

Synergistic Effect of Methyltetrahydrofolate Reductase (MTHFR) C677T and A1298C Polymorphism as Risk Modifiers of Pediatric Acute Lymphoblastic Leukemia

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ABSTRACT

Background and Purpose: ALL is the most common pediatric cancer. The causes of the majority of pediatric acute leukemia are unknown and are likely to involve an interaction between genetic and environmental factors. Therefore, unfavourable gene-environmental interactions might be involved in the genesis of ALL. The aim of this work was to evaluate, in a case-control study, whether the common polymorphisms in 5, 10-methylenetetrahydrofolate reductase (MTHFR) namely (C677T and A1298C) and methionine synthase (MS) (A2756G) genes may play a role in altering susceptibility to pediatric ALL as individual genes and in combination.

Patients and Methods: DNA of 88 ALL patients (age ≤ 18 years) and 311 healthy control subjects was analyzed for the polymorphisms of MTHFR and MS genes using PCR-RFLP method.

Results: The frequencies of the wild types of MTHFR 677CC, MTHFR 1298AA and MS 2756AA, the homozygous genotypes of MTHFR 677TT, MTHFR 1298CC and MS 2756GG and heterozygous genotypes of MTHFR 677CT and MS 2756AG showed no statistically significant differences between patients and controls. The frequency of the MTHFR 1298AC heterozygous genotype was 25% among patients compared to 45.0% among controls; the difference was found to be statistically significant (p value = 0.001, O.R = 0.382 & 95% C.I = 0.222-0.658).

The frequency of the MTHFR 1298AC heterozygous genotype plus 1298CC homozygous genotype was 34% among patients compared to 54.3% among controls and the difference was statistically significant (p value = 0.001). A synergistic effect of 677CT and 1298AC (CTAC) was observed, (p value = 0.002) with 3.65 fold protection (OR 0.273 & 95% C.I = 0.155-0.9) compared to 2.6 folds for MTHFR 1298AC alone. This protective effect of CTAC

polymorphism was abolished when combined with MS 2756AA or AG.

Conclusion: The present study provided further evidence for the protective role of MTHFR 1298AC mutant alleles in acute lymphoblastic leukemia in children (2.6 fold protection). This suggests that folate and methionine metabolism play an important role in the pathogenesis of pediatric ALL. In contrast to the main bulk of literature, we did not find any protective role of either MTHFR C677T or MS A2756G polymorphisms. This may reflect the ethnic variation in both the polymorphism frequencies, variation in plasma level of folate, in addition to the possible role of gene-environment interaction mainly dietary availability of folate. The synergistic effect of MTHFR 1298AC and 677CT and its abolishment by MS 2756AA or AG further emphasizes that the interaction of genes, rather than the polymorphism in any single one, determines risk susceptibility to disease.

Key Words: MTHFR – Polymorphism – Pediatric ALL.

INTRODUCTION

Acute lymphoblastic leukaemia (ALL) is the most common pediatric leukemia accounting for 25-30% of all cases of childhood malignancies [1]. Although the clinical, pathological and immunophenotypic features of the disease are well documented, little is known about leukemogenesis [1]. The causes of the majority of pediatric acute leukemias are unknown and likely to involve an interaction between genetic and environmental factors. Unfavourable gene-environment interactions might be involved in the genesis of ALL [2]. Leukemia commonly arises as a result of DNA translocations, inversions or deletions in genes regulating blood cell development or homeostasis. Folate deficiency has been associated with uracil misincorporation

