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Original Article

MTHFR A1298C and *C677T* gene polymorphisms and susceptibility to chronic myeloid leukemia in Egypt

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Abstract: Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme regulating the intracellular folate metabolism which plays an important role in carcinogenesis through DNA methylation. We aimed to evaluate the association between *MTHFR* A1298C and C677T polymorphisms and the risks of chronic myeloid leukemia (CML). Eighty-five patients with CML and a control group containing 100 healthy, age and sex matched individuals were examined for *MTHFR* C677T and A1298C polymorphisms using polymerase chain reaction-restriction fragment-length (PCR-RFLP) method. The frequency of 677TT genotype in patients with CML was significantly higher compared to controls (OR = 2.513, 95% CI: 0.722-4.086, *P* = 0.025). No such association was shown for heterozygous *677CT* (OR = 1.010, 95% CI: 0.460-2.218, *P* = 0.981). Moreover, for A1298C genotype, a statistically significant higher frequency of 1298CC was also detected in CML patients compared to control group (OR = 1.1816, 95% CI: 0.952-3.573, *P* = 0.036), 0.036). No such statistical significance was demonstrable for heterozygote *1298AC* (OR = 1.046, 95% CI: 0.740-1.759, *P* = 0.092). In addition, patients with joint 677CT/1298AC or 677TT/1298CC genotypes showed an association with increased risk of CML (OR = 1.849, 95% CI: 0.935-2.540, *P* = 0.024; OR = 1.915, 95% CI: 1.202-3.845, *P* = 0.020 respectively). A statistically significant increased risk of resistant to therapy was observed with *677CT* and *1298AC* genotypes (*P* = 0.001, *P* = 0.002 respectively). We conclude that both *MTHFR* 677TT and 1298CC polymorphisms have been associated with risk of CML and both 677CT and 1298AC genotypes are associated with higher risk of resistant to therapy.

Keywords: MTHFR, CML, polymorphism

Introduction

The etiology of most types of leukemia remains unknown. The established causes of leukemia such as ionizing radiation and cancer chemotherapy account for only a small percentage of the total cases [1, 2]. Leukemias commonly arise as a result of DNA translocations, inversions, or deletions in genes regulating blood cell development or homeostasis. Folate deficiency has been implicated in increasing the risk of chromosomal aberrations because it is associated with uracil misincorporation into DNA. *MTHFR* is an enzyme gene located on the short arm of chromosome 1 at 1p36.3. The cDNA sequence of this gene is 2.2kb long and is composed of 11 exons (103 to 432 bp) [3]. It is a key enzyme in folate and homocysteine (Hcy) metabolism which catalyzes the reduction of 5, 10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, the predominant circulatory form of folate and carbon donor for the remethylation of homocysteine to methionine [4].

There are two common genetic polymorphisms that have been shown to decrease the activity of *MTHFR*, which are the C677T mutation at codon 222, and the A1298C mutation at codon 429. Hyperhomocysteinemia is a consequence of single nucleotide polymorphisms (SNPs) in *MTHFR* 677 C>T that can cause homocysteine levels in the blood to increase, usually exceeding 15 μmol/L. *MTHFR* 677 C>T is the result of a missense mutation occurring at position 222, where alanine is substituted with valine. The mutation results in a thermolabile *MTHFR* that has lower enzyme activity at 37°C, leading to increased total plasma homocysteine [5].

Table 1. Age distribution according to C677T and A1298C genotypes

Polymorphism		CML (n = 85)				Control (n = 100)		
		No.	Mean	SD	P*	No.	Mean	SD
Total		85	46.69	12.717	0.500	100	48.22	11.870
C677T	CC	30	45.85	14.479	0.592	45	47.51	13.600
	CT	44	48.87	11.868	0.097	49	39.71	14.496
	TT	11	38.00	14.259	0.937	6	42.00	19.519
A1298C	AA	32	45.00	16.820	0.917	40	44.50	14.787
	AC	38	48.13	11.071	0.294	51	45.00	14.365
	CC	15	42.50	15.175	0.418	9	37.07	15.633

P: Significance between AML versus control group. P*: Significance between CML versus control group.

