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POLYMORPHISMS OF *MTHFR* AND *MTR* GENES ARE NOT RELATED TO SUSCEPTIBILITY TO CHILDHOOD ALL IN NORTH INDIA

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Background: **Acute lymphoblastic leukemia (ALL) is the most worldwide common type of childhood cancer. Methylenetetrahydrofolate reductase (MTHFR) and 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR) participate in folate pathways and are known as critical factors for DNA integrity as well as DNA hypomethylation. The aim of this work is to investigate frequency of MTHFR (677C→T and 1298A→C) and MTR (2756A→G) polymorphisms and their interaction with respect to possible effect on risk of childhood ALL among North Indian population.** ***Procedure:*** **A case control study from has been conducted on bone marrow and peripheral blood samples from 125 ALL patients and 100 sex-age matched healthy controls using PCR-RFLP method.** ***Results:*** **No statistically significant differences were observed for different genotypes between patients and controls (p>0.05). Significant difference for the risk of ALL in individuals having genotype of MTHFR 677TT (OR=0.61, 95% CI=0.21–1.77) and MTHFR 1298CC (OR=0.56, 95% CI=0.18–1.68) was not observed. The correlation of SNP of MTR gene and risk of ALL was not observed, too.** ***Conclusions:*** **The differences in distribution of possible combined genotypes of MTHFR (677C→T, 1298A→C) and MTR (2756A→G) between ALL patients and controls were statistically insignificant.**
Key Words: **methylenetetrahydrofolate reductase; 5-methyltetrahydrofolate-homocysteine methyltransferase; acute lymphoblastic leukemia; hypomethylation.**

Acute lymphoblastic leukemia (ALL) is a malignant neoplasm of hematopoietic stem cells, which is the most common cancer among children with a peak incidence in 4–5 years of age [1]. It accounts for 23% of childhood cancers (children younger than 15 years) [2]. It is still the most common malignant disorder in childhood and principal cause of death due to disease in the pediatric age group, especially prevalent in males [3]. Interaction of genetic and environmental factors together may enhance the risk of leukemogenesis [3, 4].

Folate metabolism plays crucial role in cellular functioning and it serves as donor of one carbon for the synthesis of purines and pyrimidines which are used for the synthesis of RNA and DNA [3, 5–7]. Normal metabolism of folate might be affected by imbalance of nutrition, cellular transport interruption and mutation in folate-related genes. Several mutations in folate-related gene sequences have been reported. However, the effect of these mutations on folate related genes still is unclear [3, 4]. Deficiency of folate or non-homogenous distribution of folate vitamins may influence the risk of cancer by misincorporation of uracil into DNA and leading to double-strand breaks and chromosomal damage, DNA hypomethylation/demethylation of some tumor suppressor genes by which it modulate the leukemogenesis [8–11].

The methylenetetrahydrofolate reductase (*MTHFR)* gene is located on chromosome 1p36 [12] and translated to enzyme which catalyzes the reduction of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. 5-methyltetrahydrofolate acts as a carbon donor for *de novo* methionine synthesis and DNA methylation [13–15]. MTHFR influences the process of DNA methylation and distribution of uridylates and thymidylates bases for DNA synthesis and repair. Therefore due to its role in folate pathways, it makes the MTHFR an important candidate for study of cancer predisposing gene [12, 15–17]. Two frequently reported and well-studied mutations in sequence of methylenetetrahydrofolate reductase gene are (*MTHFR* 677C→T and 1298A→C) which reduce the activity of encoded enzyme [16, 17]. The folate used for synthesis of purine and pyrimidine is accumulated in the form of 5-methyltetrahydrofolate (the most abundant form in wild-type *MTHFR* 677 CC subjects) [18, 19].

5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR*) gene is located on 1q43. It is the enzyme which catalyzes the transfer of methyl base from 5-methyl THF to homocysteine [20]. It is reported to have a polymorphism in locus 2756 (A→G *i.e.* glycine→aspartic acid), resulting in reduced enzyme activity and it is considered as a prime cause for elevation of homocysteine and subsequently DNA hypomethylation [20, 21],

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Abbreviations used: ALL – acute lymphoblastic leukemia; MTHFR – methylenetetrahydrofolate reductase; MTR – 5-methyltetrahydrofolate-homocysteine methyltransferase.