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into DNA and DNA double strand breaks during uracil excision repair, thus increasing the risk of chromosomal aberrations [3]. Defects or polymorphisms in the genes of the folate-dependent enzymes and deficiencies of micronutrients may influence cancer susceptibility (4-7). The 5, 10-methylenetetrahydrofolate reductase (MTHFR) directs 5, 10-methylenetetrahydrofolate (5, 10-methylene-THF) towards methionine synthesis at the expense of DNA synthesis. Two MTHFR polymorphisms namely C677T and A1298C are associated with reduced enzyme activity and C677T with altered distribution of intracellular folate [8]. A lower MTHFR activity leads to increased plasma levels of homocysteine and decreased 5-methyl-THF formation. This contributes to the pool of methylene-THF, the methyl group donor for the conversion of dUMP to dTMP by thymidylate synthase enzyme. Therefore, it is thought that lower MTHFR activity might favour optimal DNA synthesis by reducing the uracil misincorporation rate, a potential cause of double stranded breaks during excision repair processes [5,9].

The importance of MTHFR in cancer susceptibility arises from its involvement in 2 pathways of folate metabolism. One leads to numerous methylation processes that are dependent on S-adenosyl-methionine (SAM), while the other, via thymidylate synthesis, contributes to DNA replication and cell division. Reduced activity of MTHFR may decrease the methylation of homocysteine to methionine and in turn the level of SAM, resulting in DNA hypomethylation. On the other hand, the reduced level of MTHFR substrate, 5,10-methylene-THF, required for thymidylate synthesis could lead to uracil misincorporation into DNA, diminished DNA repair and increased frequency of chromosomal breaks and damage. Malignancies that are derived from rapidly proliferating tissues, which have a higher requirement for DNA synthesis should be more susceptible to folate deficiency and resultant DNA damage. The DNA variants causing the reduced MTHFR activity were found to be associated with the reduced risk of leukemia, lymphoma and colorectal carcinoma. The mechanism proposed to explain these associations was the shunt of folate metabolism versus thymidine and purine synthesis, which would slow the incorporation of uracil into DNA and protect against carcinogenesis [10].

Investigations on mutations in the MTHFR gene focused on the catalytic domain and the two polymorphisms C677T and A1298C, which slightly change enzymatic activity. In case of C677T polymorphism, the cytosine base at position number 677 changes to thymidine base, which in turn affects the amino acid sequence at position number 222 (Alanine → Valine). The MTHFR C677T variants are MTHFR 677CC denotes wild type (most common), MTHFR 677CT denotes heterozygous genotype and MTHFR 677TT denotes homozygous genotype. The MTHFR enzyme with such polymorphism becomes thermolabile, causing a loss of its activity with increased temperature. The modified protein loses its cofactor FAD more quickly and has a lower stability. The mutation effect can be suppressed by addition of folate, which causes a higher FAD affinity and an increase in MTHFR stability. The MTHFR A1298C polymorphism is localized in the coding regulatory region domain [11].

Increased levels of homocysteine can also be the result of improper methionine synthase (MS) function. The substrate of this enzyme is 5-methyltetrahydrofolate, produced in reactions catalyzed by MTHFR and is the donor of methyl group for methionine (Met) synthesis from homocysteine (Hcy) [12].

The most common polymorphism in the MS gene is substitution A2756G, which leads to a change of aspartic acid to glycine (D919G). This D919G polymorphism probably leads to decreased methionine synthase activity and increases the cellular homocysteine level [12]. Methionine synthase (MS) catalyses the transfer of the methyl group from 5-methyl-THF to homocysteine generating tetrahydrofolate and methionine, thus playing a critical role in maintaining adequate intracellular methionine concentrations. The commonly identified polymorphism (MS A2756G) is thought to affect enzymatic activity [13] and to induce modest homocysteine reduction [14] and DNA hypomethylation [15].

Apart from gene polymorphisms, change in folate supply influences nucleic acid synthesis, DNA repair and methylation.

A relationship between plasma folate levels, the content of uracil and DNA damage in dividing cells renders the MTHFR and MS genes a

suitable candidate for studies of leukemia susceptibility.

