Submitted 12.10.09 | Revision Received 2.20.10 | Accepted 2.22.10

Methylenetetrahydrofolate Reductase Gene Polymorphisms and Risk of Myeloid Leukemia

Pazhakh Vahid, MSc, Razmkhah Farnaz, MSc, Farhad Zaker, PhD, Atashrazm Farzaneh, MSc, Rasighaemi Parisa, MSc

(Department of Hematology, Iran University of Medical Sciences, Molecular and Cellular Research Center, Tehran, Islamic Republic of Iran)

DOI: 10.1309/LMA6H9RW9CKNLNDV

Abstract

Objective: 5,10-Methylenetetrahydrofolate reductase (MTHFR) involved in folate metabolism has an important role in a cell because folate availability is critical for DNA integrity. This research aims to evaluate, in a case-controlled study, if the polymorphisms in MTHFR gene contribute to altering susceptibility to leukemias of acute myeloid leukemia (AML) and chronic myeloid leukemia (CML).

Materials and Methods: Thirty-eight CML patients and 106 AML patients were diagnosed based on detection of BCR-ABL fusion gene by reverse transcription polymerase chain reaction (RT-PCR) and immunophenotyping by flow cytometry. A control group containing 97 healthy, age- and sex-matched individuals participated in this study. Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms in the patient and control groups were evaluated by using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique.

We assessed the relationship between the MTHFR genotype and the risk of hematologic malignancies by calculating the odds ratio (OR) with a 95% confidence interval (CI) using conditional logistic regression.

Results: The frequencies of CT and TT genotypes (of 677 allele) and AC and CC genotypes (of 1298 allele) among AML patients did not show a statistically significant difference when compared with those of the controls. Also, among CML patients, the frequencies of above stated genotypes did not show statistically significant differences compared with those of the controls.

Conclusions: The data indicate that because of no statistical difference in the frequencies of MTHFR gene polymorphisms (C677T and A1298C) in the patient and control groups, these polymorphisms do not contribute to an inherited genetic susceptibility of AML and CML.

Keywords: MTHFR, polymorphism, AML, CML

Leukemias are clonal diseases that commonly arise due to genetic lesions disturbing the regulation of blood cell development and hematopoiesis. Proven reasons for leukemia, such as ionizing radiation, benzene, and cancer chemotherapy, are responsible for only a small portion of the total cases.[1] The enhancing risk factors for leukemias are quantity and quality changes in folate metabolism.[2]

As a carrier of single-carbon fragments, folate is a vital nutrient for normal growth of mammalian cells. Folate plays an essential role in promoting purine and pyrimidine synthesis, especially in converting dUMP to dTMP (by 5-10-methylen tetrahydrofolate [THF] form). Folate is also essential for converting homocysteine to methionine and supplying methyl groups for methylation of DNA, RNA, proteins, neurotransmitters, and phospholipids (by 5-methyl THF form).[3] 5,10-Methylenetetrahydrofolate reductase enzyme (MTHFR: EC 1.5.1.20) catalyzes the irreversible reduction of 5-10-methylen THF to 5-methyl THF.

Methylenetetrahydrofolate reductase 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).[4] The major product of MTHFR locus in human is a 77-kilodalton protein, which is catalytically active, although a smaller 70-kilodalton isoform has also been observed in some tissues.

Several polymorphisms have been identified in this gene so far, among which 2 cases are shown to be of great significance in the literature. C677T polymorphism in exon 4 leading alanine to be substituted by valine at codon 222 is the most frequently studied polymorphism in this gene. This allele is commonly named thermolabile because its activity decreases above 37°C.[5] People with homozygous MTHFR 677TT genotype have 30% enzyme activity compared with those having wild-type allele, while the enzyme activity is 60% in people with heterozygous MTHFR 677 CT allele.[4] A C677T polymorphism causes amino acid change at the binding site of the MTHFR enzyme co-factor, flavin adenine dinucleotide (FAD). This polymorphism facilitates the separation of the enzyme from its co-factor, and, as a result, the enzyme activity decreases.[6]

