Common Gene Polymorphisms in the Metabolic Folate and Methylation Pathway and the Risk of Acute Lymphoblastic Leukemia and non-Hodgkin’s Lymphoma in Adults

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Abstract

Folate and methionine metabolism is involved in DNA synthesis and methylation processes. Polymorphisms in the genes of folate metabolism enzymes have been associated with some forms of cancer. In a case-control study, we evaluated whether four common polymorphisms in methylenetetrahydrofolate reductase (MTHFR C677T and A1298C), methionine synthase (MS A2756G), and methionine synthase reductase (MTRR A666G) genes may have a role in altering susceptibility to adult acute lymphoblastic leukemia (ALL) and non-Hodgkin’s lymphoma (NHL). We analyzed DNA of 120 adult ALL, 200 NHL, and 257 healthy control subjects. Individual carrying the MTHFR 677TT genotype showed a 3.6-fold decreased ALL risk (odds ratio (OR) 0.28, 95% confidence interval (95% CI) 0.12–0.72) than wild-types. Similarly, MS 2756GG individuals showed a 5.0-fold decreased ALL risk (OR 0.20, 95% CI 0.02–1.45) than wild-types. In combined results, subjects with the MTHFR 677CT/TT and MS 2756AG/GG genotypes revealed a 3.6-fold ALL risk reduction (OR 0.28, 95% CI 0.14–0.58) and those with the MTHFR 677TT and MTRR 66AG genotypes revealed a 4.2-fold ALL risk reduction (OR 0.24, 95% CI 0.06–0.81). Finally, those with the MS 2756AG/GG and MTRR 66AG/GG genotypes revealed a 2.2-fold ALL risk reduction (OR 0.45, 95% CI 0.10–0.85). Single analysis for NHL did not show any significant difference for all the polymorphisms investigated, but in the low-grade NHL subgroup, we found a 2.0-fold risk reduction for the MTRR 66GG homozygous genotype (OR 0.50, 95% CI 0.25–0.99), which was higher (OR 0.37, 95% CI 0.14–0.85) when analyzed in combination with MS 2756AA genotype. These data are in accordance with the hypothesis that polymorphisms in the genes for folate and methionine metabolism might play a greater role in the occurrence of ALL than NHL by influencing DNA synthesis and/or DNA methylation. [Cancer Epidemiol Biomarkers Prev 2004;13(5):787–94]

Introduction

The biological mechanisms and etiology of malignant lymphoma and leukemia are not yet completely understood and ionizing radiation, benzene, and cancer chemotherapy account for only a small percentage of the total cases (1, 2). Single genetic defects are unlikely to be responsible for hematological malignancies and a modest familial link is observed. Therefore, unfavorable gene-environment interactions might be involved in the genesis of these tumors. Several genetic alterations, due to incorrect DNA synthesis or altered methylation status of oncogenes and/or tumor suppressor genes, have been identified in the pathogenesis of lymphoid malignancies (3–5). Folate and methionine metabolism plays an essential role in both DNA synthesis and methylation processes. Defects or polymorphisms in the genes of the folate-dependent enzymes and deficiencies of micro-nutrients may influence cancer susceptibility (5–8). Methylenetetrahydrofolate reductase (MTHFR) reduces 5,10-methylenetetrahydrofolate (methylene-THF) to 5-methyl-THF, the main circulatory form of folate and one-carbon donor for the remethylation of homocysteine to methionine (Fig. 1). Two common polymorphisms (C677T and A1298C) in the MTHFR gene (9, 10) have been described which reduce enzyme activity, the latter to a lesser extent than C677T. A lower activity leads to increased plasma levels of homocysteine and decreased 5-methyl-THF formation. This contributes to the pool of methylene-THF, the methyl group donor...
DNA methylation

\[
\begin{align*}
\text{SAM} \rightarrow \text{SAH} \\
\text{Methionine} \rightarrow \text{Homocysteine} \\
m_{\text{MS}} \rightarrow \text{Col}-\text{MS} \rightarrow \text{Col}-\text{III} \\
\end{align*}
\]

