

Genetic Polymorphism of Folate and Methionine Metabolizing Enzymes and their Susceptibility to Malignant Lymphoma

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ABSTRACT

Background: Folate and methionine metabolism is involved in DNA synthesis and methylation. Polymorphisms in the genes of folate metabolism enzymes have been associated with some forms of cancer. In the present study, 2 polymorphisms were evaluated for a folate metabolic enzyme, methylene-tetrahydrofolate reductase (MTHFR), and one was evaluated for methionine synthase (MS).

The 2 polymorphisms MTHFR 677 C→T and MTHFR 1298 A→C, are reported to reduce the enzyme activity, which causes intracellular accumulation of 5, 10-methylene-tetrahydrofolate and results in a reduced incidence of DNA double strand breakage. The MS 2756 A→G polymorphism also reduces the enzyme activity and results in the hypomethylation of DNA.

Patients and Methods: To test this hypothesis, genetic polymorphisms in the folate metabolic pathway were investigated using the DNA from a case-control study on 31 patients having malignant lymphoma from the Oncology Outpatient Clinic of the New Children's Hospital, Cairo University and 30 controls who were actually normal children attending for vaccination to the same hospital.

Results: We found that there is a higher susceptibility with the MTHFR 677CC and MTHFR 1298 AA genotypes (OR=4.3, 95% CI 1.12-16). When those harbor at least one variant allele in either polymorphism of MTHFR they were defined as reference. For the MS 2756 AG genotype polymorphism there was also a higher susceptibility to developing malignant lymphoma (OR=2.6; 95% CI 1.1-6.4).

Conclusion: Results suggest that folate and methionine metabolism may play an important role in the pathogenesis of malignant lymphoma. Further studies to confirm this association and detailed biologic mechanisms are now required.

Key Words: Polymorphism - Folate - Methionine - Lymphoma.

INTRODUCTION

Biologic mechanisms underlying the pathogenesis of lymphoid malignancies remain to be

clarified in detail. However, accumulated evidence suggests that certain genetic events during cell differentiation, such as chromosomal translocations, play an important role [1].

DNA methylation is a feature of DNA influencing cellular development, and function. Aberrations of DNA methylation are a candidate mechanism for the development of cancer [2]. 5, 10-methylene-tetrahydrofolate reductase (MTHFR) is one of the main regulatory enzymes of homocysteine (Hcy) metabolism that catalyzes the reduction of 5, 10- MTHF to 5-MTHF, the methyl donor of Hcy to methionine (Fig. 1). A common 677 C→T transition in the MTHFR gene is a well-established genetic determinant of hyperhomocysteinemia, and results in a thermo labile protein, with a decreased enzymatic activity. A missense mutation in the exon 4 of the MTHFR gene, which is located on chromosome 1 p36, is associated with a cytosine to thymine substitution at nucleotide 677, which converts an alanine to a valine codon in the N-terminal catalytic domain of the protein, which is the molecular basis of this thermolability which in turn results in an oncogenic potential [3].

A second polymorphism associated with decreased enzymatic activity but not with thermolability was discovered in the MTHFR gene. This genetic variant corresponds to an adenosine to cytosine transversion at nucleotide 1298, in exon 7 leading to glutamate to alanine substitution within the C-terminal regulatory domain of the MTHFR protein. Subjects harboring the 1298 CC genotype have reduced enzyme activity but to a lesser extent than those bearing the 677 TT genotype, probably because of the distinct

locations of the two polymorphisms, the 677 C→T and 1298 A→C mutations are found in regions encoding the N-terminal and the C-terminal regulatory domains of the protein respectively; which, in turn, also results in an oncogenic potential [4].

Methionine synthase (MS) is a cobalamin-dependant enzyme that catalyzes the methyl transfer from homocysteine to methionine, thus playing a critical role in maintaining adequate interacellular S-adenosyl-methionine (SAM) levels for DNA methylation having a cancer suppressor effect. The MS 2756 A→C polymorphism converts an aspartic acid to a glycine residue and has been predicted to alter enzyme activity that may affect DNA methylation processes, yet its function remains controversial [5].

Given the importance of folate in cancer risk, chromosomal integrity, and immune function, examination of the association of the folate metabolic polymorphisms (MTHFR 677, 1298 & MS 2756) with development of malignant lymphoma will be done in the present study.