Another important, well-studied polymorphism in the MTHFR gene is A1298C in exon 7, which causes glutamate to be substituted by alanine at codon 249.[7] This polymorphism is located in the S-adenosyl methionine (SAM) regulatory domain. The binding of SAM to the MTHFR enzyme

Corresponding Author

Farhad Zaker, PhD
farhad_zaker@yahoo.com

Abbreviations

MTHFR, Methylenetetrahydrofolate reductase; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; RT-PCR, reverse transcription polymerase chain reaction; PCR-RFLP, polymerase chain reaction restriction fragment length polymorphism; OR, odds ratio, CI, confidence interval; THF, tetrahydrofolate; FAD, flavin adenine dinucleotide; SAM, S-adenosyl methionine; ALL, acute lymphoid leukemia; IBTO, Iranian Blood Transfusion Organization; IUMS, Iran University of Medical Sciences; RFLP, restriction fragment length polymorphism; dNTPs, deoxynucleotide triphosphates; FPGS, Folypolyglutamate synthetase; SHMT, serine hydroxymethyltransferase; MS, methionine synthase

leads to conformational changes within the structure of the enzyme and inhibits its activity, so the A1298C polymorphism also decreases the MTHFR activity though not so much as C677T.[4] People with a 1298CC genotype do not show higher levels of serum homocysteine in comparison to the wild type people. However, like people with the 677TT genotype, those with the compound homozygous genotype for alleles C677T and A1298C show higher homocysteine and decreased folate in their serum.[5] Locations of C677T and A1298C polymorphisms in the MTHFR gene are as far as 2.1kb from each other and are in tight negative linkage disequilibrium.[8]

Of course, other polymorphisms have been identified in the MTHFR enzyme coding gene such as the T1317C allele, which does not change any amino acid into protein[7] or G1793A allele resulting in argentine-to-glutamine substitution at codon 594. The association between these polymorphic alleles and enzyme activity has not yet been clarified.

The correlation of the 2 main polymorphic alleles of the MTHFR gene, C677T and A1298C, with increased serum homocysteine, presents a higher risk of cardiovascular disease and birth defects, especially neural tube defects, coronary artery disease,[4] cerebrovascular disease, venous thrombotic disease,[9] squamous cell carcinoma,[10] breast cancer,[11] colorectal cancer,[12,13] Turner Syndrome,[14] endometrial cancer,[9] schizophrenia,[15] and hypertension,[16] which have been studied so far. Also 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.[17,8,18] However, the findings about this correlation in other acute and chronic leukemias are controversial.[2,19-22]

Since the majority of the previous studies have assessed the role of these polymorphisms in lymphoid leukemias, we decided to evaluate the 2 major MTHFR gene polymorphisms, C677T and A1298C, in myeloid leukemias (acute myeloid leukemia [AML] and chronic myeloid leukemia [CML]).

Materials and Methods

Our study population consisted of blood or bone marrow samples from 38 CML patients (male/female: 1.05, mean age 45.0 years, SD ± 16.7) and 106 AML patients (male/female: 1.17, mean age 45.9 years, SD ± 14.7) who were diagnosed based on the detection of the BCR-ABL fusion gene by RT-PCR and immunophenotyping by flow cytometry, respectively. Also 97 healthy age- and sex-matched individuals (male/female: 0.94, mean age 44.8 years, SD ± 18.6) participated in this experiment as the control group. All patients had been referred to the Iranian Blood Transfusion Organization (IBTO) and Hematology-Oncology and Stem Cell Transplantation Research Center of Shariati Hospital (Tehran, Iran) from 2007 through 2008. The Medical Ethics Committee of the Iran University of Medical Sciences (IUMS) approved the study (300MT) and written informed consent was obtained from all patients who participated in this study.