In the last few years there has been a growing interest in the possible association between folate-related polymorphisms and the risk of lymphoid malignancies. Skibola et al. [16] and Matsuo et al., [15] independently reported that individuals with either MTHFR polymorphism had a significant lower susceptibility to adult ALL and malignant lymphoma. Similar findings for both ALL and NHL in adults were reported by Gemmati et al. [2]. Comparable results were obtained by Franco et al. [17] and Wiemels et al. [18] in pediatric leukaemia.

In this work, we analysed genetic polymorphism of MTHFR 677 and 1298 and MS in 88 pediatric ALL patients and 311 healthy controls to evaluate, in a case-control study, the impact of these polymorphisms as risk modifiers in pediatric ALL.

PATIENTS AND METHODS

The study included 88 precursor B ALL Egyptian pediatric patients. All patients presented to the Pediatric Oncology Department, NCI, Cairo University during the period from January to September 2003. They included 56 males and 32 females with an age range of 1.5 to 18 years and a median of 6 years. All patients were diagnosed according to standard methods including blood picture, bone marrow, cytochemistry and immunophenotyping.

The control group consisted of 311 Egyptian healthy individuals randomly selected from blood donors with ages ranging from 18 to 48 years. Informed consent was obtained from donors and patients' parents and the protocol was approved by the Institutional Research Board.

Genotype analysis: Five mL peripheral blood samples, anticoagulated by EDTA were obtained. The mononuclear cell layer was separated on Ficoll Hypaque [19]. DNA was isolated from peripheral blood at diagnosis, as described by Davis et al. [20] using the salting out technique. Cell pellets were mixed with proteinase K buffer, 20% sodium dodecyle sulfate and proteinase K enzyme (20 mg/mL), incubated at 56°C for 1 hour or at 37°C overnight, equal volume of 4M ammonium acetate was added, mixed thoroughly and an equal volume of isopropanol was then added. With a gentle swirl, the formed precipitate was removed and washed with 70% cold ethanol then dried and the precipitate resuspended in Tris/EDTA (TE) buffer. DNA concentration was determined by spectrophotometry, measuring the optical density at 260 nm and 280 nm of the prepared diluted DNA (1 O.D at 260 nm unit =50 µg/mL) and 260nm/280nm of 1.1-2 was obtained.

PCR amplification was performed using specific primers for each locus (Table 1), the PCR conditions were as follows: initial denaturation at 95°C for 10 minutes, 35 cycles of 45 seconds at 95°C, 45 seconds at 59°C, and 1 minute at 72°C and a last elongation step at 72°C for 7 minutes [16].

The amplicons were digested with restriction enzymes HinfI for MTHFR 677 (Fig. 1-A), MboII for MTHFR 1298 (Fig. 1-B) and HaeIII for MS (Fig. 1-C) according to the manufacturer's instructions.

The amplified digested products were separated on 3% agarose gel at 100 volts for 30 min. A 50 bp DNA marker was included in each run. The obtained band sizes for different polymorphisms are shown in Table (2).

Table (1): The oligonucleotide primer pairs used for amplification of the candidate genes.

Primer	Primer sequence	Specificity	Ref
<i>MTHFR677:</i>		MTHFR C677T	(16)
Forward	TGA AGG AGA AGG TGT CTG CGG GA		
Reverse	AGG ACG GTG CGG TGA GAG TG		
<i>MTHFR1298:</i>		MTHFR A1298C	(16)
Forward	CTT TGG GGA GCT GAA GGA CTA CTA C		
Reverse	CAC TTT GTG ACC ATT CCG GTT TG		
<i>MS2756:</i>		MS A2756G	(15)
Forward	TGT TCC AGA CAG TTA GAT GAA AAT C		
Reverse	GAT CCA AAG CCT TTT ACA CTC CTC		

Table (2): Restriction endonucleases used for determination of the polymorphisms of candidate gene and the characteristic patterns of respective DNA fragments.