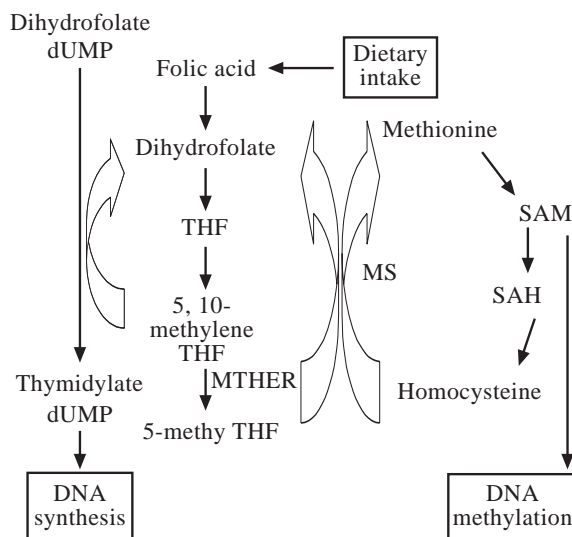


Fig. (1): Overview of folate-and methionine-metabolizing pathways. MTHFR catalyzes the reduction of 5, 10-methylene THF. Reduced activity of MTHFR results in the accumulation of 5, 10-methylene THF, which accelerates methylation of dUMP to dTMP. MS catalyzes the transfer of the methyl base from 5-methyl THF to homocysteine. Reduced activity of MS leads to hypomethylation of DNA. SAM: S-adenosyl-methionine; SAH: S-adenosyl-homocysteine. (Adapted from reference [1]).

SUBJECTS AND METHODS

Sixty-one cases were included in this study; 31 of them with malignant lymphoma and 30 age matched controls. All patients were taken from the outpatient Oncology Clinic of the New Children's Hospital Cairo University; whereas the 30 age matched controls were assigned from the internal medicine outpatient clinic cases attending for vaccination in the same hospital.

Therapy:

All patients with de novo or relapsed disease were treated according to the pediatric oncology unit protocols for malignant lymphoma.

Methods:

All patients were subjected to a full history taking and clinical examination.

Routine laboratory work consisted of complete blood counts (CBC), liver function tests. Lymph node biopsies were performed to all patients to diagnose the subtype of lymphoma.

Genotype Analyses of the MTHFR 677, MTHFR 1298 and MS 2756:

DNA of each case was extracted from the whole blood (5 ml sample) with a QIAamp DNA blood Mini Kit (Qiagen, Valencia, CA). Genotyping was performed according to the previously described methods for MTHFR 677 [6], and MTHFR 1298 [7] of the MTHFR gene and MS 2756 of the MS gene polymorphisms in detail [8,9]. For the C→T polymorphism, extracted DNA was amplified with the forward primer 5'-TGA AGG AGA AGG TGT CTG CGG GA-3' and reverse primer 5'-AGG ACG GTG CGG TGA GAG TG -3'. Polymerase chain reaction (PCR) thermal cycling conditions were 2-minute denaturation at 94°C, then 40 cycles at 94°C for 30 seconds, 62°C for 30 seconds and finally 72°C for 30 seconds. This was followed by 7-minute extension at 72°C. Amplified 198-bp PCR products were digested with Hinf 1 (Fermentas) and were visualized under electrophoresis on 4% agarose gel with ethidium bromide. The C allele produced 198-bp band, and the T allele produced 175- and 23-bp fragments (Fig. 2-A). For the MTHFR 1298 A→C polymorphism, DNA was amplified with the forward primer 5'-CTT TGG GGA GCT GAA GGA CTA CTA C -3' and the reverse primer 5'-CAC TTT GTG ACC ATT CCG GTT TG-3'. PCR was 2-minute denaturation at 94°C

followed by 35 cycles at 92°C for 1minute, 60°C for 1minute, and 72°C for 30 seconds, with 7-minute extension at 72°C. Mbo II (Fermentas) digestion produced 56-, 31-, 28-, and 18-bp bands for the A allele and 84-, 31-, 30- and 18-bp bands for the C allele (Fig. 2-B). For the MS 2756 A→G polymorphism, DNA was amplified with the forward primer 5'-TGT TCC AGA CAG TTA GAT GAA AAT C-3' and the reverse primer 5'-GAT CCA AAG CCT TTT

ACA CTC CTC-3'. PCR thermal cycling conditions were 2-minute denaturation at 95°C followed by 35 cycles at 95°C for 1 minute, 60 °C for 1.5 minutes and 72°C for 1minute, with a 7-minute extension at 72 °C. PCR products were digested with Hae III (Fermentas). Resulting in a 211-bp band for the A allele and 131- and 80-bp fragments for the G allele (Fig. 2-C), after 4% agarose gel electrophoresis. Heterozygotes produced bands for each allele.

