Association of the 5,10-Methylenetetrahydrofolate Reductase (MTHFR C677T and A1298C) Polymorphisms in Korean Patients with Adult Acute Lymphoblastic Leukemia

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Abstract. Background: Methylenetetrahydrofolate reductase (MTHFR) plays a central role in converting folate to methyl donor for DNA methylation. Because MTHFR is a key enzyme in folate metabolism, changes in its activity resulting from polymorphisms in the MTHFR gene could modify the susceptibility to cancer. Recently, the C677T and A1298C mutations of MTHFR were discovered to be associated with susceptibility in acute lymphoblastic leukemia (ALL). Patients and Methods: The association between MTHFR polymorphisms and susceptibility and clinical outcome in ALL was studied in 118 adult ALL patients and matched healthy controls (n=427). DNA samples taken from patients with ALL and controls were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays to detect the MTHFR C677T and A1298C mutations. Results: No significant difference was found in the development of adult ALL among those with different MTHFR genotypes of the C677T or A1298C polymorphisms. However, the MTHFR 677CT+TT genotype showed a tendency to be associated with adult ALL (crude odds ratio (OR), 0.67; 95% confidence interval (CI), 0.44-1.02; adjusted OR, 0.74 95% CI, 0.47-1.14). Conclusion: The MTHFR C677T and A1298C polymorphisms are not significant risk factors in adult acute leukemia in the Korean population. A key enzyme in folate metabolism and DNA synthesis is 5,10-methylenetetrahydrofolate reductase (MTHFR). MTHFR catalyses the reduction of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, a major circulating form of folate. Moreover, 5-methyltetrahydrofolate provides a methyl group for homocysteine methylation to methionine, while 5,10-methylenetetrahydrofolate and its derivatives are essential cofactors for both thymidylate and de novo purine synthesis. Two common polymorphisms (MTHFR C677T and A1298C) are associated with a 50% to 60% decrease in the catalytic activity of MTHFR (1, 2).

Changes in folate metabolism influence nucleic acid synthesis, and DNA repair and methylation; folate deficiency induces chromosomal damage and the formation of fragile sites and micronuclei, which are often associated with tumorigenesis (3, 4). Because MTHFR is a key enzyme in folate metabolism, changes in its activity resulting from polymorphisms in the MTHFR gene could modify susceptibility to cancer. However, associations between...
polymorphisms of \textit{MTHFR} and a variable risk of neoplasia have been shown by several studies (5-12). For example, the homozygous \textit{MTHFR} 677TT mutant was found to be associated with a reduced incidence of colorectal cancer (10), whereas an increased frequency of 677TT homozygotes has been reported in cervical, endometrial, ovarian, breast, stomach, lung and esophageal cancer (5-9, 11, 13-16).

Skibola \textit{et al.} (13) have reported an association between susceptibility to adult acute lymphoblastic leukemia (ALL) and polymorphisms in the gene encoding \textit{MTHFR}. They suggested a lower risk of acute lymphoblastic leukemia in individuals carrying at least one T allele, which they believed was caused by more reliable DNA synthesis because of a greater availability of 5,10-methylenetetrahydrofolate for DNA synthesis, and reduced uracil misincorporation instead of thymidine due to the greater availability of methyl donors as a result of reduced \textit{MTHFR} activity.

However, studies performed in different populations have often produced inconsistent results and doubts about these associations have been raised (17-26). Therefore, in this study, we investigated the association between \textit{MTHFR} polymorphisms and the risk of adult ALL in Koreans.

\section*{Patients and Methods}

\textit{Study population.} This was a retrospective case controlled study. The study population constituted of 118 patients (mean age±SD, 37.93±17.33 years; age range, 23-91 years) diagnosed with ALL at 10 university hospitals including the HOGS study group, and was composed of 63 men and 55 women. Age- and sex-matched (mean age±SD, 48.40±16.52 years; age range, 23-91 years) controls (total number 427) were recruited from among healthy individuals who were randomly selected following health screening to exclude those with a history of malignant neoplastic or thrombotic disease.