Position and mutation of genes	Restriction enzyme	Restriction condition	Fragment patterns	Reference
MTHFR C677T	Hinf I	6U at 37°C overnight	198 bp, 175bp, 23bp	(16)
MTHFR A1298C	Mbo II	5U at 37°C overnight	56bp, 31bp, 30bp, 28, 18bp, 84bp, 31bp, 30bp, 18bp.	(16)
MS A2756G	Hae III	4U at 37°C overnight	211 bp.131bp, 80bp	(15)

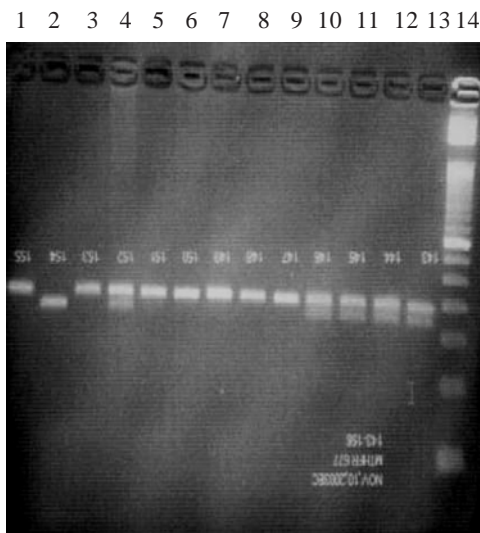


Fig. (1-A): Pattern of MTHFR C677T polymorphism by HinfI digest.

Lane 1, 3, 5-9: Wild (198 bp).
 Lane 2: Homo (175, 23 bp).
 Lane 4 & 10-13: Hetero (98, 175, 23 bp).
 Lane 14: 50bp marker.



Fig. (1-B): Pattern of MTHFR A1298C polymorphism by MboII digest.

Lane, 1 & 7-9: Hetero (84, 56, 31, 30, 28, 18bp).
 Lane, 2 & 3: Wild (56bp, 31bp, 30bp, 28, 18bp).
 Lane, 4 & 5: Homo (84bp, 31bp, 30bp, 18bp).
 Lane 6: Empty.
 Lane 10: 50 bp Marker.

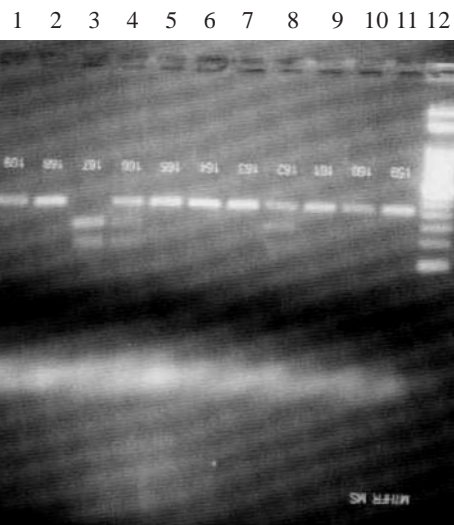


Fig. (1-C): Pattern of MS A2756G polymorphism by Hae III digest.

Lane 1, 2, 5-7 & 9-11: Wild (211bp).
 Lane 3: Homo (131, 80 bp).
 Lane 4 & 8: Hetero (211, 131, 80 bp).
 Lane 12: 50 bp Marker.

Statistical analysis:

The level of significance was calculated by the Chi Square or Fisher's exact test. Odds Ratio (OR) was calculated as an estimate of the relative risk of having the disease according to the relative frequency of different genotypes among patients and controls. ORs are given with 95% Confidence Interval (CI). *p*-value was considered significant at ≤ 0.05 . All of the statistical tests were performed using the SPSS version 12 software.

RESULTS

We studied 88 precursor B ALL pediatric Egyptian patients and 311 individuals, randomly selected from Egyptian healthy blood donors, as control group.

The frequency of MTHFR C677T, MTHFR A1298C and MS polymorphism in patients and controls is shown in Table (3).