We analyzed MTHFR C677T and A1298C polymorphisms by the restriction fragment length polymorphism (RFLP) technique after PCR. Blasts and mononuclear cells were purified by Ficoll-Hypaque (Pharmacia LKB, Uppsala, Sweden) centrifugation, and their DNA was extracted through the standard method.[23] The primers used to amplify the MTHFR gene were as follows:

for C677T; forward primer 5 -TGA AGG AGA AGG TGT CTG CGG GA-3 and reverse primer 5 -AGG ACG GTG CGG TGA GAG TG-3 [18] and for A1298C; forward primer 5 -GCA AGT CCC CCA AGG AGG-3 and reverse primer 5 -GGT CCC CAC TTC CAG CAT C-3 .[8] The total reaction volume of 50 µL contained approximately 100 ng of DNA, 10 pmol of each primer, deoxynucleotide triphosphates (dNTPs), (10 mM each), 2.5 U of Prime Taq polymerase and reaction buffer (GENET BIO, Chungnam, South Korea), and ddH_2O. Samples were amplified (Techne TC-512) using the following PCR conditions: 95°C for 5 minutes, 40 cycles of 94°C for 30 seconds, 60°C for 1 minute, 72°C for 1 minute, and finally 72°C for 7 minutes. Amplification success was monitored by agarose electrophoresis. Afterwards the PCR products were subjected to digestion by Hinf1 and MboII (Fermentase) for C677T and A1298C polymorphisms, respectively. The enzymatic mixture was composed of 0.1 µL restriction enzyme, 1 µL buffer, 13 µL PCR products, and 18 µL distilled water and was incubated overnight at 37°C for both polymorphisms. After digestion, the enzymatic mixture was subjected to electrophoresis on 2% agarose.

In the case of C677T polymorphism, an undigested 198 bp fragment showed wild type CC genotype, while 2 digested fragments of 175 and 23 bp showed mutant TT genotype, and 3 fragments of 198, 175, and 23 bp indicated heterozygous CT genotype.[18]

With regards to the A1298C polymorphism, this enzyme has 1 and 2 restriction sites on mutant and wild-type alleles, respectively. So wild-type AA genotype produces 3 digested fragments of 79, 37, and 29 bp, whereas mutant CC genotype generates 2 digested fragments of 108 and 37 bp, and heterozygous AC genotype produces 4 fragments of 108, 79, 37, and 29 bp.[8]

Statistical Analysis

We assessed the association between the MTHFR genotype and the risk of hematologic malignancies by calculating the OR with a 95% CI using conditional logistic regression. A 95% CI excluding 1.0 was considered statistically significant. Statistical analyses were carried out with the SPSS version 16 (Chicago, IL) statistical software package.

Results

All 241 DNA samples (38 CML patients, 106 AML patients, and 97 healthy controls) were successfully genotyped by RFLP technique. Methylenetetrahydrofolate reductase genotyping among 97 healthy controls indicated a polymorphic allele frequency of 23.19% for C677T and 41.23% for A1298C.

Among the 106 adult AML patients in our study, the allele frequency of 677T was 26.88%. The frequencies of CT and TT genotypes in these patients were 36.79% and 8.49%, respectively, which did not show a statistically significant difference compared with those of the controls (OR=1.02, 95% CI: 0.57–1.8; OR=2.2, 95% CI: 0.65–7.6, respectively). Also, the 1298C allele frequency was 39.14% among AML patients. The frequencies of AC and CC genotypes in these

patients were 46.22% and 16.03%, respectively, which did not show a statistically significant difference compared with those of the controls (OR=1.33, 95% CI: 0.7–2.5; OR=0.75, 95% CI: 0.34–1.6, respectively).

For MTHFR 677 and 1298 among 38 CML patients participating in our study, we observed allele frequencies of 22.36% and 43.42%, respectively. The frequencies of CT and TT genotypes (for 677 allele) and AC and CC genotypes (for 1298 allele) in CML patients were 28.94%, 7.89%, 50%, and 18.42%. None of the above-stated frequencies showed any statistically significant difference when compared to those of the controls (OR=0.7, 95% CI: 0.3–1.6; OR=1.75, 95% CI: 0.36–8.4; OR=1.7, 95% CI: 0.7–4; OR=1.03, 95% CI: 0.4–3). Distributions of genotype frequencies are listed in **Table 1**.