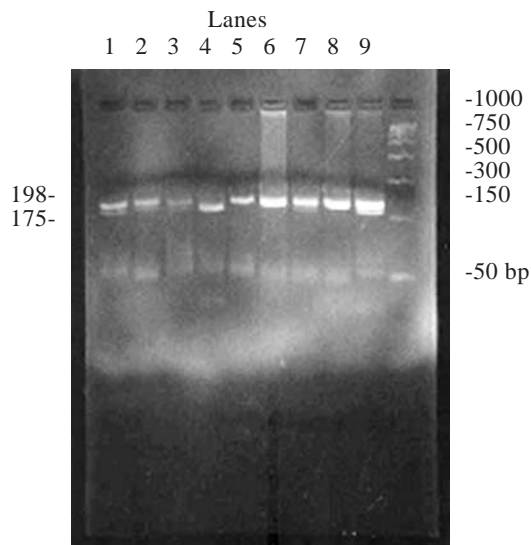


Fig. (2-A): Polymorphisms of MTHFR677CC genotype in lane 2, 3, 5, 6, 8, CT genotype in lane 1, 7, 9 and TT genotype in lane 4.

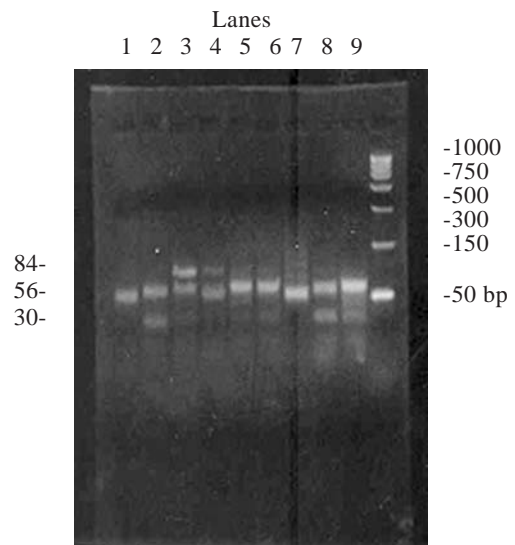


Fig. (2-B): Polymorphisms of MTHFR 1298 AA genotype in lane 1, 2, 5, 6, 8, 9 and AC genotype in lane 3, 4.

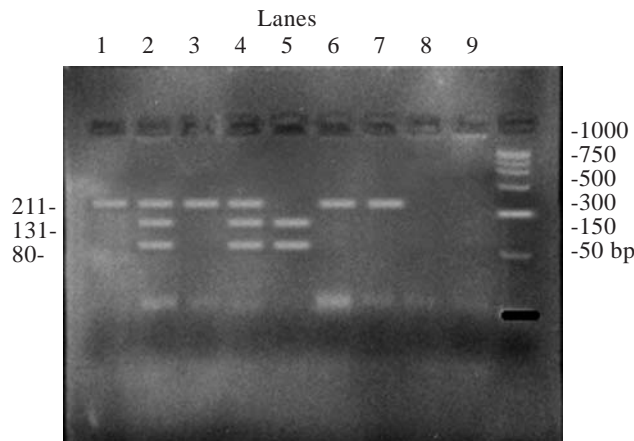


Fig. (2-C): Polymorphisms of MS 2756 AA in lane 1, 3, 6, 7, AG genotype in lane 2, 4 and lane 5 GG genotype.

Statistical Analysis:

SPSS software was used for data analysis, Chi-Square, was used for comparing differences in proportions of genes among cases and controls. Odds ratio & 95% confidence intervals (95% CI) were calculated with $p < 0.05$ as a significance level.