The frequency of the MTHFR 1298AC heterozygous genotype was lower among patients (22/88, 25%) compared to controls (140/311,

45%) and the difference was statistically significant (*p* value =0.001, O.R=0.382 & 95% C.I =0.222-0.658) imposing a 2.6 fold protection. Also, the frequency of the MTHFR 1298AC heterozygous genotype plus 1298CC homozygous genotype was significantly lower among patients (*p* value =0.001 O.R=0.432 & 95% C.I =0.263-0.707) compared to controls imposing a 2.3 fold protection (Table 3).

When the combined genotype of MTHFR A1298C and C677T polymorphisms was compared with the reference genotype CCAA (Table 4), a synergistic effect of MTHFR 677CT and MTHFR 1298AC (CTAC) was observed (*p* value=0.002, OR 0.273 & 95% C.I=0.155-0.900) with 3.65 fold protection compared to 2.6 folds for MTHFR 1298AC alone. This protective effect of MTHFR CTAC polymorphism was abolished when combined with MS 2756AA or AG (Table 5).

The difference in the frequency of MTHFR C677T between our study population and various ethnic groups is shown in Table (6).

Table (3): The frequency of MTHFR C677T, MTHFR A1298C and MS polymorphism in childhood ALL compared to normal controls.

Genotype	Controls		ALL cases		<i>p</i> value	Odds ratio	95% confidence interval
	No	(%)	No	(%)			
MTHFR677	311	(100)	88	(100)			
CC	156	(50.2)	39	(44.3)		1	Reference
CT	135	(43.4)	42	(47.7)	0.451	0.848	0.576-1.246
TT	20	(6.4)	7	(8)	0.456	0.771	0.384-1.548
CT+TT	155	(49.8)	49	(55.6)	0.398	0.837	0.577-1.214
MTHF1298	310	(100)	88	(100)			
AA	141	(45.5)	58	(65.9)	–	1	Reference
AC	140	(45.2)	22	(25)	0.001	0.382	0.222-0.658
CC	29	(9.4)	8	(9.1)	0.428	0.904	0.747-1.095
AC+CC	169	(54.5)	30	(34)	0.001	0.432	0.263-0.707
MS	306	(100)	87	(100)			
AA	194	(63.4)	55	(63.3)			
AG	97	(31.7)	29	(33.3)	0.793	1.016	0.905-1.141
GG	15	(4.9)	3	(3.4)	0.772	0.939	0.756-1.166
AG+GG	112	(36.6)	32	(36.7)	0.94	1.006	0.902-1.122

p value in bold face: Significant difference (≤ 0.05).

Table (4): MTHFR C677T and A1298C polymorphism among Egyptian pediatric ALL cases and controls.

Sequence of MTHFR 677/1298	ALL cases (n=88)		Controls (n=306)		p value	Odds ratio	95% confidence interval
	No	(%)	No	(%)			
CCAA	18	(20.5)	55	(17.9)		R G*	
CCAC	14	(15.9)	75	(24.5)	0.170	0.894	0.763-1.048
CCCC	6	(6.8)	24	(7.8)	0.798	0.942	0.754-1.176
CTAA	32	(36.3)	68	(22.2)	0.08	1.441	0.912-2.412
CTAC	8	(9.1)	62	(20.2)	0.002	0.273	0.155-0.900
CTCC	1	(1.2)	5	(1.6)	0.663	0.816	0.557-1.196
TTAA	7	(7.9)	17	(5.5)	0.883		

*Reference group. The combined effect of A1298C and C677T polymorphisms was studied. All combinations abolished the protective effect of 1298AC. Only CTAC gave a synergistic effect where it provided a 3.6 fold protection.
p value in bold face: Significant difference (≤ 0.05).

Table (5): MTHFR C677T & A1298C and MS A2756G polymorphism interaction among ALL cases and controls.