The C677T polymorphism occurs in exon 4 and results in an alanine-to-valine substitution at codon 222. The polymorphism lies at the base of the binding site for the MTHFR cofactor, flavin adenine dinucleotide (FAD) [6]. Individuals with the *MTHFR* C677T TT genotype have been shown to have 30 percent in vitro *MTHFR* enzyme activity compared with the wild type, whereas those with the heterozygous (CT) genotype have been found to have 60 percent wild-type MTHFR enzyme activity [7]. A1298C in exon 7, results in a glutamate-to-alanine substitution at codon 429. A1298C polymorphism leads to diminished enzyme activity and the allele frequencies up to 33% have been reported previously for this polymorphism [8, 9]. Thus, different leukemias are most likely the result of an adverse gene environment interaction, with susceptibility being related to polymorphisms in multiple genes. Two *MTHFR* polymorphisms, C677T and A1298C, have been associated with reduced enzyme activity.

CML is a clonal hematopoietic malignancy that is characterized by translocation between chromosomes 22 and 9 drives the leukemic changes in CML [10]. Although the t [9, 22] translocation is the primary cause, findings of recent studies suggest that single nucleotide polymorphisms in various critical genes may affect the risk of CML. Examples for polymorphic genes associated with increased risk for CML include the *MTHFR* (A1298C) [11], *FLT3* (D324N) [12], *NQO1* [13], and *p53* (codon 72) [14].

Several studies have been completed regarding the correlation between the presence of C677T and A1298C polymorphisms of the *MTHFR* gene and the risk of different leukemias that mostly indicate a lower risk of acute lymphoid leukemia (ALL) in people possessing 677TT variant [4, 15]. However, the findings about this correlation in other acute and chronic leukemias are controversial [16, 17]. In the present study, we investigated whether C677T and A1298C polymorphisms might affect the risk for CML.

Materials and methods

Study population

This study consisted of blood or bone marrow samples 85 adult CML outpatients of both genders, who were diagnosed by RT-PCR and immunophenotyping. Patients were treated in hematology Oncology Centre, Mansoura, Egypt. The control group consisted of 100 healthy volunteers of both genders. All procedures involving human subjects were approved by the Medical Ethical Committee of Mansoura School of Medicine. Written informed consent was obtained from all registered volunteers.

MTHFR genotyping

DNA from patients and controls was extracted from peripheral blood samples collected in EDTA tubes using the DNA purification kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Amplification of the C677T region was performed using the forward primer: 5-TGAAGGAGAAGGTGTCTGCGGGA-3 and the reverse primer: 5 AGGACGGTGCGGTGAGAGTG-3. Amplification of the A1298C region was performed using; forward primer 5-GCA AGT CCC CCA AGG AGG-3 and reverse primer 5-GGT CCC CAC TTC CAG CAT C-3. Each PCR reaction of 25 μL contains the components: 10 Mm Tris HCL, 1.5 Mm $MgCl_2$, 50 mM KCl, 1U Taq polymerase, 400 uM dNTP and 0.5 mM primer. Samples were amplified using the following PCR conditions: 5 min of initial denaturation at 95°C, followed by 40 cycles of 95°C for 30 sec, 60°C for 60 sec, and 72°C for 60 sec, with a final extension at 72°C for 7 min. The amplified PCR products of C677T and A1298C were separately digested with HinfI and MboII restriction enzymes (New England Biolabs, USA), respectively. After digestion, products were subjected to electrophoresis on 2% agarose. The digestion fragment sizes for C677T genotypes were: a single 198 bp band

Table 2. Sex distribution according to C677T and A1298C genotypes

Polymorphism		Case					Control					P
		Total	Male (n = 40)		Female (n = 45)		Total	Male (n = 49)		Female (n = 51)		
			No	%	No	%		No	%	No	%	
C677T	CC	30	14	46.6	16	53.3	45	23	51.1	22	48.8	0.592
	CT	44	20	45.4	24	54.5	49	23	46.9	26	53.0	0.531
	TT	11	6	54.5	5	45.4	6	3	50.0	3	50.0	0.486
		p	0.379				P	0.820				
			Male (n = 37)		Female (n = 48)			Male (n = 50)		Female (n = 50)		
			No	%	No	%		No	%	No	%	
A1298C	AA	32	17	53.1	15	46.8	40	16	40	24	60.0	0.149
	AC	38	16	42.1	22	57.8	51	29	56.8	22	43.1	0.215
	CC	15	4	26.6	11	73.3	9	5	55.5	4	44.4	0.380
		p	0.310				p	0.206				