Several reports have indicated that substitutions of C→T at locus 677, A→C at locus 1298 of *MTHFR* gene and A→G at locus 2567 of MTR gene reduce the enzyme activity and result in hypomethylation of DNA and subsequently reduced incidence of DNA double-strand breakage [21]. Hence, the study of these SNPs can rationally assist to determine the risk of group to ALL and also can be used for study of development and progression of childhood ALL in the population study. The aim of this work is to investigate frequency of MTHFR and MTR polymorphisms and their interaction with respect to possible effect on risk of childhood ALL among North Indian population.

METHODS

Specimens. Total 125 bone marrow aspirates were collected from the patients at initial time of diagnosis and prior to any treatment during admission to Hemato-oncology ward (2007–2009), Advanced Pediatric Center, PGIMER, Chandigarh, India. Informed consent was obtained from all patients in accordance with the institutional guidelines and the declaration of PGIMER. The ethical committees of PGIMER approved the protocol.

Diagnosis was based on morphological, cytogenetic and immunophenotypic criteria of WHO classification [39] by Department of Hematology, PGIMER. The clinical characteristic of the ALL patients are shown in Table 1.

Table 1. Demography of study

Variable	Patients	Controls
Gender		
Male	97 (77.6%)	77 (77%)
Female	28 (22.4%)	23 (23%)
Age		
Mean (±SD)	6.46 (±3.34)	6.55 (±3.37)
Range	1–14	
Age group		
<2 years	12 (9.6%)	11 (11%)
2–5 years	43 (34.4%)	33 (33%)
>5 years	70 (56%)	56 (56%)
NCI Risk group		
Standard	103 (82.4%)	-
High	22 (17.6%)	-

Control samples (n=100) were obtained from blood of healthy donors with respect of median and mean of age and sex of patients. The mononuclear cell fraction was separated from bone marrow aspirate and blood by Ficoll density gradient centrifugation (Ficoll Hystopaqu, Sigma Aldrich, USA) and was used for DNA isolation. All samples contained at least 40% blast cells.

DNA isolation. DNA was extracted from mononuclear cells of bone marrow and blood by standard proteinase K digestion followed by phenol-chloroform method [27]. DNA quantity and quality was determined by spectrophotometry via measuring the optical density at 260 nm and 280 nm of the prepared diluted DNA (1 OD at 260 nm unit =50 µg/mL) and 260 nm/280 nm.

Detection of MTHFR 677C→T, 1298A→C and MTR 2756 A→G SNPs. Genomic DNA containing the polymorphic sites was amplified by polymerase chain reaction (PCR) with specific primers for each gene (Table 2) using 15 ng genomic DNA, 0.5 µM of each of the primers, 100 µM deoxyribonucleotide triphosphates (dNTPs), 10 mM Tris (tris-(hydroxymethyl) aminomethane)–HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, and 0.5 units of Taq polymerase (Sigma–Aldrich, USA) in a total volume of 50 µl.

The PCR conditions were as mentioned in Table 2. RFLP was performed with restriction enzymes according to the manufacturer's instructions (MBI, Fermentas, Burlington, ON, Canada). The restriction digestion was visualized after electrophoresis on 2.5% agarose gel. The amplified PCR product of MTHFR *677C→T,* digested by *HinfI* restriction enzyme. Those patients carrying genotype CC showed a single band of 198 bp, whereas carrier of genotype TT showed bands of 175 and 23 bp, and three bands of 198, 175 and 23bp appeared with genotype CT. After digesting with *MboII* for detecting MTHFR 1298A→C*,* the bands 56, 31, 30, 28 and 18 bp were observed for genotype AA, whereas, the expected bands for genotype CC were 84, 31, 30 and 18 bp and for heterozygous (AC), the size of expected bands were 84, 56, 31, 30, 28 and 18 bp. The amplified product of MTR 2756A→G gene followed by digestion by *HaeIII*, the carrier of genotype AA showed the single band at 211 bp. Those homozygous for mutation (GG) showed bands of 131 and 80bp. Heterozygous (AG) represented bands of 211, 131 and 80 bp.