\textit{Sample and data collection.} The institutional review board of Bundang CHA Hospital approved the research protocol and informed consent was obtained from all participating individuals. All cases and controls were restricted to Koreans. Peripheral blood or bone marrow slides for DNA assays, combined with data on age, gender and chromosomal status were taken from the 118 adult ALL patients. All the samples were collected and processed according to a standardized protocol from May 2001 to January 2002.

\textit{MTHFR genotyping.} DNA was extracted from leukocytes using DNA extraction kits (QI Amp blood kit, Qiagen, Hilden, Germany) according to the manufacturer’s protocol. The \textit{MTHFR} C677T and A1298C genotypes were identified as previously described (1, 17). The regions containing the two polymorphisms were amplified separately. For the 677 nucleotide polymorphism, the primers 5'--GCA CTT GAA GAG AAG GTG TC-3' (forward) and 5'--AGG ACG GTG CGG TGA GAG TG-3' (reverse) were used, and for the 1298 nucleotide polymorphism, 5'--CTT TGG GGA GCT GAA GGA CTA CTA C-3' (forward) and 5'--CAC TTT GTG ACC ATT CCG GTT TG-3' (reverse) were used. Human genomic DNA (200 ng) was amplified with 100 pmol of each forward and reverse primer, 1.5 mM MgCl$_2$, 0.2 M each deoxynucleotide triphosphate and 1 unit \textit{Taq} polymerase (Takara, Seongnam, South Korea) in a total volume of 100 \textmu l. Polymerase chain reaction (PCR) conditions were as follows: denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 51°C for 30 s and 72°C for 30 s, and finally by a terminal elongation at 72°C for 5 min. The PCR products were digested with \textit{Hin}II (for nucleotide 677) or \textit{Fnu}4HI (for nucleotide 1298) for 2 hours at 37°C.

Amplification success was monitored by 3.0% agarose electrophoresis. For the 677 nucleotide, an undigested PCR product (203 bp) indicated a homozygous wildtype, three bands of 203, 173 and 30 bp indicated the heterozygous genotype, and two bands of 170 and 30 bp indicated the homozygous genotype. For the 1298 nucleotide, a single band of 138 bp indicated a wild-type and two fragments of 119 and 19 bp indicated the homozygous genotype.

\textit{Statistical analysis.} The distributions of genotypes among cases and controls were compared using the Chi-square test. Odds ratios (ORs) and confidence intervals (CIs) were used to estimate the relative risks associated with adult ALL and in particular with the presence of different \textit{MTHFR} genotypes. Logistic regression was performed including sex and age as covariates. Probability values of less than 0.05 were accepted as significant. Statistical analysis was performed using Statistical Analysis System software 8.2 (SAS Institute, Inc., NC, USA).

\section*{Results}

\textit{MTHFR C677T and A1298C genotype distributions and allele frequencies in adult leukemia patients and healthy controls.} The distributions of the \textit{MTHFR} C677T and A1298C genotypes in cases and controls are summarized in Table I. The genotype distributions of the \textit{MTHFR} C677T and A1298C polymorphic loci did not deviate significantly from the Hardy-Weinberg equilibrium in either group. The 677TT genotype and 677TT allele frequencies were 0.1186 and 0.3516, in the ALL group and 0.1405 and 0.4090 in the control group, respectively (Table I). The frequencies of the 1298CC genotype and 1298C allele were 0.0187 and 0.1729, respectively, in the ALL cases (0.0187 and 0.1662, respectively). The distributions of the \textit{MTHFR} C677T and A1298C genotypes were compared using the Chi-square test. Odds ratios (ORs) and confidence intervals (CIs) were used to estimate the relative risks associated with adult ALL and in particular with the presence of different \textit{MTHFR} genotypes. Logistic regression was performed including sex and age as covariates. Probability values of less than 0.05 were accepted as significant. Statistical analysis was performed using Statistical Analysis System software 8.2 (SAS Institute, Inc., NC, USA).