Discussion

The 677T allele of the MTHFR gene polymorphisms shows remarkable ethnic and geographical variation. In a study[24] on 7000 newborns from 16 areas worldwide, the 677T allele varied from 26.6% to 46% in Italy and 25.7% in the Middle East to 44.2% in northern China. Furthermore, in a study in Lebanon, the frequency of the MTHFR 677TT homozygous genotype was 7.66% and 13.08% in Muslims Arabs and Christians, respectively.[25] These results indicate the presence of a selective pressure that causes such a geographical-ethnical pattern about the MTHFR 677T allele. In our study, 4.12% of the control samples had a mutant homozygous MTHFR 677TT genotype, and the frequency of allele T was 23.19%.

Analysis of the control group in our samples showed a rather low frequency of MTHFR C677TT polymorphism among the Iranian population when compared to other Asian populations, especially eastern countries.[5,11,26] Like C677T, the MTHFR A1298C polymorphism also shows remarkable geographical-ethnical variation. Most of the Asian studies showed a frequency range of 15% to 20% for the 1298C allele.[9,10,20,27] Our results showed the allele frequency of 41.23% for MTHFR 1298C in the Iranian population, which seems much higher than other Asian reports. Such a high frequency for the A1298C allele has already been reported among Iranians[28] as well as among the Lebanese and the Jewish population of the Middle East.[29,30] In addition, the frequency of homozygous 1298CC genotype in our study was calculated to be 22.68%, which seems very high and is comparable with the results in the Lebanese population of 23.9% by Sabbagh and colleagues.[29]

Multiple studies about the role of the 2 C677T and A1298C polymorphisms of the MTHFR enzyme gene in leukemia have been completed. Skibola and colleagues[18] observed that people with MTHFR 677 TT, 1298AC, and 1298CC genotypes, compared with wild-type genotypes, have a lower risk for developing ALL (a 4.3-, 3-, and 14-fold decrease, respectively, the last of which was not statistically significant). No significant difference between MTHFR genotypes distribution among AML patients and control groups was observed in this study, suggesting that MTHFR gene polymorphisms have not influenced the risk of AML.

In another study, Wiemels and colleagues[8] assessed the influence of MTHFR polymorphisms on 3 subgroups of pediatric leukemias including infant lymphoblastic or myeloblastic leukemias with MLL rearrangements and childhood lymphoblastic leukemias with either TEL-AML1 fusions or hyperdiploid karyotypes. They found that MTHFR 677 variants (CT and TT genotypes) were associated with a lower risk of leukemias with MLL rearrangements, while the 1298CC genotype induced a lower risk of hyperdiploid leukemias. Also in this study, no association was observed between MTHFR polymorphisms and myeloid leukemia. A smaller study in Brazil[17] on pediatric ALL also showed the MTHFR 677T allele conferred a 2.4-fold decrease in the risk of ALL, while the A1298C genotype did not affect the risk of ALL.

There are few studies about the role of MTHFR polymorphisms in developing chronic leukemias, especially CML. In the Moon and colleagues study[20] the 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. In this study the MTHFR 1298CC genotype was higher than the controls in AML and multiple myeloma patients, although the difference was not statistically significant. In Brazil, Barbosa and colleagues[2] also did not find any significant association between MTHFR polymorphisms and CML/AML M3 leukemias.

Table 1_Association Between MTHFR C677T and A1298C Genotypes and AML/CML

Polymorphism	AML cases	OR 95% CI	CML cases	OR 95% CI	Control
C677T					
CC	58 (54.71%)	1 —	24 (63.15%)	1 —	56 (57.73%)
CT	39 (36.79%)	1.02* I (0.57–1.8)	11 (28.94%)	0.7 I (0.3-1.6)	37 (38.14%)
TT	9 (8.49%)	2.2 I (0.65–7.6)	3 (7.89%)	1.75 I (0.36-8.4)	4 (4.12%)
Allele frequency of T	26.88%		22.36%		23.19%
A1298C					
AA	40 (37.73%)	1 —	12 (31.57%)	1 —	39 (40.2%)
AC	49 (46.22%)	1.33 I (0.7-2.5)	19 (50%)	1.7 I (0.7-4)	36 (37.11%)
CC	17 (16.03%)	0.75 I (0.34-1.6)	7 (18.42%)	1.03 I (0.4-3)	22 (22.68%)
Allele frequency of C	39.14%		43.42%		41.23%
Total	106		38		97

I, insignificant.