Table 3. Association between patients' laboratory findings and MTHFR genotypes

		Geno type	CML (n = 85)			*p*	Contro (n = 100)			*p*
			No.	Mean	SD		No.	Mean	SD	
C677T	TLC ($\times 10^9$/L)	Total	85	68.1747	77.71733		100	7.70	2.24	
		CC	30	83.4408	97.22829	0.266	45	7.918	2.30	0.301
		CT	44	42.1200	24.75076		49	7.50	2.17	
		TT	11	66.6500	39.14916		6	6.00	1.00	
	Hemoglobin (g/dL)	Total	85	10.4533	1.77221		100	13.4750	.87359	
		CC	30	10.5077	1.70972	0.294	45	13.4265	.90341	0.669
		CT	44	10.7067	1.74088		49	13.5179	.84110	
		TT	11	9.1500	2.22486		6	13.8667	.83267	
	Platelet count ($\times 10^9$/L)	Total	85	1.90.11	154.79		100	299.95	76.82	
		CC	30	179.16	151.66	0.786	45	297.73	77.33	0.636
		CT	44	179.55	91.71		49	307.54	78.15	
		TT	11	300.90	320.24		6	265.33	66.34	
A1298C	TLC ($\times 10^9$/L)	Total	85	68.1747	77.71733		100	7.7000	2.23550	
		AA	32	77.8786	126.42145	0.586	40	8.3125	2.22069	0.122
		AC	38	56.4783	34.02699		51	7.3824	2.13222	
		CC	15	84.8200	60.75070		9	7.0714	2.33582	
	Hemoglobin (g/dL)	Total	85	10.4533	1.77221		100	13.4750	0.87	
		AA	32	11.0429	1.26291	0.058	40	13.3219	0.79	0.313
		AC	38	10.5304	1.81094		51	13.5088	0.97	
		CC	15	9.2000	1.99069		9	13.7429	0.78	
	Platelet count ($\times 10^9$/L)	Total	85	190.11	154.79	0.522	100	299.95	76.82	0.344
		AA	32	153.86	99.48		40	315.38	61.89	
		AC	38	214.27	147.79		51	288.97	85.24	
		CC	15	184.10	242.89		9	291.36	85.45	

for CC, 198, 175 and 23 bp for CT, and 175 bp and 23 bp for TT. Digestion of PCR product with MboII for 1298AA genotype produces fragments of 72, 37, and 28 bp, whereas mutant CC genotype generates fragments of 100 and 28 bp, and heterozygous AC genotype produces fragments of 100, 72, and 28 bp.

Statistical analysis

Allele frequencies were tested from Hardy-Weinberg equilibrium (HWE) before analysis. Qualitative data were presented as frequency and percentage. Chi square test was used to compare groups. Quantitative data were pre-

Table 4. C677T and A1298C genotypes and risk of CML

Polymorphism			Cases (N = 85)		Control (N = 100)		*OR*	95% CI		P
			N.	%	N.	%				
C677T	Genotype	CC	30	35.2	45	45.0	1	-	-	-
		CT	44	51.7	49	49.0	1.010	0.460	2.218	0.981
		TT	11	12.9	6	6.0	2.513	0.722	4.086	0.025
	Allele	C	64	75.9	75	75.0	0.786	0.429	1.442	0.436
		T	21	24.7	25	25.0				
A1298C	Genotype	AA	32	37.6	40	40.0	1	-	-	-
		AC	38	44.7	51	51.0	1.046	0.740	1.759	0.092
		CC	15	17.6	9	9.0	1.816	0.952	3.573	0.036
	Allele	A	56	65.8	68	68.0	0.827	0.490	1.398	0.478
		C	29	34.1	32	32.0				
C677T/A1298C		CC/AA	11	12.9	36	36.0	0.470	0.264	0.738	0.562
		CC/AC	14	16.4	19	19.0	1.258	0.547	1.052	0.937
		CC/CC	10	11.7	5	5.0	0.694	0.452	0.939	0.583
		CT/AA	18	21.1	13	13.0	1.050	0.853	2.059	0.459
		CT/AC	26	30.5	24	24.0	1.849	0.935	2.540	0.024
		TT/AA	4	4.7	2	2.0	1.204	0.895	1.630	0.095
		TT/CC	2	2.3	1	1.0	1.915	1.202	3.845	0.020