RESULTS

Clinical Picture and Diagnosis:

The 31 children included in the study were proved to have malignant lymphoma. Twenty-two were males (71%) and 9 were females (29%). The mean age at the time of enrollment

in the study was 7.5 ± 3.6 years. Their mean age at presentation was 4.06 ± 2.9 years, with a mean duration of follow up of 5.5 ± 2.8 years. Twenty-four children had B symptoms (77.4%) (which is considered to be a high risk criterion) and their performance status was assessed using the Karnofsky scale (Table 1). Thirty (96.7%) of the enrolled patients were already diagnosed at arrival, being diagnosed 17.7 ± 28.9 days earlier. The diagnosis of the affected patients, stage of the disease and sites of extra-nodal affection are listed in table (1).

Table (1): Performance status, the assigned diagnosis, stage of disease, sites of extra-nodal affection and received chemotherapy in the enrolled infants.

Characteristic	Cases n=31		Age in years (mean±SD)
	n	%	
<i>Performance Status:</i>			
60%	4	12.9	5.7 ± 1.7
70%	6	19.4	6.6 ± 1.7
80%	11	35.5	6.4 ± 3.4
90%	10	32.3	9.8 ± 4.2
<i>Pathological Diagnosis:</i>			
Abdominal Burkitt Lymphoma	15	48.4	7.66 ± 3.8
Large cell Lymphoma	9	29	6.5 ± 2.2
T cell Lymphoma	4	12.9	6.7 ± 3.8
African Burkitt Lymphoma	2	6.5	13.5 ± 2.1
Lymphoblastic Lymphoma	1	3.2	4
<i>Stage of Disease:</i>			
Stage 1	2	6.5	13.1 ± 2.1
Stage 2	17	54.8	7.4 ± 3.6
Stage 3	9	29	6.5 ± 3.2
Stage 4	3	9.7	6.6 ± 1.1
<i>Affected Extranodal Sites:</i>			
None	14	45.2	8.9 ± 4.1
Intestine	13	41.9	6.5 ± 2.7
Pleura	1	3.2	8
Bone marrow	1	3.2	6
Parotid	1	3.2	3
Para-spinal mass	1	3.2	6
<i>Received Chemotherapy</i>			
<i>Protocol:</i>			
BFM86	13	41.9	7.4 ± 2.9
BFM90	14	45.2	7.3 ± 4
COMP	2	6.5	10.5 ± 4.9
T-cell lymphoma protocol	2	6.5	6.5 ± 4.9

The apparent age differences in the affected patients according to diagnosis, stage of the disease and sites of extra-nodal affection did not reach statistical significance with *p* values =0.1, 0.086 and 0.4 respectively.

Therapeutic Intervention and Outcome:

All patients received a total of 172 cycles of chemotherapy with a mean of 5.5 cycle/child (the minimum was 2 cycles and the maximum was 8 cycles and the mode was 6 cycles). Description of received chemotherapy is presented in (Table 2). None received radiotherapy. The mean duration of therapy was 6.4 ± 2.7 months having a range of 1-12.3 months. Twenty-seven (87.1%) of the enrolled patients were alive and free of disease at the end of the study, 3 (9.7%) were alive with disease and 1 (3.2%) patient had died. The 3 patients who were alive and with disease were undergoing salvage chemotherapy with favorable responses up till September 2005. The outcome of the enrolled patients according to received therapy is shown in (Table 3).

Table (2): Number of chemotherapy cycles received and duration of therapy in the enrolled patients.

Characteristic	Cases n=31		Age in years (mean±SD)	Number of Received cycles.	Duration of therapy in months.
	n	%			
<i>Given Chemotherapy</i>					
BFM86	13	41.9	7.4 ± 2.9	6.2 ± 0.6	6.5 ± 2.8
BFM90	14	45.2	7.3 ± 4	4.9 ± 1.5	6.4 ± 2.9
COMP	2	6.5	10.5 ± 4.9	6	4.2 ± 1.8
T-cell protocol	2	6.5	6.5 ± 4.9	5	8.1 ± 2.1

Table (3): Outcome in relation to received chemotherapy in the enrolled patients.