Table 2. Primer sequences and PCR Conditions

Gene	Primer Sequence (5'- 3')	Annealing temp. (°C) / time / cycle
MTHFR 677		
F	TGA AGG AGA AGG TGT CTG CGG GA	59 / 1 min / 35
R	AGG ACG GTG CGG TGA GAG TG	
MTHFR1298		
F	CTT TGG GGA GCT GAA GGA CTA CTA C	59 / 1 min / 35
R	CAC TTT GTG ACC ATT CCG GTT TG	
MTR2756		
F	TGT TCC AGA CAG TTA GAT GAA AAT C	60 / 1 min / 35
R	GAT CCA AAG CCT TTT ACA CTC CTC	

Statistical analysis. Distribution of *MTHFR 677C→T and MTHFR 1298A→C* and *MTR 2756A→G* variants among patients and controls were tabulated for cases and controls (Table 3). With respect to assumption which indicates that *MTHFR* and *MTR* genotypes may be associated with the risk of ALL, we tested genotypes interaction in models for acute lymphoblastic leukemia and the interaction of these genes with age, sex and hematological classification, risk group. The χ^2 test was used to examine differences in frequencies between *MTHFR 677C→T, MTHFR 1298A→C* and *MTR 2756A→G* genotypes in cases and controls. Fisher exact test was used when cell numbers were less than 5. The relationship between *MTHFR* and *MTR* genotypes and risk of ALL was assessed by means of the odds ratio (OR) with 95% confidence interval (95%CI). It was calculated using both conditional and unconditional logistic regression (adjusting for age and sex) by use of software version 11.5 (SPSSs, Chicago, IC), and EPI software version 3.2.

Table 3. Frequencies of MTHFR 677C→ T, 1298A→ C and MTR 2756A→ G genotypes in case and control

Genotype	Patients (n = 125)	Controls (n = 100)	OR	CI (95%)	P-Value
MTHFR C677T					
CC	54 (43.2%)	40 (40%)	1		
CT	62 (49.6%)	49 (49%)	0.94	0.52–1.69	0.93
TT	9 (7.2%)	11 (11%)	0.61	0.21–1.77	0.30

Genotype	Patients (n = 125)	Controls (n = 100)	OR	CI (95%)	P-Value
CT+TT	71 (56.8%)	60 (60%)	0.88	0.50–1.55	0.72
MTHFR A1298C					
AA	52 (41.6%)	40 (40%)	1		
AC	65 (52%)	49 (49%)	1.02	0.56–1.85	0.94
CC	8 (6.4%)	11 (11%)	0.56	0.18–1.68	0.37
AC+CC	73 (58.4%)	60 (60%)	0.94	0.53–1.66	0.91
MTR A2756G					
AA	74 (59.2%)	58 (58%)	1		
AG	44 (35.2%)	35 (35%)	0.99	0.54–1.80	0.92
GG	7 (5.6%)	7 (7%)	0.78	0.23–2.66	0.87
AG+GG	51 (40.8%)	42 (42%)	0.95	0.54–1.68	0.96

RESULTS

Demographic data of cases and controls has been summarized in Table 1. The variables have also been categorized for ALL immunophenotypes. The blast lineage, NCI risk groups of ALL patients have been tabulated in Table 1. 94.4% patients were diagnosed with B-lineage ALL and 5.6% with T-lineage ALL. The distribution of ALL patients on the basis of NCI risk groups were 82.4% in standard risk group and 17.6% in high risk group.