	ALL cases (n=88)		Controls (n=306)		p value	Odds ratio	95% confidence interval
	No	(%)	No	(%)			
CCAAAA	13	(14.9)	33	(10.5)		R G*	
CCAAAG	2	(2.2)	18	(5.8)			
CCAAGG	1	(1.1)	1	(0.3)			
CCACAA	9	(10.3)	47	(15)	0.11	2.05	0.952-3.254
CCACAG	4	(4.3)	25	(8)			
CCACGG	1	(1.1)	3	(1.0)			
CCCCAA	2	(2.2)	14	(4.5)			
CCCCAG	4	(4.3)	9	(2.9)			
CTAAAA	19	(21.8)	43	(13.7)	0.834	0.922	0.509-1.669
CTAAAG	10	(11.4)	19	(6.1)			
CTAAGG	1	(1.1)	4	(1.3)			
CTACAA	6	(6.8)	40	(12.8)	0.121	2.167	0.902-5.207
CTACAG	2	(2.2)	18	(5.8)	0.124	2.826	0.702-11.381
TTAAAA	3	(3.2)	9	(2.9)			

*Reference group. The protective effect of CTAC polymorphism was abolished when combined with MS 2756AA or AG.
p value in bold face: Significant difference (≤ 0.05).

Table (6): Comparison of the frequencies of MTHFR C677T among healthy Egyptians and various Ethnic groups.

Ethnic group	Wild type CC	Hetero-zygous CT	Homo-zygous TT	N	Reference	P.value compared to Egyptians
Egyptians	156 (49.8%)	135 (43.1%)	20 (6.4%)	311	Current study	
French-canadian	126 (42.0%)	128 (42.7%)	46 (15.3%)	300	(10)	0.001
Italian	35 (31.8%)	55 (50%)	20 (18.2%)	110	(22)	<0.001
Italy caucasian	78 (30.3%)	128 (49.8%)	51 (19.8%)	257	(2)	<0.001
German caucasian	184 (48.5%)	152 (40.1%)	43 (11.3%)	379	(23)	0.08
Japanese	81 (33.3%)	126 (51.9%)	36 (14.8%)	243	(15)	<0.001
U.K	89 (44.5%)	79 (39.5 %)	32 (16%)	200	(18)	0.002
Brazilian	22 (30.9%)	36 (50.7%)	13 (18.3%)	71	(17)	<0.001

p value in bold face: Significant difference (≤ 0.05).

DISCUSSION

ALL in children offers a unique opportunity to examine the effect of carcinogen-metabolism genes in the risk of pediatric cancers. The young age of patients and, thus, a short latency period between the appearance of the initiating mutation and the detection of tumor cells should facilitate the identification of risk factors, as compared with adult cancer patients in whom many factors come into play because of long latency periods [21].

The difference in the frequency of MTHFR C677T between our study population and various ethnic groups is shown in Table (6). The frequency of MTHFR 677T allele among our population was statistically significantly lower than those reported for Caucasians, only the German population [23] did not show a statistically significant difference when compared to our population.

As regards the frequencies of MTHFR 1298C allele, our results are similar to those of

other populations (Table 7), but significantly higher than the Japanese population (p value = <0.001) [15]. The differences among different groups, including our data, reflect the expected ethnic variability between different populations.

In the current study population, all individuals with the MTHFR 677TT (homozygous mutant) genotype had the MTHFR 1298AA (wild) genotype and those with the MTHFR 1298CC (homozygous mutant) genotype had the MTHFR 677CC (wild) genotype. Also no homozygous carriers of both T677 and C1298 alleles were observed. Similar findings were reported by other investigators [10,16]. Although there was no evidence of statistical interaction between MTHFR 677 and MTHFR 1298, a linkage between the two was observed indicating linkage disequilibrium between the 2 site variants [10,16].

The frequencies of MS polymorphisms A2756G among our population and the available data from the Italian Caucasian [2] and Japanese population [15], were comparable (Table 8).

Table (7): Comparison of the frequencies of MTHFR A1298C among healthy Egyptians and various ethnic groups.