Received Chemotherapy	Complete Response		Partial Response		Stationary Disease		Progressive Disease			
	n	%	n	%	n	%	n	%		
BFM86	13	41.9	11	84.6	2	15.3	1	7.1	-	-
BFM90	14	45.2	12	85.7	1	7.1	-	-	-	-
COMP	2	6.5	2	100	-	-	-	-	-	-
T-cell protocol	2	6.5	1	50	-	-	-	-	1	50

Genotyping of MTHFR 677, MTHFR 1298, and MS 2756:

Among the 61 patients analyzed, the frequency of the MTHFR677 mutant allele was 29% for patients and 33.3% for controls. Frequencies of the MTHFR 677CC, CT & TT genotypes were 71%, 22.6% and 6.4% respectively for patients and 66.7%, 33.3%, and 0%

for controls ($p=0.44$). As for MTHFR 1298, the mutant allele frequency was 45.1% for patients and 73.3% for controls. Frequencies of MTHFR 1298 AA, AC, and CC genotypes were 54.8%, 41.9% and 3.3% respectively for patients and 26.7%, 53.3% and 20% for controls ($p=0.08$). For MS 2756, the mutated allele frequency was 54.8% for patients and 26.6% for controls. Frequencies of the MS2756 AA, AG, GG genotypes were 45.2%, 41.9% and 12.9% respectively for patients and 73.4%, 13.3% and 13.3% for controls respectively ($p=0.01$).

Calculation of the risk estimation for genotypes by the unconditional logistic model is demonstrated in (Table 4). It shows the frequency of genotypes for patients, and controls, with sex and age adjusted OR for each polymorphism. When the MTHFR 677CC genotype was defined as the reference, the MTHFR 677CT/TT genotype showed a reduced OR (OR 0.8; 95% CI, 0.45-1.3). When the MTHFR 1298 AA genotype was defined as the reference, the adjusted Odds Ratios for the MTHFR AC, CC genotypes were 0.58 and 0.37 respectively. The MS 2756 GG genotype showed a higher adjusted OR=1.2; 95% CI 0.6-2.5, $p = 0.7$.

Table (4): Number of patients and controls, sex-and age adjusted odds ratios with 95% CI for MTHFR 677, 1298, and MS 2756.

Genotype	Patients n=31	Controls n=30	OR	95%CI	p value
MTHFR677					
CC	22(71%)	20(66.7%)	1.0	Reference	-
CT	7(22.6%)	10(33.3%)	0.8	(0.45-1.3)	0.44
TT	2(6.4%)	0	NE		-
MTHFR 1298					
AA	17(54.8%)	8(26.7%)	1.0	Reference	0.08
AC	13(41.9%)	16(53.3%)	0.58	(0.3-1.12)	0.04
CC	1(3.3%)	6(20%)	0.37	(0.2-0.7)	-
MS 2756					
AA	14(45.2%)	22(73.4%)	1.0	Reference	0.01
AG	13(41.9%)	4(13.3%)	2.6	(1.1-6.4)	0.7
GG	4(12.9%)	4(13.3%)	1.2	(0.6-2.5)	-

OR: Odds ratio.
Confidence intervals (CI).
Methionine synthase (MS).
NE: Not estimated.

The description of the distribution for the MTHFR 677 and MTHFR 1298 polymorphisms are demonstrated in table (5). Risk estimation showed lower than unity for each combination of alleles when the MTHFR 677 CC/1298 AA

was defined as the reference, the adjusted OR for the other genotypes showed a markedly reduced risk (OR=0.39; 95% CI 0.15-1.06, $p=0.06$), so those with full enzyme activity had higher susceptibility than those with the normal allele.

Table (5): Number of patients and controls, sex and age-adjusted OR with 95% CI intervals for MTHFR 677 and 1298.

Mthfr 677	Mthfr 1298	Patients n=31	Controls n=30	OR	95% CI	p value
CC	AA	13(41.9%)	4(13.3%)	1	Reference	-
CC	AC	8(25.8%)	10(33.3%)	0.42	0.16-1.09	0.05
CC	CC	1(3.2%)	6(20%)	0.27	0.11-0.68	0.009
CT	AA	3(9.7%)	4(13.3%)	0.41	0.14-1.2	0.17
CT	AC	4(12.9%)	6(20%)	0.39	0.15-1.06	0.06
TT	AA	1(3.2%)	0	NE	-	-
TT	AC	1(3.2%)	0	NE	-	-
TT	CC	0	0	-	-	-

The description of the distribution of the two genes is demonstrated in table (6); MTHFR and MS in combination. In this analysis, the patients having MTHFR 677 CT/TT with MTHFR 1298 AC/CC and MS 2756 AA/AG were defined as the reference group (group A). The OR for MTHFR 677 CT/TT with MTHFR 1298 AC/CC and MS 2567 AA/GG (group C) was the same for MTHFR 677 CT/TT with MTHFR 1298 AC/CC and MS 2567 GG (group B) and it could not to be estimated because none of the controls had this combination and also for MTHFR 677 AA, MTHFR 1298 CC and MS 2567.