The mean age (±SD) of all cases and controls were 6.46 (±3.34) and 6.55 (±3.37) years (p=0.245) respectively. Around 9.6% of patients were in the age-group of age ≤2 years and 34.4% were in the age-group of 2< age ≤ 5 and 56% were in age of more than 5 years, whereas it was 11, 33 and 56% respectively for healthy controls. The number of males and females among cases and controls were almost similar (77.6: 77 for males and 22.4: 23 for females, respectively). The distribution pattern of ALL patients and controls according to place of living were divided in to two groups (a) urban (b) rural. 35.2% of patients were living in urban and 64.8% were living in rural areas. The values for controls were not different from cases (38% urban and 62% rural). The distribution of ALL cases and controls among different states of North India showed the most of the patients in present study were from Punjab (44%), followed by Haryana (27.2%), Uttar Pradesh (12.8%), Himachal Pradesh (8%), Chandigarh (4.0%) and J&K (4%). Similar distribution was seen among the controls.

The prevalence of genotypes of MTHFR and MTR genes in patients and controls were depicted in Table 3. There is no significant differences between patients and controls in this study population. The frequencies of MTHFR C677T/A, MTHFR A1298C, MTR A2756G variants in childhood ALL and healthy controls did not deviate significantly from the Hardy — Weinberg equilibrium. Interestingly, all genotypes of MTHFR 677TT, MTHFR 1298CC and MTR 2756GG show slight decreasing of the risk disease, but no significant difference (p>0.05) was observed between patients and controls in all these studied genes.

The frequencies of *MTHFR 677C→T, MTHFR 1298A→C* and *MTR 2756A→G* in stratified risk group is shown in Table 4. Significant difference of distribution of genotypes between standard and high risk group was not observed.

Table 4. Frequencies of genotypes in standard and high risk group patients

Genotype	Standard Risk Group 103 (%)	High Risk Group 22 (%)	OR	CI (95%)	P-Value
MTHFR C677T					
CC	45 (43.7)	11 (50)	Ref.	-	-
CT	51 (49.5)	9 (40.9)	1.39	0.48–4.05	0.67
TT	7 (6.7)	2 (9)	0.97	0.67–1.04	1
CT+TT	58 (56.3)	11 (50)	1.29	0.47–3.65	0.76
MTHFR A1298C					
AA	43 (41.7)	9 (41)	Ref.	-	-
AC	53 (51.4)	11 (50)	1.01	0.34–2.93	0.81
CC	7 (6.7)	1 (4.5)	1.06	0.79–1.41	1
AC+CC	60 (58.2)	12 (54.5)	1.05	0.37–2.97	0.88
MTR A2756G					
AA	60 (58.2)	14 (63.6)	Ref.	-	-
AG	38 (36.9)	7 (31.8)	1.27	0.43–3.85	0.82
GG	5 (4.8)	1 (4.5)	1.03	0.71–1.49	1
AG+GG	43 (41.7)	8 (36.3)	1.25	0.44–3.62	0.82

The comparison of the frequency distribution of *MTHFR 677C→T, MTHFR 1298A→C* and *MTR 2756A→G* polymorphisms between case and control on the bases of sex showed that the distribution of MTHFR 677 and MTHFR 1298 and MTR2756 variants among the both sex were not significantly different (p>0.05). Also on the basis of age-group (the age of the children at the time of diagnosis), distribution of genotypes of *MTHFR 677C→T, MTHFR 1298A→C* and *MTR 2756A→G* were calculated. However, no significant difference was detected (Table 5).

The effect of gene-gene interaction between MTHFR polymorphisms on susceptibility of childhood ALL was investigated further; significant association between the interaction of MTHFR variants and de-