\textit{Association between \textit{MTHFR} genotypes and adult leukemia.} The genotype frequencies of 677CT (adjusted odds ratio (AOR), 0.72; 95% CI, 0.45-1.14) and \textit{MTHFR} 677TT (AOR, 0.81; 95% CI, 0.40-1.63) showed no significant decrease in the risk of ALL over \textit{MTHFR} 677CC. Moreover, the combined \textit{MTHFR} 677CT+TT genotype (AOR, 0.74; 95% CI, 0.47-1.15) showed no significant difference in frequency versus \textit{MTHFR} 677CC.

Similarly, the genotype frequencies of \textit{MTHFR} 1298CC (AOR, 0.70; 95% CI, 0.14-3.61) and 1298AC (AOR, 0.96; 95% CI, 0.59-1.56) showed no significant impact on the risk of \textit{MTHFR} 1298AA. Moreover, the combined \textit{MTHFR} 1298AA+AC genotype (AOR, 0.94; 95% CI, 0.59-
1.51) showed no significant difference in frequency versus MTHFR 1298AA. No significant differences in frequencies of combined MTHFR C677T and A1298C genotypes were observed between the two groups (data not shown), and no significant differences in genotype frequencies were evident for the MTHFR C677T and A1298C polymorphisms in Ph(+) ALL patients (data not shown).

Haplotype analysis. Four haplotypes (C-A, T-A, C-C, T-C) were distinguished by the four alleles of MTHFR C677T and A1298C polymorphisms. When the cases of adult leukemia were compared with the controls, C-A (p=0.3208), T-A (p=0.2379) and C-C (p=0.8172) showed statistically insignificant differences (Table III).

Discussion

In the present study, we studied the impact of MTHFR C677T and A1298C polymorphisms on the development of adult acute lymphoblastic leukemia in a Korean population. Associations between the MTHFR polymorphisms and ALL have been studied in several populations but results obtained have not been consistent (Table IV; 13, 17, 20, 25, 27).

Skibola et al. (13) reported that individuals with the MTHFR genotypes 677TT, 1298AC and 1298CC had 4.3-, 3-, and 14-fold reduced risks, respectively, of developing adult ALL, but not acute myeloid leukemia in an English population. They also suggested that folate inadequacy may play a role in the development of adult ALL. Subsequently, several researchers reported that the MTHFR C677T polymorphism, but not A1298C, was linked to a decreased risk of developing childhood ALL in English (14), Brazilian (17) and Italian populations (27). Pereira et al. (28) also reported that the MTHFR 677T allele plays a protective role in adult ALL, whereas the A1298C genotype did not appear to significantly affect the risk of adult ALL when meta-analysis methods were applied.

Krajinovic et al. (20) found that the MTHFR 1298CC, 677TT/1298AA, 677CC/1298CC genotypes, but not 677CT or 677TT genotypes were associated with a reduced risk of adult ALL in the Canadian population. Zanrosso et al. (25) also reported finding that the 677T allele was linked to a reduced risk, whereas the 1298AC genotype was found to be associated with an elevated risk of ALL in Brazilian non-white children. On the other hand, polymorphisms in the MTHFR gene were not found to be associated with ALL in Italian, Japanese, Portuguese or German populations (19, 21, 23, 24).

The present study was also not able to demonstrate a clear association between 677CT or 677TT and a reduced risk of developing ALL, though the ORs tended to be lower for 677CT+TT as compared with 677CC (Table II). This result is similar to those reported in Japanese, Italian and
German ALL patients. It is to be noted that many Korean foods contain folic acid and Koreans usually have a higher folate-containing diet than in that of Western populations. This result, combined with the findings of the recent Canadian study by Krajinovic et al. (20), suggested that the role of MTHFR genotypes in the development of ALL may differ among populations due to different dietary and racial backgrounds (4). The Canadian investigators also determined that folic acid supplementation offset the risk effect of developing ALL, by stratifying patients before and after the time that folate supplementation was recommended during pregnancy. In the present study, the association of adult leukemia with allele haplotypes was delineated (Table III). However, against our expectations, in adult ALL, none of the allele haplotypes significantly increased the risk of cancer development in the Korean population.

