Methylenetetrahydrofolate Reductase (MTHFR) Genotype Association with the Risk of Chronic Myelogenous Leukemia

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Abstract

The metabolism of folate is essential in DNA synthesis, and polymorphisms of genes involved in this metabolism have been implicated in many types of cancer. One such gene is the Methylenetetrahydrofolate Reductase (MTHFR) gene, which encodes an enzyme that converts folate to a methyl donor used for DNA methylation. In this report, we studied the association between the different genotypes of the two most common MTHFR polymophisms, C677T and A1298C, and the risk of Chronic Myelogenous Leukemia (CML). For this purpose, 149 of previously diagnosed CML patients and 170 normal controls were examined using PCR followed by Restriction Fragment Length Polymorphism (RFLP). Results showed that the frequency of the C677T TT homozygous mutant genotype in patients with CML was significantly higher compared to controls (OR = 2.84, 95% CI: 1.24-6.50, *P*-value = 0.014). No such association was shown for the heterozygous C677T CT genotype (OR = 1.52, 95% CI: 0.95-2.41, *P*-value = 0.081). As for the A1298C genotypes, a statistically significant higher frequency of the mutant homozygous genotype 1298CC was also detected in CML patients compared to the control group (OR = 2.18, 95% CI: 1.01-4.69, *P*-value = 0.046). No such statistical significance was demonstrable for the heterozygote genotype 1298AC (OR = 1.08, 95% CI: 0.68-1.73, *P*-value = 0.743). This is the first report to suggest that both mutated MTHFR genotypes, specifically the homozygous 677TT and 1298CC polymorphisms, can be associated with a higher risk of developing CML.

Keywords: CML, MTHFR, C677T, A1298C.

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Introduction

Methylenetetrahydrofolate Reductase (MTHFR) is the key enzyme in folate metabolism. It irreversibly reduces 5, 10-methylenetetrahydrofolate (5,10-methylene-THF) to 5-methyl-THF, which is the main form of folate in serum that is involved in amino acid synthesis where it remethylates homocysteine into methionine at the expense of nucleotide synthesis. [1] 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. Both mutations are quite common. For example, in Caucasians, the homozygous 677TT genotype frequency is 10-15%, [2] and that

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for the 1298CC genotype is 5-10%. [3] In-vitro experiments showed that the homozygous C677T genotype retains only 30% of the normal MTHFR activity, while the heterozygous CT genotype retains about 60% of that activity. [4] Although to a lesser extent compared to the C677T alleles, the A1298C mutations were also shown to result in a decrease in MTHFR enzyme activity. [5, 6]

The decrease in MTHFR activity caused by the two polymorphisms, C677T and A1298C, results in the accumulation of 5,10-methylene-THF, which is then used in nucleotide synthesis, thus shifting away from homocysteine metabolism. The 5,10-methylene-THF donates a methyl group to uracil, converting it to thymine. In cases of folate deficiency, higher levels of uracil can induce misincorporation during DNA replication, causing DNA double strand breaks during DNA excision repair and thus genetic instability. [7,8] Also, the resulting hypomethylation can sometimes lead to genomic instability and activation of certain oncogenes. [9]

Since the MTHFR gene has an essential role in folate metabolism, genetic polymorphisms in this gene could alter the susceptibility to different cancers including hematological malignancies, as it was shown that an appropriate supply of folate is crucial specially for rapidly replicating cells such as hematopoietic cells. [9] Indeed, several previous studies have noticed a change in cancer risk in individuals with mutated MTHFR genotypes. [10] However, only a small number of reports have studied the effect of MTHFR gene alterations on the risk of developing leukemia. Those reports have focused on acute leukemias such as acute lymphoblastic leukemia (ALL), and acute myelogenous leukemia (AML), where they described a lower risk of ALL in individuals with mutated MTHFR alleles, but not in the case of AML. Still, the results were not always consistent, and many studies where performed on specific ethnicities such as Cucasians and did not include other ethnic groups. [10-14]

Chronic myelogenous leukemia (CML) is characterized by the bcr-abl fusion transcripts resulting from translocation t (9;22) seen in more than 95% of affected patients. Only very few studies addressed the association between MTHFR genotypes and the risk of CML, and yet their results were not only inconsistent, but sometimes also contradicting even though they were conducted on the same ethnic group. [10, 15, 16] In this study, the association between MTHFR polymorphism and the risk of CML is thoroughly investigated in the Jordanian population.

Materials and Methods

Study Population

Blood samples from 149 patients diagnosed with CML were collected. The patients were diagnosed as bcr-abl positive between the years 2003-2007 by RT-PCR, FISH or both. The control group consisted of 170 healthy individuals.

