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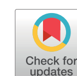
C677T and A1298C polymorphisms of Methylenetetrahydrofolate reductase (*MTHFR*) gene: Effect and risk to develop chronic myeloid leukemia: A study on Syrian patients



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ABSTRACT

Methylenetetrahydrofolate reductase (*MTHFR*) gene is essential in folate metabolism. Genetic variations in this gene like 677 C > T and 1298 A > C affect its activity; the latter could act efficiently the DNA methylation and may give susceptibility to different malignancies. Not surprisingly, different studies have described a relationship between the presence of 677 C > T and 1298 A > C *MTHFR* polymorphisms and the leukemia risk development. However, only few studies have reported this issue in CML.

For this work, 118 CML patients and 217 controls were studied. The *MTHFR* 677 C > T and 1298 A > C polymorphisms were investigated by polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) technique. The frequency of CT and TT genotypes of the *MTHFR* gene (677 C > T) polymorphism in CML patients was significantly higher compared to controls. Moreover, for the AC and CC genotypes of the *MTHFR* 1298 A > C polymorphism, a statistically highly significant frequency of 1298 CC genotype was also detected in CML patients when compared to control group (OR = 1.9, 95%, CI = 1.15–3.16, p = 0.01, and OR = 106.92, 95% CI = 14.17–806.64, $p = 1.7 \times 10^{-16}$, respectively). In addition, CML patients with compound 677CT/1298AC, 677TT/1298AA, 677 CC/1298 AC and 677 CC/1298 CC genotypes were related to a high risk of CML (OR = 5.71, 95% CI: 2.751–11.864, p = 0.000001; OR = 59.5, 95% CI: 12.565–282.071, $p = 9.8 \times 10^{-12}$, OR = 1.9, 95% CI: 0.8678–4.549, p = 0.07; OR = 206.8, 95% CI: 26.284–1627.034, $p = 1.15 \times 10^{-19}$ respectively). The frequency of *MTHFR* 677 C > T and 1298 A > C genotypes was no significantly increased in patients with others phases of CML (accelerated or blastic transformation phases) when compared to the patients in the chronic phase of the disease. We found that both *MTHFR* 677TT and 1298CC genotypes have been in a high risk to develop a CML in Syrian patients.

1. Introduction

Chronic myeloid leukemia (CML) is a, myeloproliferative disorder presented by accesses of myeloid clone precursors in the bone marrow, blood. It is considered relatively a rare disease. It accounts for approximately 14% of all leukemias worldwide [Karkucak et al., 2012]. CML is also marked by the presence of Philadelphia (Ph) chromosome, which results from the translocation involved both long arms of chromosomes 9 and 22 [t(9;22)(q34.1;q11.2)]; this abnormality involves the breakpoint cluster region (*BCR*) gene (22q11.2) and c-abl proto-oncogene 1 (*ABL1*) (9q34). This results in the hybrid *BCR/ABL1* gene, which is found in more than 95% of the CML patients [Faderl et al., 1999; Deininger et al., 2000]. Clinical and biological aspects of CML are well documented, but little information is known about factors that influence an individual's susceptibility to CML. Since leukemias are formed by rapid proliferating tissues that have the highest requirement for DNA synthesis, it is thought that CML are affected by the metabolic fate of folic acid [Skibola et al., 1999].

Folate metabolism influences the methylation processes and DNA synthesis, so any change in its flux could give genomic instability. The lack of Folate has been found affecting the DNA replication by hypomethylation and uracil misincorporation, which increase the risk of

Abbreviations: AP, accelerated phase; ALL, acute lymphoblastic leukemia; *BCR*, breakpoint cluster region gene; BC, blastic crises phase; *ABL1*, c-abl proto-oncogene 1; CML, chronic myeloid leukemia; CP, chronic phase; CI, confidence intervals; HWE, Hardy–Weinberg equilibrium; *MTHFR*, Methylenetetrahydrofolate reductase gene; OR, odds ratios; Ph, Philadelphia chromosome; PCR-RFLP, polymerase chain reaction and restriction fragment length polymorphism; SNPs, single nucleotide polymorphisms

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chromosomal aberrations formation and facilitating the onset of oncogenic activation presumably [Blount et al., 1997]. One of the important enzymes in Folate metabolism; 5,10-Methylenetetrahydrofolate reductase (MTHFR: EC 1.5.1.20) and it affects both nucleotide synthesis and methylation reactions. This enzyme catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the initial form of folate and carbon donor for the re-methylation of homocysteine to methionine. The *MTHFR* gene is assigned on the chromosome 1p at 1p36.3 and it encodes MTHFR enzyme [Krajinovic et al., 2004]. Many *MTHFR* single nucleotide polymorphisms (SNPs) have been found, these cause an activity reduction of the enzyme. Two common polymorphisms are in the *MTHFR* gene: (i) 677 C > T (exon 4 at codon 222), which is a C to T substitution at position 677 this results in an alanine to valine substitution, and (ii) 1298 A > C (exon 7 at codon 429), which causes a glutamate to alanine (A to C) substitution [Hanson et al., 2001]. However, the MTHFR enzyme is known as affecting the folate distribution and gives leukemia susceptibility, by presence of *MTHFR* polymorphisms [Wiemels et al., 2001]. Many studies have described an association between the presence of 677 C > T and 1298 A > C *MTHFR* polymorphisms and the risk of various types of leukemia [Wiemels et al., 2001; Frazer et al., 2007; Franco et al., 2001; Zanrosso et al., 2006]. Most of these studies have aimed on the role as protector of 1298C and 677T alleles however conflicting results from studies performed in different populations have been often observed. But few studies have focused on the protective role of these alleles in CML [Moon et al., 2007; Barbosa et al., 2008; Aly et al., 2014; Jakovljevic et al., 2012; Jankovic et al., 2011; Ismail et al., 2009; Dorgham et al., 2014; Bănescu et al., 2015; Khorshied et al., 2014].