Table 5. Frequencies of genotypes in different age groups

	Age <2					2 < Age < 5					Age > 5				
Genotype	Patients (n=12) (%)	Controls (n=11) (%)	OR	CI (95%)	P-Value	Patients (n=45) (%)	Controls (n=32) (%)	OR	CI (95%)	P-Value	Patient 68 (%)	Control 57 (%)	OR	CI (95%)	P-Value
MTHFR C677T															
CC	6 (50)	4 (36.3)	-	-	-	19 (42.2)	14 (43.7)	-	-	-	29 (42.6)	22 (38.5)	-	-	-
CT	5 (41.6)	6 (54.5)	0.76	0.33–1.72	0.6	23 (51.1)	16 (50)	1.06	0.37–1.52	0.9	34 (50)	25 (43.8)	1.03	0.45–2.36	0.91
TT	1 (8.3)	1 (9)	0.83	0.19–3.64	1	3 (6.6)	4 (12.5)	0.74	0.3–1.84	0.67	5 (7.3)	7 (12.2)	0.73	0.36–1.49	0.52
CT+TT	6 (50)	7 (63.6)	0.77	0.34–1.67	0.68	26 (57.7)	20 (62.5)	0.96	0.35–2.6	0.89	39 (57.3)	32 (56.1)	0.95	0.43–2.11	0.95
MTHFR A1298C															
AA	5 (41.6)	4 (36.3)	-	-	-	17 (37.7)	12 (37.5)	-	-	-	30 (44.1)	24 (42.1)	-	-	-
AC	7 (58.3)	5 (45.4)	1.05	0.49–2.23	1	24 (53.3)	17 (53.1)	1	0.34–2.29	0.81	34 (50)	27 (47.3)	1.01	0.45–2.25	0.86
CC	1 (8.3)	1 (9)	0.90	0.20–4.05	1	3 (6.6)	3 (9.3)	0.85	0.36–2.01	1	4 (5.8)	7 (12.2)	0.65	0.29–1.48	0.40
AC+CC	8 (66.6)	6 (54.5)	1.03	0.49–2.16	1	27 (60)	20 (62.5)	0.95	0.34–2.70	0.88	38 (55.8)	34 (59.6)	0.89	0.41–1.93	0.89
MTR A2756G															
AA	7 (58.3)	6 (54.5)	-	-	-	28 (62.2)	20 (62.5)	-	-	-	39 (57.3)	32 (56.1)	-	-	-
AG	4 (33.3)	4 (36.3)	0.93	0.39–2.19	1	16 (35.5)	11 (34.3)	1.04	0.36–3.02	0.86	24 (35.2)	19 (33.3)	1.02	0.72–1.43	0.91
GG	0 (0)	0 (0)	-	-	-	2 (4.4)	2 (6.2)	0.86	0.31–2.35	1	5 (7.3)	5 (8.7)	0.91	0.47–1.75	1
AG+GG	4 (33.3)	4 (36.3)	0.93	0.39–2.19	1	18 (40)	13 (40.6)	1	0.68–1.46	0.83	29 (42.6)	24 (42.1)	0.99	0.46–2.16	0.87

velopment and progression of ALL among the children in the present study was on observed (Table 6).

As it is shown in Table 3, individuals who carried the genotype of MTHFR 677TT, did not show and difference on risk of ALL (OR=0.61; 95% CI=0.21–1.77). The adjusted OR for carriers of MTHFR 1298CC genotype was calculated. It was 0.56 (95% CI=0.18–1.68) which statistically did not show significance reduction on susceptibility against of childhood ALL. Moreover, this trace was not observed for variants of *2756A→G* gene. The significance was not altered when these 3 polymorphisms were evaluated in combination (Table 7).

Table 6. Interaction of MTHFR genotypes between case and control

Combined genotypes	Patients (n = 125)	Controls (n = 100)	OR	CI (95%)	P-Value
677CC/1298AA	22(17.6%)	16 (16%)	-	-	-
677CT/1298AA	40 (32%)	20 (20%)	0.52	0.1–1.54	0.18
677TT/1298AA	8 (17.6%)	12 (12%)	1.35	0.34–5.47	0.63
677CC/1298AC	20 (6.4%)	22 (22%)	0.51	0.17–1.48	0.16
677CT/1298AC	17(13.6%)	18 (18%)	0.49	0.16–1.5	0.16
677TT/1298AC	0 (0%)	0 (0%)	NA	NA	NA
677CC/1298CC	13(10.4%)	12 (12%)	0.73	0.2–2.55	0.57
677CT/1298CC	5 (4%)	7 (7%)	0.72	0.35–1.48	0.51
677TT/1298CC	0 (0%)	0 (0%)	NA	NA	NA

Table 7. Frequencies of combined genotype in childhood ALL and control. Genotype is in respect of MTHFR 77C→ T,1298A→ C and MTR 2756A→ G