In another study about the relationship between MTHFR polymorphisms and the risk of relapse after hematopoietic cell transplantation for CML, a 2- to 4-fold decrease was observed in the relapse risk of patients with variants of both polymorphisms (which was statistically significant for only the 1298C allele).[19] This decreased risk seems to be independent of the undesired effects of the 1298C allele of CML mentioned in the Moon and colleagues study.[20] Larger nucleotide sources (as a result of the lower activity of the MTHFR enzyme) may have caused more genetic stability in transformed cells. These may affect the acquiring additional genetic changes and lead to the progression from BCR-ABL positivity to clinical relapse.

Among the results standing in contrast with the aforementioned studies, the Hur and colleagues study[21] took notice that no relation was found between MTHFR C677T variants (CT and TT) and CML, AML, and ALL development, while the MTHFR A1298C variants (AC and CC) were reported to decrease the risk of AML and CML. However, Deligezer and colleagues[22] observed a similar pattern in the distribution of the MTHFR C677T polymorphism in myeloid and lymphoid leukemias, in such a way that the frequency of the MTHFR 677TT genotype in all 3 malignancies of CML, AML, and ALL was lower than those of the controls, although the differences were not statistically significant. In our study, we did not find any statistical significant association between the distribution of MTHFR polymorphisms (C677T and A1298C) in myeloid leukemias (AML and CML) and the control group. This indicates that the presence of the polymorphisms did not affect the risk of AML and CML among our samples.

Despite some incompatible results, the hypothesis can be suggested that imbalanced intracellular distribution of folate metabolites caused by MTHFR variants may affect the risk of lymphoid leukemias but not myeloid leukemias. Acute lymphoid leukemia and AML are clinically different diseases arising from distinct cell lineages and also show differences in response to treatment. Methotrexate as an antifolate is an effective chemotherapy agent for the treatment of ALL, while it is not as successful with AML. It is due to a higher expression of Folypolyglutamate synthetase (FPGS) in lymphoid cells than myeloid ones. Folypolyglutamate synthetase catalyzes the formation of long-chain folate and antifolate polyglutamate derivatives. This different expression is also seen in normal lymphoid and myeloid progenitor cells.[18] In fact, these differences are indicators of the probable higher folate requirement of lymphoid cells and subsequently more susceptibility to folate deficiency and DNA damages than myeloid cells. Of course, it should be considered that most of the studies done so far about the relationship between MTHFR gene polymorphisms and leukemias have not assessed the dietary folate intake in their samples, so the effect of folate level has not been completely clarified yet.

In experimental models of folate shortage, the misincorporation of uridylate in the DNA structure increases to as many as 100 times. This situation enhances the probability of the occurrence of double strand breaks in the DNA molecule up to 50 times.[9] This model can justify the role of genetic instabilities in malignancies.

However, in a study about the effects of MTHFR polymorphisms and dietary folate intake in colorectal cancer risk, it has been found that people with the MTHFR 677TT genotype with a normal folate level in their plasma have a lower risk of colorectal cancer compared with those with 677CC genotype.[31] Interestingly, consuming alcohol 5 or more times per week diminishes the lower cancer risk.[12] In fact, the associated risk of malignancies with MTHFR polymorphisms shows a gene-nutrient pattern that strongly depends on the folate intake level. Since MTHFR variants are risk factors for some cancers, such as cervical, breast, esophageal, and gastric malignancies,[20,27] it is well understood that MTHFR polymorphisms can play different roles in these disorders depending on the organs involved and the environmental situations.

Decreased activity of MTHFR enzyme causes an elevation in its substrate levels (5-10-methylen THF) and subsequently promotes more methylation of uridylate to thymidylate. On the other hand, a decreased 5-methyl THF pool (the product of enzymatic reaction) may affect DNA methylation and thereby contribute to tumor genesis.