MTHFR genotyping

DNA from patients and controls was extracted from peripheral blood samples collected in EDTA tubes using the Wizard DNA purification kit (Promega, USA). Genotyping was performed for both polymorphisms, C677T and A1298C by two separate uniplex PCR reactions using the thermal cycler PTC-100 (MJ Research, Inc., USA). Amplification of the C677T region was performed using the forward primer: TGAAGGAGAAGGTGTCTGCGGGA and the reverse primer: AGGACGGTGCGGTGAGAGTG yielding a 198 bp band, whereas for A1298C, the forward primer: CAAGGAGGAGCTGCTGAAGA, and the reverse primer: CCACTCCAGCATCACTCACT, were used yielding a 128 bp band. The PCR conditions, as described by Yi et al., [17] for both amplifications, were: 8 min of initial denaturation at 95°C, followed by 40 cycles of 95°C for 60 sec, 63 °C for 60 sec, and 72°C for60 sec, with a final extension at 72°C for 7 min.

The PCR products of C677T and A1298C were separately digested with HinfI and MboII restriction enzymes (Promega, USA), respectively. Resulting fragment were visualized using ethidium bromide staining and 3% agarose (Promega, USA) gel electrophoresis. The digestion fragment sizes for C677T genotypes were: a single 198 bp band for CC, 198, 175 and 23 bp for CT, and 175 bp and 23 bp for TT. For A1298C genotypes the fragments were: 72, 28 and 28 bp for AA, 28, 72 and 100 bp for AC, and 100 and 28 bp for CC.

Statistical analysis

The two-tailed Chi-squared test was used to examine the differences in genotype distribution between patients and controls. The difference was considered significant in case of a two-tailed *P* value less than 0.05. The association between CML and MTHFR C677T and MTHFR A1298C polymorphisms was evaluated by multiple logistic regression analysis. Adjusted odds ratios and their confidence intervals (95% CI) were calculated. All analyses were performed using the Statistical Package for Social Science (SPSS ver. 15.0, SPSS Science, Chicago, IL).

Results

The frequency for the MTHFR C677T CC wild type homozygous genotype was higher among controls (55.3%) when compared to CML patients (42.2%). Additionally, for CML patients, the frequencies for the C677T CT heterozygous genotype (45.0%) and the C677T TT homozygous mutated genotype (12.8%) were higher compared to controls (38.8%) and (5.90%), respectively.

As for the MTHFR A1298C mutation, the percentages of individuals carrying the wild type A1298C AA genotype (44.7%) and the heterozygous MTHFR A1298C AC genotype (47.65%) were higher among controls compared to CML patients, which were (39.6%) and (45.6%), respictively. However, the frequency of individuals carrying the homozygous A1298C CC polymorphisms was higher among CML patients (14.8%) compared to controls (7.65%). While the difference in frequency of the heterozygous C677T CT genotype between CML patients and controls was not statistically significant (OR = 1.52, 95% CI: 0.95-2.41, *P*-value = 0.081), the frequency of the homozygous C677T TT genotype in patients with CML was significantly higher compared to the control group (OR = 2.84, 95% CI: 1.24-6.50, *P*-value = 0.014). Similarily, for the A1298C genotypes, the relatively higher frequency of the mutant homozygous genotype 1298CC was statistically significant in patients with CML compared to controls (OR = 2.18, 95% CI: 1.01-4.69, *P*-value = 0.046). No such statistical significance was demonstrable for the heterozygote genotype 1298AC (OR = 1.08, 95% CI: 0.68, 1.73, *P*-value = 0.743).

When considering the joint effect of the two polymorphisms, the following combinations showed an association with increased risk of CML: the 677CC/1298CC genotype (OR = 4.20, 95% CI: 1.69-10.46, *P*-value = .002), the 677TC/1298AA genotype (OR = 2.70, 95% CI: 1.22-5.98, *P*-value = 0.015), the 677TC/1298AC genotype (OR = 2.60, 95% CI: 1.24-5.46, *P*-value =0.012), and finally the 677TT/1298AA genotype (OR = 4.16, 95% CI: 1.55-11.20, *P*-value = 0.005). Distributions of all genotype frequencies are summarized in (Table1).

Table (1): Association between different MTHFR genotypes and the risk of CML.

Genotype	*Patients (n=149, %)*	*Controls (n=170, %)*	*OR (95% CI)*	*P-value*
C677T				
CC	*63 (42.2%)*	*94 (55.3%)*	*1.00*	
CT	*67 (45.0%)*	*66 (38.8%)*	*1.52 [0.95,2.41]*	*0.081*
TT	*19 (12.8%)*	*10 (5.90%)*	*2.84 [1.24,6.50]*	*0.014*
A1298C				
AA	*59 (39.6%)*	*76 (44.7%)*	*1.00*	
AC	*68 (45.6%)*	*81 (47.65%)*	*1.08 [0.68,1.73]*	*0.743*

CC	*22 (14.8%)*	*13 (7.65%)*	*2.18 [1.01,4.69]*	*0.046*
C677T/A1298C				
CC/AA	*15 (10.0%)*	*39 (22.94%)*	*1.00*	
CC/AC	*27 (18.1%)*	*42 (24.71%)*	*1.67 [0.78,3.60]*	*0.189*
CC/CC	*21 (14.1%)*	*13 (7.65%)*	*4.20 [1.69,10.46]*	*0.002*
TC/AA	*28 (18.8%)*	*27 (15.9%)*	*2.70 [1.22,5.98]*	*0.015*
TC/AC	*39 (26.2%)*	*39 (22.90%)*	*2.60 [1.24,5.46]*	*0.012*
TC/CC	-	-	*0.00 [0,00]*	*1.00*
TT/AA	*16 (10.70%)*	*10 (5.9%)*	*4.16 [1.55,11.20]*	*0.005*
TT/AC	*2 (1.30%)*	-	*0.00 [0,00]*	*1.00*
TT/CC	*1 (0.80%)*	-	*0.00 [0,00]*	*1.00*