Ethnic group	Wild type AA	Hetero-zygous AC	Homo-zygous CC	N	Reference	p value compared to Egyptians
Egyptians	141 (45.5%)	140 (45.2%)	29 (9.4%)	310	Current study	
French-canadian	150 (50%)	119 (39.7%)	31 (10.3%)	300	(10)	0.39
Italian	56 (50.9%)	49 (44.5%)	5 (4.6%)	110	(22)	0.24
Italy caucasian	126 (49.0%)	110 (42.8%)	21 (8.2%)	257	(2)	0.68
German caucasian	153 (40.4%)	174 (45.9%)	52 (13.7%)	379	(23)	0.15
Japanese	159 (65.4%)	75 (30.8%)	9 (3.7%)	243	(15)	<0.001
U.K	93 (47%)	83 (42%)	23 (11%)	200	(18)	0.62
Brazilian	41 (57.7%)	28 (39.4%)	2 (2.8%)	71	(17)	0.07

p value in bold face: Significant difference (≤ 0.05).

Table (8): Comparison of the frequencies of MS A2756G among healthy Egyptians and various ethnic groups.

Ethnic group	Wild type AA	Hetero-zygous AC	Homo-zygous CC	N	Reference	p value compared to Egyptians
Egyptians	194 (63.4%)	97 (31.6%)	15 (4.9%)	306	Current study	
Italy caucasian	158 (61.5%)	89 (34.6%)	10 (3.9%)	257	(2)	0.68
Japanese	1156 (64.2%)	81 (33.3%)	6 (2.5%)	243	(15)	0.33

p value in bold face: Significant difference (≤ 0.05).

Several case-control studies have been conducted to investigate the presence of a relationship between gene variants and the impact of MTHFR polymorphisms as a risk modifier in

susceptibility to ALL. Most of the studies showed a protective effect of the enzyme variants [2,10,16-18], while others failed to detect such results [22,23].

Data in this study documented the protective effect of MTHFR 1298AC genotype (2.6 fold protection), while the homozygous MTHFR 1298CC genotype did not show this protective effect which may be due to the smaller number of homozygous cases. However, combined analysis of MTHFR 1298AC and MTHFR 1298CC showed a protective effect of 2.3 folds. Also the MTHFR 677CT and TT lacked the protective effect. In this work, the combined effect of MTHFR C677T and MTHFR A1298C polymorphisms was studied. All combinations abolished the protective effect of MTHFR 1298AC, except for MTHFR 677CT and MTHFR 1298 AC (CTAC) which gave a synergistic effect where it provided a 3.6 fold protection compared to a 2.6 fold protection for 1298 AC alone. This is in agreement with the results of Skibola et al. [16] who reported that the 1298AC polymorphism was associated with a 3.0 fold protection (OR=0.33; 95% CI=0.15-0.73). They also reported, in contrast to our data, that the MTHFR 677TT was associated with a 4.3 fold protection (OR=0.23; 95% CI=0.06-0.81) and that 677CT/1298AC individuals were at a 5.6 fold decreased risk of developing ALL.

Also in contrast to our data, four more studies reported a reduced risk of ALL associated with MTHFR variants [2,10,15,17]. A case-control study conducted on a French-Canadian origin population sample (270 pediatric ALL patients and 300 healthy controls) reported that the TT677/AA1298 and CC677/CC1298 individuals were associated with reduced risk of ALL of 2.5 and 3.3 (OR=0.4; 95% CI=0.2-0.9 and OR=0.3; 95% CI, 0.1-0.6); respectively [10]. Also, an Italian study [2] demonstrated that individuals carrying the MTHFR 677TT genotype showed a 3.6 fold decreased risk of ALL (OR=0.28 and 95% CI=0.12-0.72). A study, performed by a Brazilian group [17] reported a 2.4 fold decreased risk of ALL linked to MTHFR 677T whereas the 1298AC polymorphism did not significantly affect the ALL risk. However, these calculations were based on small numbers of compound carriers (71 cases). Similarly, a United Kingdom study (18) on 253 pediatric ALL cases and 200 controls, reported 3.2 fold protection for carriers of C677T from MLL positive leukemias (OR =0.36, 95% CI=0.15-0.85 and $p=0.017$) and not others (TEL-AML1 positive leukemias). Though we did not specifically discriminate by molecular typing, yet the incidence of MLL gene in