Concerning these facts, in the case of colorectal cancer decreased activity of the MTHFR enzyme leads a shifting of the normal pattern of intracellular folate storage toward more DNA stability (protective role), while in the cases of cervical or breast cancers, decreased activity of the MTHFR enzyme in some tissues likely shifts more toward hypomethylation resulting in oncogenes activation and susceptibility to malignancies (adverse effects).[20] Supporting this hypothesis, it has been shown that MTHFR knockout mice have neuropathological lesions, aortic lipid deposition, and DNA hypomethylation.[32] In addition, these incompatible results can be associated with the presence or lack of folate deficiency, different types of malignant cells (solid tumors/hematologic malignancies or acute/chronic leukemias or lymphoid/myeloid lineages), and ethnical differences.

As stated in previous studies,[4] MTHFR enzyme activity should not be considered as the only effective factor in folate metabolism pathways. Intake of several elements in a nutrient diet, such as vitamins B6 and B12, which are co-factors of serine hydroxymethyltransferase (SHMT) and methionine synthase (MS) enzymes, respectively, 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. Excessive use of alcohol, which causes folate and vitamin B family deficiencies, must also be considered as an effective environmental factor. Different variants of all the above-stated enzymes may contribute to the risk of DNA instability and tumor genesis.

In conclusion, the present study showed no relation between MTHFR polymorphisms and myeloid leukemias (AML and CML), but further studies on larger samples with determined biochemical profiles (such as folate situation and homocysteine level) are necessary before absolute judgment about the role of MTHFR polymorphisms in the development of myeloid leukemias. Also, the high frequency of the 1298C allele in the Iranian population can be considered interesting points of our results. LM

Acknowledgments: We would like to acknowledge our colleagues from the following departments: the Hematology Department and Cellular and Molecular Research Center of Iran University of Medical Sciences, Tehran, Iran.

1. Smith, MT, Zhang L. Biomarkers of leukemia risk: Benzene as a model. *Environ Health Perspect*. 1998;106 (suppl 4):937–946.

2. Barbosa CG, Souza CL, Neto JPM, et al. Methylenetetrahydrofolate reductase polymorphisms in myeloid leukemia patients from Northeastern Brazil. *Genet and Mol Biol*. 2008;31:29–32.