Table (6): Number of patients and controls, sex and age-adjusted OR with 95% CI intervals for MTHFR 677 and 1298.

MTHFR 677 & 1298	MS 2756	Patients n=31	Controls n=30	OR	95% CI	p value
CT/TT & AC/CC						
Group A	AA/AG	3(9.7%)	6(20%)	1	Reference	-
CT/TT & AC/CC						
GroupsB,C	-	13(42%)	2(6.7%)	0.39	0.15-1.02	0.18
Group B						
CC&AA	GG	2(6.5%)	0	NE	-	-
Group C						
AA/AG	11(35.5%)	2(6.7%)	4.33	1.12-16.8	0.03	

DISCUSSION

Folate metabolism plays an essential role in DNA synthesis and methylation processes. Deviation in the flux of folate due to genetic variation could result in selective growth and genomic instability. In addition, this affects susceptibility to various cancers, for example: malignant lymphomas [10]. In the present study, 31 patients and 30 controls were included, and we report an association between susceptibility to malignant lymphoma and polymorphisms in the genes encoding the enzyme MTHFR. Specifically, we found that individuals with at least one MTHFR mutation at 677(C→T) or 1298 (A→C) were less likely to acquire lymphoma.

We observed that MTHFR 677 mutant allele CT (carriers) showed a risk reduction although it was not statistically significant to a decreased risk of 1.2. Whereas, a statistically significant reduction was noted for homozygous MTHFR CC (OR= 0.37, 95% CI 0.2-0.7) which was equivalent to a decreased risk of 2.7. We also examined the joint effects of the two polymorphisms. We found that double heterozygotes (677CT/1298AC) were approximately 2.5 times less likely to develop lymphoma than 677CC/1298 AA individuals would.

Analyses in combination with MTHFR 677, MTHFR 1298 & MS 2756 also showed a positive association, revealing the lowest susceptibility for MTHFR 677, MTHFR 1298 with the MS 2756 AA/AG (OR=4.33; 95% CI 1.11-16.8). Among our controls, the prevalence of T variant of the MTHFR 677 was 33%, lying within the published range of 23-41% for persons of European descent [11]. In the present study, the frequency of the mutant allele of MTHFR 1298 in patients was 45.1%. However, results observed were higher when compared to results of other investigators [1]. It is likely that discrepancies in allele frequency result from ethnic or regional differences.

For MS 2756 polymorphism, the allele frequency observed in this study was 54.8% for patients which is also higher than the results of other investigators [9,12,13,14].

As these enzymes are active in related metabolic pathways, their polymorphisms were evaluated in a combined setting. Results of combined analysis of MTHFR 677 & 1298

illustrated a clear association between folate metabolism and susceptibility to lymphoid malignancies. Folate is an essential nutrient for DNA synthesis, and mutations of MTHFR 677 and MTHFR 1298 reduce enzyme activity. The resultant inhibition of methyl THF leads to increased levels of methylene THF, and this elevation accelerates the methylation of uridylate to thymidylate. Uracil is normally only an RNA base, but it is incorrectly incorporated into DNA if the methylation of uridylate to thymidylate is insufficient during DNA synthesis [15]. Higher rates of occurrence of mis-incorporation raised the incidence of two closely spaced uracils on opposite strands, and excision repair of these close sites may induce double-strand breakage of DNA and this in turn results in carcinogenesis. Approximately 1,000 times higher rates of mis-incorporation of uracil were observed in an experimental model of folate deficiency, and this led to 50 times higher rates of double-strand breakage [15], a possible explanation for genetic instability and occurrence of malignant disease [16].