Table 5. Clinical characteristics of CML patients according to MTHFR genotypes

Groups	Total	C677T						P	A1298C						P
		CC		CT		TT			AA		AC		CC		
		No	%	No	%	No	%		No	%	No	%	No	%	
Total	85	30	35.2	44	51.7	11	12.9		32	37.6	38	44.7	15	17.6	
Phases															
Chronic	60	26	43.3	25	41.6	9	15.0	0.037	27	45.0	20	33.3	13	21.6	0.049
Accelerated	16	3	18.7	11	68.7	2	12.5		3	18.7	12	75.0	1	6.2	
Blastic	9	1	11.1	8	88.8	0	0.0		2	22.2	6	66.6	1	11.1	
Therapy															
Response	35	25	71.4	9	25.7	1	2.8	0.001	20	57.1	13	37.1	2	5.7	0.002
Resistant	50	5	10.0	35	70.0	10	20.0		12	24.0	25	50.0	13	26.0	

sented as mean and standard deviation. For comparison between groups; ANOVA and Kruskal Wallis (for non-parametric data) were used. Odds ratios (ORs) were calculated using unconditional logistic regression, and were given within 95% confidence intervals (CI). Kaplan-Meier test was used for survival analysis and the statistical significance of differences among curves was determined by Log-Rank test. A *P* value less than 0.05 was considered statistically significant. Analyses were performed using SPSS (statistical package for social science) program (SPSS, Inc, Chicago, IL) version 16.

Results

Table 1 shows that there was no statistical difference for age when compared CML patients with C677T and A1298C genotypes to control subjects.

Table 2 shows no significant difference between the control and CML groups for sex as there was no difference for sex distribution in respect to C677T and A1298C between CML patients and control subjects. Moreover, no statistical differences between males and females in respect to both C677T and A1298C genotypes versus each other.

Table 3 shows no correlations between patients or control subjects and various laboratory parameters as there was no statistical difference for Hemoglobin level, Total leukocyte and platelet counts in respect to C677T and A1297C genotypes among patients with CML and control subjects.

Table 4 shows distribution of C677T and A1298C genotypes and alleles in CML patients and control group. The frequency of the MTHFR 677CC genotype was higher among controls (45.0%) when compared to CML patients (35.2%). In addition, for CML patients, the frequency of the 677CT genotype (51.7%) was higher than control group (49.0%) but with no statistical significance (P = 0.981). However, the frequency of C677T TT genotype was significantly higher (OR = 2.513, 95% CI: 0.722-4.086, P = 0.025) among cases (12.9%) when compared to control group (6.0%), while in MTHFR A1298C genotypes, the frequencies of control subjects who were carrying 1298AA genotype (40.0%) and the heterozygous MTHFR 1298AC genotype (51.0%) were higher among controls compared to CML patients, which were (37.6%) and (44.7%), respectively. In addition, the frequency of individuals who were carrying the 1298CC polymorphisms was significantly higher (OR = 1.1816, 95% CI: 0.952-3.573, P = 0.036), 0.036) in CML patients (17.6%) when compared to controls (9.0%). When considering the combined effect of the two polymorphisms, 677CT/1298AC genotype (OR = 1.849, 95% CI: 0.935-2.540, P = 0.024) and 677TT/1298CC genotype (OR = 1.915, 95% CI: 1.202-3.845, P = 0.020) showed an association with increased risk of CML.

Table 5 shows that there was a significant difference in CML phase's distribution among patients according to C677T and A1298C genotypes. Higher trend of chronic phase was present among cases with 766CC genotype but higher frequency of blastic crises was present in cases with 677CT genotype. In addition, Patients with 1298AA genotype showed higher trend of chronic phase but cases with 1298AC genotype showed higher trend toward blastic crises. There was a statistically significantly trend toward increased risk of resistant to therapy with 677CT and 1298AC genotype who were associated with worse response to therapy (P = 0.001, P = 0.002, respectively).

Discussion

Leukemias are clonal diseases which commonly arise as a result of genetic damage deregulating blood cell development. The risk of leukemia may be increased by both quantitative and qualitative changes in folate metabolism [11]. Folate metabolism plays an important role in carcinogenesis due to its involvement in DNA methylation by 5-methyl THF [18]. 5, 10-MTHFR catalyzes the irreversible reduction of 5-10-methylen THF to 5-methyl THF [19]. MTHFR activity plays an important role in DNA synthesis and its reduced activity has been associated with abnormal DNA synthesis and repair, chromosomal instability, and alterations of the key pathway of the methylation resulting in an increased cancer risk [20]. The association of *MTHFR* variants with an increased or decreased risk for different neoplasia was reported in several studies [21]. The variant MTHFR 677T and 1298C alleles have been associated with increased plasma homocysteine levels [22-24], decreased risk of certain cancers, especially acute leukemias [25] and colorectal cancer [26, 27]. We were therefore interested to establish the distribution and risk association of the two most common polymorphisms, *MTHFR* A1298C and *MTHFR* C677T in CML.