Combined genotypes	Patients (n = 125)	Controls (n = 100)	OR	CI (95%)	P-Value
CCAAAA	14	7	Ref.	-	-
CCAAAG	6	5	0.82	0.44–1.52	0.70
CCAAGG	5	4	0.83	0.43–1.61	0.68
CCACAA	15	8	0.98	0.64–1.50	0.82
CCACAG	12	7	0.86	0.19–3.81	0.92
CCACGG	0	0	NA	NA	NA
CCCCAA	2	2	0.75	0.27–2.09	0.60
CCCCAG	2	1	1	0.43–2.35	1
CTAAAA	18	9	1	0.25–3.97	1
CTAAAG	8	1	1.33	0.91–1.95	0.37
CTAAGG	2	1	1	0.43–2.35	1
CTACAA	15	10	1.33	0.34–5.32	0.64
CTACAG	13	5	0.77	0.16–3.71	0.7
TTAAAA	1	1			

DISCUSSION

Polymorphism of genes which regulate the metabolism of folate is the important phenomenon which may play role in cancer susceptibility by decreasing folate status or by interference in distribution of folate in cells could influence the risk of ALL [22]. Childhood ALL is more prone to environmental exposures during fetal and infant stage [10]. Reduction in the level of 5, 10-methylene–THF (MTHFR substrate), which is essential for synthesis of thymidylate, could lead to uracil misincorporation into DNA [23] and further diminished the DNA repair system and increased the susceptibility to double-strand breakage there by leading to the chromosomal damage and translocation [24–27]. Folate availability is critical for DNA integrity, required for the transfer of methyl groups in the biosynthesis of thymidilate. In this study we tried to find out whether MTHFR variants and MTR polymorphism play a protective role in a childhood ALL.

The decrease in activity of MTHFR could affect the nucleotide synthesis by increasing availability of the 5, 10-methylene–THF which is required for DNA synthesis and cell division. Folate distribution decreases the transmethylation capacity and thereby decreasing S-adenosylmethionine / *S*-adenosylhomocysteine ratio and methylation of homocysteine to methionine. Diminished folate availability increases the probability of mis-incorporation of uracil in DNA strand at the time of replication and subsequently increasing the frequency of chromosomal breakage in human leukocyte [28–31].

A hematological malignancy like ALL is more vulnerable to DNA and chromosomal damage because folate deficiency in them cannot accomplish the demand of hasty DNA synthesis in uncontrolled and rapidly proliferating hematopoitic cells. DNA hypomethylation and uracil misincorporation are considered important factors in carcinogenesis [32, 11].

Several groups have reported that the polymorphisms which reduce the MTHFR activity were associated with the reduced risk of leukemia and lymphoma [10, 33, 34]. ALL patients with polymorphisms in serine hydroxymethyl transferase gene and thymidylate synthase genes are considered as low risk group [15, 35]. ALL cases with a polymorphisms in MTR 2756A→G genotype) showed reduction by 5.6-fold in adult ALL risk [35]. Not only MTR 2756A→G variants were considered as a risk allele for ALL but also in combination with the MTHFR variants [14].

In this study, attempts were made to evaluate the associations between the MTHFR 677C→T, MTHFR *1298A→C* and MTR 2756A→G polymorphisms and the risk of ALL in the 125 cases of the childhood ALL in north India compared with the sex and age matched controls. It has been reported that both MTHFR polymorphisms reduce the susceptibility to adult and childhood lymphoid leukemia however not in myeloid leukemia [10, 34, 36–38].