DNA synthesis

\[
\begin{align*}
\text{Folic Acid} \rightarrow \text{DHF} \rightarrow \text{THF} \rightarrow 5\text{-CH}_2\text{-THF} \rightarrow 10\text{-CHO-THF} \rightarrow 5,10\text{-CH}_2\text{-THF} \rightarrow \text{MTHFR} \\
\text{Purines} \rightarrow \text{dTMP} \rightarrow \text{dUMP} \\
\end{align*}
\]

Figure 1. Overview of folate and methionine metabolism pathways. TS, thymidylate synthase; DHF, dihydrofolate; THF, tetrahydrofolate; 5,10-CH\text{2}-THF, 5,10-methylenetetrahydrofolate; 5-CH\text{2}-THF, 5-methyltetrahydrofolate; 10-CHO-THF, 10-formyltetrahydrofolate; Col, cob(I)alamin; ColII, cob(II)alamin; ColIII, cob(III)alamin; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine.

for the conversion of dUMP to dTMP by thymidylate synthase enzyme. Therefore, it is thought that lower MTHFR activity might favor optimal DNA synthesis by reducing uracil misincorporation rate, a potential cause of double-strand breaks during uracil excision repair processes (6, 11). On the other hand, a lower availability in 5-methyl-THF might decrease the synthesis of methionine and consequently of S-adenosylmethionine, crucially involved in cellular methylation processes (12, 13). Methionine synthase (MS) catalyzes the transfer of the methyl group from 5-methyl-THF to homocysteine generating tetrahydrofolate and methionine, thus playing a critical role in maintaining adequate intracellular methionine concentrations. A recently identified common polymorphism (MS A2756G) is thought to affect the enzymatic activity (14) and to induce modest homocysteine reduction (15) and DNA hypomethylation (16). While several studies have investigated the association between folate-related polymorphisms and neural tube defect (17–19), arterial or venous vascular disease (9, 20–24), and several forms of solid tumor (25–28), only few recent studies have investigated their influence on the risk of lymphoid malignancies. Skibola et al. (29) and Matsuo et al. (16) reported independently that individuals with either MTHFR polymorphisms had a significantly lower susceptibility to adult acute lymphoblastic leukemia (ALL) and malignant lymphoma, respectively. Similar results were obtained by Franco et al. (30) and Wiemels et al. (31) in childhood leukemia. The role of MS A2756G polymorphism appears more controversial. In fact, Matsuo et al. (16) have shown that MS A2756G polymorphism may be considered a risk factor for malignant lymphoma, while Skibola et al. (32) found a protective effect of MS A2756G against ALL when analyzed with serine hydroxymethyltransferase C1420T polymorphism. Finally, methionine synthase reductase (MTRR) might be a further candidate gene in studies on lymphoid malignancies susceptibility, alone or in conjunction with MS gene, because it maintains adequate cell levels of methylcob(III)alamin, the functional cofactor form of MS enzyme (33, 34). Alterations in MTRR activity may affect MS-dependent remethylation processes (33, 35, 36); therefore, we also tested whether a newly discovered MTRR A666G polymorphism (37) might be a further modulating factor in hematological malignancies. In the present study, we analyzed the association of two folate-related polymorphisms (MTHFR C677T and A1298C) and two methylation-related polymorphisms (MS A2756G and MTRR A666G) with susceptibility to ALL and non-Hodgkin's lymphoma (NHL).

Methods

Selection of Cases and Controls. Recruitment of ALL cases consisted of 120 Italian individuals (all Caucasians) with newly diagnosed ALL according to the French-American-British classification. They were from the files of patients who received hematological diagnosis by the Unit of Hematology of the University of Ferrara (n = 71) in the period between January 1990 and December 2001 and by the Department of Hematology of the San Bortolo Hospital of Vicenza (n = 49) in the period between December 1992 and December 2001. The two hematological centers are in adjacent regions of northern Italy about 100 km distant and all the cases were resident in the same geographic area. The whole group of ALL cases were persons aged 18–75 years with a mean age of 48.5 ± 15.64 years and 57.5% of them were male. Among the ALL cases, 88 (73.3%) were diagnosed as ALL-B and 20 (16.6%) were ALL-T, and for 12 cases (10%), the immunophenotype could not be specified.