In our study, 6.0% of the control samples had a mutant homozygous *MTHFR* 677TT genotype, and the frequency of allele T was 25.0%. The 677T allele of the MTHFR gene polymorphisms showed remarkable ethnic and geographical variation. In a previous study, 677T allele varied from 26.6% to 46% in Italy and 25.7% in the Middle East to 44.2% in northern China [28]. Moreover, other studies showed that the homozygous 677TT genotype occurred in approximately 10% to 15% of Caucasian and Asian populations [29] and approximately 5% to 10% of Caucasians carry the 1298CC genotype [3]. In addition, we found that 9.0% of the control samples had a mutant homozygous 1298CC genotype, and the frequency of allele C was 32.0%. Such a high frequency for the A1298C allele had already been reported among the Lebanese and the Iranian population [19, 30]. However, our finding seemed to contradict other studies which had lower frequencies of A1298CC genotype among control samples [16, 31, 32].

Many studies have been performed to identify genetic variants associated with CML susceptibility, and among them are MTHFR [11, 13], glutathione S-transferases (GSTs) [16, 13], and haptoglobin (Hp) gene polymorphisms [33, 34]. Association studies between the *MTHFR* polymorphisms C677T and A1298C and risk of CML have been carried out in different populations, but several of them had conflicting results. Our study showed a statistically significant association between both of the two common *MTHFR* gene polymorphisms, C677T and A1298C, and the risk of CML. The association was most clear in cases of the homozygous mutant 677TT and 1298CC genotypes. Two recent studies reported inconsistent results. Barbosa and his colleagues did not find any significant association between both *MTHFR* polymorphisms and CML [16], while Robin *et al*. who investigated the relationship between *MTHFR* polymorphisms and the risk of relapse after hematopoietic cell transplantation for CML, observed a decrease in the relapse risk of patients with variants of both *MTHFR* polymorphisms [17]. A previous study by Moon and his colleagues found that *MTHFR* 1298CC genotype was strongly associated with the risk of CML in the Korean population, while the 677TT allele did not affect the risk of CML [32].

Our results demonstrated risk association between *MTHFR* 677TT and CML. In agreement with our findings, previous studies found a significant association between C766TT genotype and CML risk [35, 36]. However, there are other studies that show contradictory results to our study as they reported that the C677T variants had no effect on CML patients [11, 31, 32, 35]. In accordance to our study, other studies reported a statistically significant association between 1298CC gene polymorphism and the risk of CML [32, 36]. But Lordelo et al observed that the *MTHFR* 1298AA genotype was linked to significantly increased risk of developing CML, while 1298AC significantly decreased this risk [31]. A study that came from South Korea reported inconsistent results specifically regarding the A1298C polymorphisms where the investigators observed a protective effect for the mutated heterozygous genotype 1298AC [11].

In our study, *MTHFR* A1298C showed no significant difference between the control and CML groups for age and gender. This is in contrast to a previous study by Lordelo et al. who observed different findings in his study as he found that the 1298AA genotype frequency was higher in the CML group and the genotypes containing the variant allele (AC and CC) predominated in the control and also showed significant difference between the control and CML groups for age [31].

The combination of heterozygosis of both polymorphisms of *MTHFR* (C677T and A1298C) leads to features similar to those observed in homozygotes 677TT, and has been associated with reduced enzyme activity, decreased plasma folate levels and hyperhomocysteinemia [9, 37]. Our study showed that individuals with combined 677CT/1298AC or 677TT/1298CC genotypes were associated with risk of CML. However, Robin *et al* suggested that individuals with the 677CC/1298AA genotype were at high risk of relapse after hematopoietic cell transplantation [17]. Studies on *MTHFR* C677T and A1298C polymorphism have yielded inconsistence results that could result from the small sample number and in other cases, including this report; this might stress the existence of an element of ethnic variability.

Most of the studies that investigated the relationship between *MTHFR* gene polymorphisms and leukemias have not assessed the dietary folate intake, so the effect of folate level has not been completely clarified yet [19]. It was previously reported that MTHFR enzyme activity was not the only effective factor in folate metabolism pathways and the intake of several elements in a nutrient diet, such as vitamins B6 and B12, can influence the folate metabolism. Flavin adenine dinucleotide as MTHFR co-factor is a form of vitamin B riboflavin, and it has been suggested that the riboflavin status may play a role in the optimal function of the MTHFR enzyme [3].

In conclusion, the present study showed an association between *MTHFR* polymorphisms (677TT, 1298CC) and risk of developing CML. In addition C677T and A1298C carriers might be at risk of resistance to therapy in CML but further studies on larger samples with assessment of folate situation is necessary before absolute judgment about the role of *MTHFR* polymorphisms in the development of chronic myeloid leukemia.

Declaration of conflict of interest

None.

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