Discussion

The C677T and A1298C mutated genotypes of the MTHFR gene have been shown to alter the risk of many cancers including colorectal cancer,[18] gastric cancer, [19] esophageal cancer, [20] and cervical cancer. [21] Reports on the role MTHFR polymorphisms in hematological malignancies have mainly focused on acute leukemias, such as ALL where a mostly protective effect was observed, [22] and only very few studies have addressed this role in chronic malignancies such as CML.

Our study shows for the first time, a statistically significant association between both of the two common MTHFR gene polymorphisms, namely C677T and A1298C, and the risk of CML. The association was most clear in both cases of the homozygous mutant genotypes, that is, 677TT and 1298CC. This is the first study of its kind conducted in Jordan and the region and as mentioned earlier, one of very few in the published literature. Two recent studies that came from South Korea reported inconsistent results specifically regarding the A1298C polymorphisms where one observed a protective effect for the mutated heterozygous genotype 1298AC (10), while the other demonstrated an association between the 1298CC homozygous variant and an increased risk of CML. [16] Both studies however, agreed that the C677T variants had no effect. Generally, such inconsistence between different reports could result from the small sample number used in some of these studies which lacks significant statistical power, and in other cases, including this report; this might stress the existence of an element of ethnic variabilty. Indeed, such ethnic variation has been noticed in the association between MTHFR mutatios and other cancers.

It is well documented that the different MTHFR genotypes can have different effects depnding on the organ involved and the environment. For example, it has been shown in many studies that individuals with the 677TT genotype and adequate folate have a decreased risk of colorectral cancer. In contrast, several other studies on cervical, breast, esophageal and gastric cancers reported an increased risk for the same genotype. [16] Two machanisms, DNA instability and DNA methylation have been hypothesized to explain the association between one-carbon metabolism and tumorgenesis. [25] As for DNA instability, lower MTHFR activity could lead to accumolation of methylene-THF which would increase the methylation of dUMP to dTMP. The lower amount of uracil nucleotide could mean a lower rate of its misincorporation into DNA, and thus decreased chances of DNA breakage and increased DNA integrity. Opposite to this explanation, it has been reported that the C677T polymorphism is not associated with folic deficiency-induced uracil incorporation into DNA, [26] or chromosomal damage in vitro. [27] As to DNA methylation, it has been shown that the decreased MTHFR activity leads to reduction in 5-methyl-THF, a methyl donor for homocysteine, and thus to hypomethylation. As some oncogenes are often hypermethylated, hypomethylation is likely to be protective in related malignancies.

In conclusion, the present study demonstrates a clear association between mutated MTHFR genotypes and an increased risk of CML.

Further studies with statistically significant sample sizes, performed on different ethnic groups, and including polymorphisms of other beochemically related genes are necessary to establish the exact role of MTHFR in hematological malignacies.

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علاقة جين (MTHFR) بسرطان الدم الحبيبي المزمن

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الملخص:

يعتبر حامض الفوليك عنصراً أساسياً في عملية تصنيع الحمض النووي الـ DNA ومن المعروف أن الطفرات التي قد تؤثر في أي من الجينات المسؤولة عن عمليات استقلاب هذا الحمض قد تكون مرتبطة بالعرضة لأنواع مختلفة من الأورام. ومن أهم هذه الجينات هو الـ MTHFR والذي سيتم في هذا البحث دراسة علاقته بسرطان الدم الحبيبي المزمن والذي يتميز بوجود كروموسوم فيلادلفيا الذي ينتج عن وجود الزيغ الصبغي المتكون أجزاء من الكروموسوم التاسع والكروموسوم الثاني والعشرين.

قمنا في هذه الدراسة بفحص وجود نوعين من التغيرات النقطية في جين الـ MTHFR وعلاقتها بمرض سرطان الدم الحبيبي المزمن وذلك باستخدام تفاعل البلمرة التسلسلي. قمنا بتجميع مئة وثلاثون عينة دم من مرضى سرطان الدم الحبيبي المزمن ومقارنتهم بمئة وسبعون عينة دم من أ،اس أصحاء ليس لديهم أي نوع من الأمراض لمعرفة نسبة وجود المرض بين الفئتين.

توصلنا في نهاية هذه الدراسة الى وجود علاقة بين سرطان الدم الحبيبي المزمن وأحد هذه التغيرات النقطية وهو (A1298 C).

الكلمات الدالة: جين (MTHFR)، مرض سرطان الدم الحبيبي المزمن (CML)، تغير جيني نقطي (A1298C)، تغير جيني نقطي (C677T).