Recruitment of NHL cases consisted of 200 Italian individuals (all Caucasians) with newly diagnosed NHL according to the Revised European American Lymphoma classification. They were from the files of patients who received hematological diagnosis by the Unit of Hematology of the University of Ferrara in the period between June 1987 and December 2001. NHL cases were persons aged 18–75 years with a mean age of 54.5 ± 15.32 years and 59% of them were male. Among the NHL cases, 88 (73.3%) were diagnosed as low-grade NHL (follicular lymphoma n = 53, marginal zone B-cell lymphoma n = 21, mantle cell lymphoma n = 16, lymphoplasmocytic lymphoma n = 11, Castleman lymphoma n = 1) and the remaining 98 (49.0%) cases were classified as high-grade NHL (diffuse large B-cell lymphoma n = 79, anaplastic large cell lymphoma n = 19). Cases diagnosed with ALL or NHL within 6 months of being diagnosed with a prior hematological malignancy or within 2 years of any other cancer were considered ineligible.

The control group (n = 257) consisted of Italian healthy individuals (all Caucasians) from the same geographic area as the cases, not relatives of the cases and randomly selected from the blood donor lists and in the same proportion as the patients coming from the two hospitals considered in the study in the period between January
adapted from Jaques

Watertown, MA) and were completed with a 5 min final PCR product was digested by

The PCR conditions were modified as follows: 5 min initial at 94°C followed by 30 cycles of 94°C for 45 s, 65°C for 60 s, and 72°C for 90 s, and the PCR product was digested by Hinfl (Roche Diagnostics GmbH, Mannheim, Germany). The genotyping protocol for MTHFR C677T polymorphism detection was adapted from Weisberg et al. (10) with the following primers: 5′-TGA AGG AGA AGG TGT CTC CGG GA-3′ and 5′-AGG ACG GTG CGG TGA GAG TG-3′. The PCR conditions were modified as follows: 5 min initial at 94°C followed by 30 cycles of 94°C for 30 s, 59°C for 13 s, and 72°C for 17 s, and the PCR product was digested by Fnu4HI (New England BioLabs, Beverly, MA). The genotyping protocol for MTHFR A1298C polymorphism detection was adapted from Matsuo et al. (16) with the following primers: 5′-TGG AGG AGC TGA CCA GTG CAG-3′ and 5′-GGG GTC AGG CCA GGA GGA G-3′. The PCR conditions were modified as follows: 5 min initial at 94°C followed by 30 cycles of 94°C for 30 s, 59°C for 13 s, and 72°C for 17 s, and the PCR product was digested by HaeIII (Roche Diagnostics). The genotyping protocol for MTRR A66G polymorphism detection was adapted from Jaques et al. (38) with the following primers: 5′-CAG GCA AAG GCC ATC GCA GAA GAC AT-3′ and 5′-CAC CTT CCC CAA CAA AAT TCT TCA AAG-3′. The PCR conditions were modified as follows: 5 min initial at 94°C followed by 32 cycles at 94°C for 60 s, 58°C for 45 s, and 72°C for 60 s, and the PCR product was digested by AluIII (New England BioLabs). All PCR cycles were performed in a Peltier Thermal Cycler apparatus (PTC-200; M. J. Research, Inc., Watertown, MA) and were completed with a 5 min final extension step at 72°C. DNA digestions were performed according to suppliers’ instructions. Confirmation of genotypes was carried out by regenotyping a random selection of samples (cases and controls) for each polymorphism investigated. There were no discrepancies between genotypes determined in duplicate.