Some recent studies have reported on the response of the MTHFR C677T and A1298C polymorphisms to chemotherapy. Ulrich et al. (18) first reported that

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<tr>
<th>Investigators</th>
<th>Population</th>
<th>ALL patients (No.)</th>
<th>MTHFR 677</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skibola et al. (13)</td>
<td>English</td>
<td>71</td>
<td>CT vs. CC (OR=0.58, 95% CI: 0.27-1.28)</td>
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<td>TT vs. CC (OR=0.23, 95% CI: 0.06-0.81)</td>
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<td>AC vs. AA (OR=0.33, 95% CI: 0.15-0.73)</td>
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<td>CC vs. AA (OR=0.07, 95% CI: 0.00-1.77)</td>
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<tr>
<td>Franco et al. (17)</td>
<td>Brazilian-Caucasian</td>
<td>71</td>
<td>CT vs. CC (OR=0.5, 95% CI: 0.2-0.9)</td>
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<td>TT vs. CC (OR=0.3, 95% CI: 0.09-0.8)</td>
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<td>CT+TT vs. CC (OR=0.4, 95% CI: 0.2-0.8)</td>
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<tr>
<td>Gemmati et al. (27)</td>
<td>Italian</td>
<td>120</td>
<td>CT vs. CC (OR=0.6, 95% CI: 0.34-1.04)</td>
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<td>TT vs. CC (OR=0.28, 95% CI: 0.12-0.72)</td>
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<td>CT+TT vs. CC (OR=0.56, 95% CI: 0.35-0.90)</td>
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<tr>
<td>Krajinovic et al. (20)</td>
<td>French-Canadian</td>
<td>270</td>
<td>CT vs. CC (OR=1.1, 95% CI: 0.8-1.6)</td>
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<td>TT vs. CC (OR=0.8, 95% CI: 0.4-1.3)</td>
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<td>Zanrosso et al. (25)</td>
<td>Brazilian</td>
<td>176</td>
<td>CT vs. CC (OR=0.68, 95% CI: 0.43-1.09)</td>
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<td>TT vs. CC (OR=0.65, 95% CI: 0.29-1.46)</td>
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<td>CT+TT vs. CC (OR=0.68, 95% CI: 0.44-1.05)</td>
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<td>Present study</td>
<td>Korean</td>
<td>118</td>
<td>CT vs. CC (OR=0.72, 95% CI: 0.45-1.14)</td>
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Table IV. MTHFR polymorphism and different population in ALL patients.
individuals with the MTHFR 677TT genotype were more susceptible to the toxic effects of methotrexate than those with the wild-type. Krajinovic et al. (29) demonstrated that the MTHFR 677T variant allele was significantly associated with lower event-free survival (EFS) in Canadian childhood ALL, whereas Aplenc et al. (22) concluded that this allele was significantly associated with the relapse of childhood ALL in an American population. However, in the present study, we could not demonstrate any significant association between MTHFR genotypes and clinical outcome, or the utility of the MTHFR genotype as a prognostic factor as compared with known prognostic factors (data not shown).

In conclusion, our findings suggest that the MTHFR genotype is unlikely to affect the development of adult ALL in the Korean population. Therefore, future studies should include larger sample sizes and factors relating to both genetics and nutrition to explain the discrepancies among populations.

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Appendix

The following investigators are participating members of the Hematology Group of Seoul (HOGS) not listed in the front page: Gachon Gil University, Inchon: Soo Mee Bang, Eun Kyung Cho; Hallym University, Seoul: Jung Ae Lee, Jung Hye Kwon; Inha University, Inchon: Moon Hee Lee; Pochon CHA University, Gachon Gil University, Inchon: Soo Mee Bang, Eun Kyung Cho; Inha University, Inchon: Soyeun Oh, So Young Chong, Hee Jung Am; Seoul National University, Seoul: Inho Kim, Sung Soo Yoon.

References