The aim of this study was to elucidate for the first time the effect of the 677 C > T and 1298 A > C polymorphisms of the *MTHFR* gene on the risk of CML development in Syrian patients.

2. Materials and methods

2.1. Patients

This study consisted of 118 bone marrow or peripheral blood samples (60 males and 58 females) patients according to hematological and cytological criteria diagnosed with CML, coming from the AL-Bairuni University Hospital Hematology Department, Damascus, Syria. The diagnosis of CML was based on cytogenetics and molecular cytogenetics analyses were performed in cytogenetics lab./Human Genetics Division at the Syrian Atomic Energy Commission.

All our CML patients presented a Ph chromosome at diagnosis revealed by standard cytogenetics and/or presented the BCR-ABL fusion, as assessed by reverse-transcription polymerase chain reaction. The mean age of our CML patients group was 40 ± 15.8 years. Accordingly, patients were stratified into two distinct groups: Group A, 93 CML-chronic phase (CP) patients and Group B, 25 CML-advanced phase [accelerated phase (AP) or blastic crises (BC)] patients. All patients were subjected to medical history taking, by clinical examination and laboratory assessment. The control group contained of 217 healthy volunteers from both genders (111 males and 106 females; age 36 ± 7.7), without history of hematological malignancy. This study was approved by the Bio-Safety & Bioethics committee of the Institutional Ethical Committee of SAEC. Written informed consent from all the participants was obtained before registering them for the study.

2.2. DNA isolation

Genomic DNA was isolated from peripheral blood samples from CML patients and controls using the QIAamp DNA Blood Mini kit (Qiagen, Germany) according to the manufactures instructions and was stored at −20 °C. The total DNA of each sample was measured by using a spectrophotometer followed by quantity ultraviolet light absorbance at 260 nm.

2.3. MTHFR genotyping

Genotyping of *MTHFR* (677 C > T and 1298 A > C) alleles for controls and patients was determined based on polymerase chain reaction followed by restriction fragment length (PCR-RFLP) gene polymorphism analysis. The primers used for 677 C > T and 1298 A > C amplification in PCR were previously described [A et al., 2007; Donnelly, 2000]. The amplified products were subjected to restriction enzymes digestion at 37 °C for 16 h with Hinf1 and *Mbo*II enzymes (Thermo Fisher Scientific, MA, USA). Visualization of the fragments was performed by electrophoresis on 3% agarose gel containing ethidium bromide.

Presence or absence of different fragments was visualized under UV transilluminator. Genotyping of *MTHFR* gene mutations distinguished between homozygous and heterozygous: for *MTHFR* C677T homozygous, the T allele showed 2 bands of 171 and 94 base pair size (bp), the C allele homozygous showed a single uncut band of 265 bp, and heterozygous for both the C and T alleles showed 3 bands of 265, 171, and 94 bp. Whereas for *MTHFR* A1298C, 2 bands of 204 and 37 bp were typical found for homozygous A allele, presence of C allele showed a single uncut band of 241 bp, and the heterozygous for C and A alleles showed three bands of 241, 204, and 37 bp [A et al., 2007; Donnelly, 2000].

2.4. Statistical analysis

The differences in genotype and allelic frequencies between patients and controls were statistically evaluated using Chi-square test or Fisher's exact test. Unconditional logistic regression analysis was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for risk estimation. p-Value less than 0.05 was considered significant. Chi-square (χ^2) test was performed to assess deviations from Hardy–Weinberg equilibrium (HWE) in controls.

3. Results

Genotypes distribution and allelic frequencies (SNPs) of the CML patients and controls studied are presented in Table 1. The frequency of CT and TT genotypes of the *MTHFR* 677 C > T polymorphism in CML patients was significantly higher when compared to controls (OR = 0.62, 95% CI = 0.37–1.06, p = 0.09, and OR = 11.8, 95% CI = 3.37–41.18, p = 0.000003, respectively). The frequency of allele T was 27.5% in the CML group and 19.8% in the control group, the difference in frequency of allele T in 677 C > T between the two groups

Table 1
The distribution of the *MTHFR* 677 C > T and 1298 A > C genotypes and allelic in CML and control groups.

Genotype	CML group (n = 118) (%)	Control group (n = 217) (%)	p-Value	OR	95% CI
MTHFR 677 C > T					
CC	72 (61)	134(61.8)		1	
CT	27 (22.9)	80 (36.8)	0.09	0.62	0.37–1.06
TT	19 (16.1)	3 (1.4)	0.000003	11.8	3.37–41.18
C allele	171 (72.4)	348 (80.1)		1	
T allele	65 (27.5)	86 (19.8)	0.02	1.54	1.061–2.23
MTHFR 1298 A > C					
AA	41 (34.8)	137 (63.1)		1	
AC	45 (38.1)	79 (36.4)	0.01	1.9	1.15–3.16
CC	32 (27.1)	1 (0.5)	1.7×10^{-16}	106.92	14.17–806.64
A allele	127 (53.8)	354 (81.5)		1	
C allele	109 (46.2)	80 (18.4)	5.2×10^{-14}	3.79	2.67–5.40

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