**Statistical Analysis.** Statistical differences between case and control populations were tested using χ² and Student’s tests. Where appropriate, Yates’ correction or Fisher’s exact test were applied. P ≤ 0.05 was considered statistically significant. Odds ratios (OR) and 95% confidence intervals (95% CI) were used to estimate the risk of developing ALL or NHL. Adjusted ORs for single or combined comparisons were calculated with logistic regression models that controlled for sex, age, and other polymorphisms (i.e., MTHFR C677T and A1298C, MTRR C677T and MS A2756G mutual OR adjustments, and MTHFR 677/MS 2756 interaction test). For the test trend across genotypes, from the reference genotype to the homozygous variant, P values are reported at the bottom of Table 1. The expected frequency of control genotypes were checked by the Hardy-Weinberg equilibrium test. All analyses were performed by Systat V.5.0 (Systat Inc., Evanston, IL) and SPSS Statistical Package (SPSS Inc., Chicago, IL).

**Results**

Comparison of the allele frequency and genotype distribution between the whole ALL group and the control group showed statistically significant differences. After categorizing the 120 ALL cases according to ALL-B and ALL-T immunophenotype, no significant differences were observed between the subgroups (data not shown); therefore, the ALL cases were analyzed as single group. In contrast, after subsetting the 200 NHL cases according to high/low grade of malignancy stratification, the MTRR and MS polymorphisms gave significant differences in the low-grade subgroup compared with controls. Therefore, NHL cases were analyzed both as a whole and a subdivided group. To evaluate in younger patients possible associations between the polymorphisms and ALL or NHL, we also subsetted cases in three subgroups of age (<40, <50, and <60 years old), but the subsetted analysis did not furnished significant differences among the subgroups.

**Genotyping and Adjusted Risk Evaluation by Single Analysis for MTHFR, MS, and MTRR Polymorphisms in ALL Cases.** Table 1 shows the allele and genotype frequencies for ALL cases and controls and the adjusted OR values for each polymorphism. Genotype frequencies were in agreement with the Hardy-Weinberg expectations in all the control genotypes analyzed.

**MTHFR C677T** The frequency of the 677T polymorphic allele was 31.1% in ALL cases and 44.7% in controls (P = 0.001). Considering the MTHFR 677CC genotype as the reference, individuals with MTHFR 677TT genotype showed a 3.6-fold reduction in ALL risk (OR 0.28, 95% CI 0.12–0.72) and individuals with MTHFR 677CT genotype showed a 1.7-fold reduction in ALL risk (OR 0.60, 95% CI 0.34–1.04). The test for trend yielded a significant P value of <0.001.
**Cancer Epidemiol Biomarkers Prev 2004;13(5). May 2004**