The effects of MS 2756 A to G polymorphism on homocysteine levels remain controversial [8,17]. Yet the variant MS 2756 G allele has been inversely associated with a number of diseases [11,18,19]. To our knowledge, the hypermethylation of DNA is associated with a higher susceptibility to malignancy. Basically, hypermethylation of specific genes causes lower expression of the coding region. Resultant inactivation of 1 or 2 alleles of the tumor-suppressor genes introduced during development, aging, by carcinogens, or chemotherapeutic drugs could lead to the formation of cells with increased carcinogenic potential [20]. Studies point to associations between lymphoid malignancy and methylation status of specific genes such as p53, p15 and p16 [21,22,23]. Most of these genes have shown a higher frequency of hypermethylation in tumor suppressor genes in patients with lymphoma.

The relation between methylation status and polymorphism of MS enzyme require further investigation before a conclusion can be drawn.

The results of the present study for MTHFR polymorphisms are similar to those published by other authors who performed similar studies on lymphoma patients [24,25]. Moreover, Matsuo et al in 2001 [1] who performed a study on 98

NHL patients, observed that MTHFR 677 CC and 1298 AA genotypes were associated with an odds ratio of 3.83 and these results were not contradicted in another study performed on 111 diffuse large B-cell lymphoma cases [26]. Similarly, Gemmati et al in 2004 [27] reported that individuals carrying the MTHFR CT/TT and MS 2756 AG/GG genotypes showed a 3.6 fold decrease in ALL risk (with an OR=0.28; 95% CI 0.014-0.58) in comparison to wild types. However, single analysis for NHL did not show any significant difference for all the polymorphisms investigated. Matsuo et al in 2004 [28] reported an age-sex adjusted odds ratio for lymphoma patients and they added that subjects harboring MTHFR 677 T or 1298 C alleles relative to 677CC/1298AA genotypes had an OR of 0.58 (95% CI 0.4-0.85, $p=0.002$), and the MS 2756 GG genotype showed an OR of 1.75 (95% CI 0.87-3, $p=0.114$).

In contrast to the presented results, Gonzalez et al. 2000 [29] reported genotype frequencies of MTHFR 677 T allele in a group of 143 patients with lymphoproliferative disorders and 200 controls. The frequencies of the polymorphic allele were similar to our results 35.5% and 32% respectively ($p=0.6$), they found no difference between patients and controls. Moreover, they concluded that MTHFR 677 T allele could be an effective multiple myeloma protective factor especially for the IgG class.

In the present study, we reported a higher susceptibility to malignant lymphoma with the mutant form of MS polymorphism. Similarly Skibola et al in 2004 [10] reported that MS 2756 AG/GG genotypes were associated with an increased risk of NHL (with an OR=1.3; 95% CI 0.99-1.7). Contrary to that, there is a lower susceptibility to colorectal cancer in patients harbouring the mutant allele [18]. The reason for this discrepancy remains unclear, but the pattern of methylation in cancer-related genes and the resultant cell transformation might be different in each cancer type [20].

The threshold of susceptibility may differ among polymorphisms, depending on the biologic mechanism. In line with grouping adopted by studies, we classified the genotypes used in this study into CC versus CT/TT for MTHFR 677, AA versus AC/CC for MTHFR 1298 and AA/AG versus GG for MS 2756.

High folate, low alcohol intake and a younger age may help suppressing the amplifying effect on cancer risk. The mechanism underlying this association may involve a decreased DNA methylation because of disturbed folate metabolism [25].

Neural tube defects are reported to be associated with MTHFR polymorphisms, and folic acid supplementation reduces the risk for their development [30].

In a report concerning colorectal cancer, an insufficient plasma folate level is found to antagonize the protective effect of MTHFR 677 [31]. Greater cancer protection can be obtained from folate supplementation in a population having variant alleles for MTHFR 677 or MTHFR 1298. Nevertheless, this speculation between DNA methylation status and folate supplementation has yet to be evaluated in detail [1].

Furthermore, the present results on MTHFR, and MS polymorphism and their pathogenetic impact on malignant lymphoma are inconsistent with some previous reports, emphasizing the need for larger studies, which may be led by the international consortium on lymphoma research, which in turn will be able to provide support to truly significant findings through much larger combined data sets.

In conclusion, the present study provided evidence that MTHFR mutant alleles are associated with a lower susceptibility to malignant lymphoma and that the MS mutant alleles are associated with a higher susceptibility to it. This suggests that folate and methionine metabolism play an important role in lymphoma genesis.

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