStats: a web tool for the analysis of association studies. *Bioinformatics*. 2006, **22**:1928-9. doi: 10.1093/bioinformatics/btl268
27. Heil, S. G., Van der Put N. M., Waas E. T., den Heijer M., Trijbels F. J., Blom H. J. Is mutated serine hydroxymethyltransferase (SHMT) involved in the etiology of neural tube defects? *Mol Genet Metab*. 2001, **73**:164-72. doi: 10.1006/mgme.2001.3175
28. Yang, L., Liu L., Wang J., Qiu L., Mi Y., Ma X., et al. 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. doi: 10.3109/10428194.2011.578186
29. Vijayakrishnan, J., Houlston R. S. Candidate gene association studies and risk of childhood acute lymphoblastic leukemia: a systematic review and meta-analysis. *Haematologica*. 2010, **95**:1405-14. doi: 10.3324/haematol.2010.022095
30. Lightfoot, T. J., Skibola C. F., Willett E. V., Skibola D. R., Allan J. M., Coppede F., et al. Risk of non-Hodgkin lymphoma associated with polymorphisms in folate-metabolizing genes. *Cancer Epidemiol Biomarkers Prev*. 2005, **14**:2999-3003. doi: 10.1158/1055-9965.EPI-05-0515
31. Wang, Q., Lu K., Du H., Zhang Q., Chen T., Shu Y., et al. Association between cytosolic serine hydroxymethyltransferase (SHMT1) gene polymorphism and cancer risk: a meta-analysis. *Biomed Pharmacother*. 2014, **68**:757-62. doi: 10.1016/j.biopha.2014.08.002
32. Melnyk, S., Pogribna M., Miller B. J., Basnakian A. G., Pogribny I. P., James S. J. Uracil misincorporation, DNA strand breaks, and gene amplification are associated with tumorigenic cell transformation in folate deficient/repleted Chinese hamster ovary cells. *Cancer Lett*. 1999, **146**:35-44. doi:
33. Christman, J. K., Sheikhnejad G., Dizik M., Abileah S., Wainfan E. Reversibility of changes in nucleic acid methylation and gene expression induced in rat liver by severe dietary methyl deficiency. *Carcinogenesis*. 1993, **14**:551-7. doi:
34. Anderson, D. D., Stover P. J. SHMT1 and SHMT2 are functionally redundant in nuclear de novo thymidylate biosynthesis. *PLoS One*. 2009, **4**:e5839. doi: 10.1371/journal.pone.0005839