our cases is 1.5% [24]. Thus the majority are MLL negative. This protective effect was not evident for A1298C alleles (OR=1.14), [18].

However, this protective effect for both MTHFR C677T and A1298C was denied by other workers [22,23].

The frequencies of methionine synthase polymorphisms A2756G among our population and the available data from the Italian Caucasian and Japanese population are comparable (p value=0.68 and 0.33, respectively) (Table 8).

In our study, the MS polymorphism showed no impact either alone or in combination with the MTHFR polymorphisms; even the protective effect of MTHFR CTAC was abolished by the presence of the wild or heterozygous type of MS (MS AA or AG). Only few studies investigated the impact of MS polymorphism on ALL risk modification. In agreement with our results, Skibola et al. [25] reported no protective effect from adult ALL (OR=0.79; 95% CI, 0.38-1.7) but in contrast a 5 and 3.8 fold protection of MS 2756GG was reported [2,15].

In general, the protective effect of polymorphism supports the idea that increased availability of 5, 10-MTHF would reduce uracil incorporation and hence reduce the risk of leukemogenesis.

The controversy in the literature has been attributed to many factors including sample size, age group and ethnic variability. The most likely explanation can be withdrawn from the work of Krajcinovic et al. [10], who documented a 2.5 and 3.3 fold protection for MTHFR TT677/AA1298 and MTHFR CC677/CC1298 respectively in 270 ALL patients and 300 healthy control of French Canadian origin. The MTHFR TT677/AA1298 individuals were associated with reduced risk of ALL. However, in 1994, the Ministry of Health in Canada recommended folate supplementation during pregnancy [26]. Therefore, analysis of the impact of MTHFR genotypes on ALL risk in children born before and after January 1996 was done, assuming that choosing January 1996 allowed enough time to implement this recommendation. Indeed, the profound protective effect of MTHFR TT677/AA1298, MTHFR CC677/CC1298 and MTHFR CC677/AC1298 on individuals was only observed in the group of children who were born

before 1996. This may explain the diverging results with regard to pediatric ALL and is further supported by a recent study of Balta and colleagues from Turkey, where in 142 pediatric ALL patients diagnosed between February 2000 and February 2002, no significant association between the MTHFR 677CT polymorphism and ALL was detected [27]. Also the conflicting results among studies investigating MTHFR polymorphisms and the risk of cancer may be due to the lack of information on dietary folate intake or other measures of overall folate status. Individuals with the MTHFR 677TT genotype with normal plasma folate concentrations have been found to be at decreased risk of cancer relative to those with the CC genotype [28,29]. This shows that risk modification depends on the folate status. In situations where there is abundant intracellular folate, the folate molecule may be able to hold the variant MTHFR protein in the appropriate and functional 3 dimensional structure [30,31].

In line with that explanation, the Egyptian population is known to consume a large amount of green leaf vegetables rich in folic acid, thus abolishing the protective effects of most variants.

In conclusion, we report similarities and variabilities in the relative frequencies of MTHFR and MS genotypes as compared to other populations; a finding that reflects natural ethnic variability. In our population, decreased susceptibility to pediatric ALL was affected only by MTHFR A1298C. Accordingly, genetic contribution to risk susceptibility to cancer (pediatric ALL in our study) could vary between different ethnic groups with different environmental backgrounds. This necessitates that such studies have to be performed locally in each community in order to detect population at risk and potential environmental hazards.

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