The significant protective effect of *MTHFR 677C→T* variant but not MTHFR 1298A→C in the Brazilian population to decrease the risk of childhood ALL has been reported by de Franchis et al. [13]. Recently, Sood et al. [34] demonstrated the significant protective effect of *MTHFR 677C→T* variant but not MTHFR 1298A→C in the north Indian population to decrease the risk of childhood ALL. Wiemels et al. [33] reported the protective effect of MTHFR 677C→T polymorphism on the risk for infant leukemia in the UK population but not for MTHFR *1298A→C* polymorphism; also it was demonstrated that 677C/T frequency was higher in ALL patients with MLL rearrangements, low in ALL with hyper-diploidy indicating that impact of 677C/T variant on development and progression of leukemia might vary between different subtype of ALL [37]. Matsuo et al. [21] provided the evidence which MTHFR mutant alleles are associated with lower susceptibility to ALL. Some other studies also reconfirmed that both MTHFR polymorphisms decreased the risk for childhood ALL in French–Canadian population [5]. The recent report by Reddy et al. [39] suggested a possible protective effect of MTHFR (677C→T) and MTHFR 1298A→C) for the first time they reported gender-bias protective effect of 677CT /1298AA and

677CT/1298AC toward of female ALL patients. Yeoh et al. [40] 2010 demonstrated association between MTHFR *1298A→C* and increasing the risk of ALL among male patients.

However, other research groups reported no association between MTHFR polymorphisms and the risk for childhood ALL, from different ethnic groups [13, 15, 21]. Wang et al. [41] in their recent meta-analysis reported albeit MTHFR C677T was found previously to be associated with increased risks of colorectal cancer, leukemia, and gastric cancer, but on the bases of this meta–analysis, they could not find evidence for a main role of MTHFR C677T in the leukemogesis of childhood acute lymphoblastic leukemia.

In the present study, significant difference between patients and healthy controls was not found, but also our result did not show the significant reduction in the risk of ALL and increasing the tolerance induced by T and C alleles in position of 677 and 1298 of MTHFR gene, respectively, in ALL patients in north Indian population.

We tried to analyze the influence of each polymorphism of MTHFR and MTR individually and in combination and their effect on the risk for childhood ALL. We could not observe significant associations between the genetic polymorphisms of the folate metabolizing enzymes and MTR on the risk for childhood ALL in our population of study.

However, the contradictory results were observed in association of genetic polymorphisms in the folate metabolic pathway with the risk for adult ALL [33, 35, 36, 40, 42]. Also, Gemmati et al. [36] and Lincz et al. [43] observed that *MTR 2756A→G* polymorphism reduced the risk for adult ALL and lymphoma. On the other hand, Skibola et al. [35] and Matsuo et al. [21] reported on increased risk for different types of adult lymphoma in carriers for at least one G allele in locus 2756 of MTR gene. No association has been observed between MTR 2756A→G polymorphism and the risk for adult ALL according to [35, 42]. Our work also showed no association between MTR 2756A→G polymorphism and the risk of childhood ALL. The inconsistencies of results might be attributed to some variables in study of population like size of sampling, age and nutritional folic acid in diet. Rosenberg et al. [44] showed that the adequate folic acid consumption in a population may increase the frequency of the MTHFR 677T allele, in contrast insufficient level of folate in diet may results in decreasing the T allele frequency. This can justify the different frequency of MTHFR allele in population of this study as compared with Reddy et al. [39] may be influenced by different level of folic acid in diet of different ethnic groups in India. Thus the polymorphism of these genes did not show any effect on ALL susceptibility, so it could be interpreted that other molecular mechanisms like hypermethylation mediated gene silencing may play an important role in etiology of ALL in north Indian population. The mechanism proposed to explain these associations was the shunt of folate metabolism versus thymidine and purine synthesis, which could reduce the possibility of the incorporation of uracil into DNA and protect against carcinogenesis. It has been proposed that shunt of folate metabolism encounters the synthesis of thymidine and purine, reduces misincorpration of uracil into DNA and protect from any damage which lead to arising cancer [10, 39].

In conclusion, the present study provided the evidence that MTHFR *(677 C→T),* MTHFR (*1298 A→C)* and MTR *(2576A→C)* are not associated with decreased risk of childhood ALL and did not show significant effect on progression and development of childhood ALL. In the other words, we found that MTHFR *677C→T and 1298A→C* may could not be considered as a crucial and probable marker for ALL development and progression and also could not be used for treatment strategies for childhood ALL, as epigenetic mechanisms are more important in progression of childhood ALL. The larger studies, which in turn will be able to provide support to truly significant finding through much larger combined and comparative data sets, are necessary in this regard.

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