3. Huang S. Histone methyltransferases, diet nutrients and tumour suppressors. *Nat Rev Cancer*. 2002;2:469–476.
4. Robien K, Ulrich CM. 5,10-Methylenetetrahydrofolate Reductase Polymorphisms and Leukemia Risk: A HuGE minireview. *Am J Epidemiol*. 2003;157:571–582.
5. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: A HuGE review. *Am J Epidemiol*. 2000;151:862–877.
6. Guenther BD, Sheppard CA, Tran P, et al. The structure and properties of methylenetetrahydrofolate reductase from *Escherichia coli* suggest how folate ameliorates human hyperhomocysteinemia. *Nat Struct Biol*. 1999;6:359–365.
7. Weisberg I, Tran P, Christensen B, et al. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab*. 1998;64:169–172.
8. Wiemels JL, Smith RN, Taylor GM, et al. Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and risk of molecularly defined subtypes of childhood acute leukemia. *Proc Natl Acad Sci USA*. 2001;98:4004–4009.
9. Matsuo K, Suzuki R, Hamajima N, et al. Association between polymorphisms of folate- and methionine-metabolizing enzymes and susceptibility to malignant lymphoma. *Blood*. 2001;97:3205–3209.
10. Song C, Xing D, Tan W, et al. Methylenetetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population. *Cancer Res*. 2001;61:3272–3275.
11. Campbell IG, Baxter SW, Eccles DM, et al. Methylenetetrahydrofolate reductase polymorphism and susceptibility to breast cancer. *Breast Cancer Res*. 2002;4:R14.
12. Chen J, Giovannucci E, Kelsey K, et al. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res*. 1996;56:4862–4864.
13. Curtin K, Bigler J, Slattery ML, et al. MTHFR C677T and A1298C polymorphisms: Diet, estrogen, and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev*. 2004; 13:285–292.
14. Oliveira KC, Bianco B, Verreschi IT, et al. Prevalence of the polymorphism MTHFR A1298C and not MTHFR C677T is related to chromosomal aneuploidy in Brazilian Turner Syndrome patients. *Arq Bras Endocrinol Metab*. 2008;52:1374–1381.
15. Wei J, Hemmings GP. Allelic association of the MTHFR gene with schizophrenia. *Mol Psychiatry*. 1999;4:115–116.
16. Qian X, Lu Z, Tan M, et al. A meta-analysis of association between C677T polymorphism in the methylenetetrahydrofolate reductase gene and hypertension. *Eur J Hum Genet*. 2007;15:1239–1245.
17. Franco RF, Simoes BP, Tone LG, et al. The methylenetetrahydrofolate reductase C677T gene polymorphism decreases the risk of childhood acute lymphocytic leukaemia. *Br J Haematol*. 2001;115:616–618.
18. Skibola CF, Smith MT, Kane E, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. *Proc Natl Acad Sci USA*. 1999;96:12810–12815.
19. Robien K, Ulrich CM, Bigler J, et al. Methylenetetrahydrofolate reductase genotype affects risk of relapse after hematopoietic cell transplantation for chronic myelogenous leukemia. *Clin Cancer Res*. 2004;10:7592–7598.
20. Moon HW, Kim TY, Oh BR, et al. MTHFR 677CC/1298CC genotypes are highly associated with chronic myelogenous leukemia: A case-control study in Korea. *Leuk Res*. 2007;31:1213–1217.
21. Hur M, Park JY, Cho HC, et al. Methylenetetrahydrofolate reductase A1298C genotypes are associated with the risks of acute lymphoblastic leukaemia and chronic myelogenous leukaemia in the Korean population. *Clin Lab Haematol*. 2006;28:154–159.
22. Deligezer U, Akisik E, Dalay N. Genotyping of the MTHFR gene polymorphism, C677T in patients with leukemia by melting curve analysis. *Mol Diagn*. 2003;7:181–185.
23. Sambrook J, Fritsch EF, Maniatis T. *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1982.
24. Wilcken B, Bamforth F, Li Z, et al. Geographical and ethnic variation of the 677C-T allele of 5,10 methylenetetrahydrofolate reductase (MTHFR): Findings from over 7000 newborns from 16 areas world wide. *J Med Genet*. 2003;40:619–625.
25. Almawi WY, Finan RR, Tamim H, et al. Differences in the frequency of the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene among the Lebanese population. *Am J Hematol*. 2004;76:85–87.
26. Boduroglu K, Alikasifoglu M, Anar B, et al. 677->CT mutation on the methylenetetrahydrofolate reductase gene is not a risk factor for neural tube defects in Turkey. (Letter). *Arch Dis Child Fetal Neonatal Ed*. 1998;78:F235.
27. Shen H, Xu Y, Zheng Y, et al. Polymorphisms of 5,10-methylenetetrahydrofolate reductase and risk of gastric cancer in a Chinese population: A case-control study. *Int J Cancer*. 2001;95:332–336.
28. Atashrazm F, Zaker F, Aqaeipour M, et al. High prevalence of MTHFR gene A1298C polymorphism in Iran. *Haematologica*. 2009;94 (suppl 2):482 abs. 1199.
29. Sabbagh AS, Mahfoud Z, Taher A, et al. High prevalence of MTHFR gene A1298C polymorphism in Lebanon. *Genet Test*. 2008;12:75–80.
30. Friedman G, Goldschmidt N, Friedlander Y, et al. A common mutation A1298C in human methylenetetrahydrofolate reductase gene: Association with plasma total homocysteine and folate concentrations. *J Nutr*. 1999;129:1656–1661.
31. Ma J, Stampfer MJ, Giovannucci E, et al. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. *Cancer Res*. 1997;57:1098–1102.
32. Paz MF, Avila S, Fraga MF, et al. Germ-line variants in methyl-group metabolism genes and susceptibility to DNA methylation in normal tissues and human primary tumors. *Cancer Res*. 2002;62:4519–4524.