**Table 1. Number of patients and controls, sex- and age-adjusted OR and 95% CI, for MTHFR, MS, and MTRR polymorphisms**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases [n (%); ALL (n = 120)]</th>
<th>OR 95% CI</th>
<th>P</th>
<th>Cases [n (%); NHL (n = 200)]</th>
<th>OR 95% CI</th>
<th>P</th>
<th>Controls [n (%); n = 257]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MTHFR 677 (n = 114)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>52 (45.6)</td>
<td>1.00 Reference</td>
<td></td>
<td>60 (30.0)</td>
<td>1.00 Reference</td>
<td></td>
<td>78 (30.3)</td>
</tr>
<tr>
<td>CT</td>
<td>53 (46.5)</td>
<td>0.60 0.34–1.04 0.055</td>
<td></td>
<td>101 (50.5)</td>
<td>0.95 0.65–1.58 0.82</td>
<td></td>
<td>128 (49.8)</td>
</tr>
<tr>
<td>TT</td>
<td>9 (7.9)</td>
<td>0.28 0.12–0.72 0.008</td>
<td></td>
<td>39 (19.5)</td>
<td>0.84 0.59–1.72 0.59</td>
<td></td>
<td>51 (19.8)</td>
</tr>
<tr>
<td>CT + TT</td>
<td>62 (54.4)</td>
<td>0.56 0.35–0.90 0.022</td>
<td></td>
<td>140 (70.0)</td>
<td>0.92 0.66–1.50 0.72</td>
<td></td>
<td>179 (69.6)</td>
</tr>
<tr>
<td>C</td>
<td>157 (68.8)</td>
<td>0.57 0.41–0.79 0.001</td>
<td></td>
<td>221 (55.2)</td>
<td>0.93 0.74–1.29 0.64</td>
<td></td>
<td>284 (55.3)</td>
</tr>
<tr>
<td>T</td>
<td>71 (31.3)</td>
<td></td>
<td></td>
<td>179 (44.8)</td>
<td></td>
<td></td>
<td>230 (44.7)</td>
</tr>
<tr>
<td><strong>MTHFR 1298 (n = 114)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>47 (41.2)</td>
<td>1.00 Reference</td>
<td></td>
<td>96 (48.0)</td>
<td>1.00 Reference</td>
<td></td>
<td>126 (49.0)</td>
</tr>
<tr>
<td>AC</td>
<td>55 (48.2)</td>
<td>1.00 0.59–1.77 0.97</td>
<td></td>
<td>90 (45.0)</td>
<td>1.20 0.74–1.57 0.40</td>
<td></td>
<td>110 (42.8)</td>
</tr>
<tr>
<td>CC</td>
<td>12 (10.5)</td>
<td>0.90 0.36–2.20 0.81</td>
<td></td>
<td>14 (7.0)</td>
<td>0.78 0.40–1.71 0.36</td>
<td></td>
<td>21 (8.2)</td>
</tr>
<tr>
<td>AC + CC</td>
<td>67 (58.8)</td>
<td>1.07 0.65–1.77 0.78</td>
<td></td>
<td>104 (52.0)</td>
<td>1.15 0.73–1.49 0.62</td>
<td></td>
<td>131 (60.0)</td>
</tr>
<tr>
<td>A</td>
<td>149 (65.3)</td>
<td>1.27 0.89–1.73 0.16</td>
<td></td>
<td>282 (70.5)</td>
<td>0.98 0.71–1.36 0.93</td>
<td></td>
<td>362 (70.4)</td>
</tr>
<tr>
<td>C</td>
<td>79 (34.6)</td>
<td></td>
<td></td>
<td>118 (29.5)</td>
<td></td>
<td></td>
<td>152 (29.6)</td>
</tr>
<tr>
<td><strong>MS 2756 (n = 118)</strong></td>
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<td></td>
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<tr>
<td>AA</td>
<td>88 (74.6)</td>
<td>1.00 Reference</td>
<td></td>
<td>129 (64.5)</td>
<td>1.00 Reference</td>
<td></td>
<td>158 (61.5)</td>
</tr>
<tr>
<td>AG</td>
<td>29 (24.6)</td>
<td>0.56 0.32–0.90 0.03</td>
<td></td>
<td>65 (32.5)</td>
<td>0.98 0.62–1.35 0.33</td>
<td></td>
<td>89 (34.6)</td>
</tr>
<tr>
<td>GG</td>
<td>18 (1.0)</td>
<td>0.20 0.02–1.45 0.14</td>
<td></td>
<td>6 (3.0)</td>
<td>0.78 0.23–2.11 0.67</td>
<td></td>
<td>10 (3.9)</td>
</tr>
<tr>
<td>AG + GG</td>
<td>30 (25.4)</td>
<td>0.52 0.30–0.80 0.011</td>
<td></td>
<td>71 (35.5)</td>
<td>0.96 0.58–1.27 0.85</td>
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<td>99 (38.5)</td>
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<td>A114</td>
<td>205 (86.8)</td>
<td>0.55 0.37–0.87 0.008</td>
<td></td>
<td>123 (60.7)</td>
<td>0.94 0.61–1.22 0.76</td>
<td></td>
<td>405 (78.8)</td>
</tr>
<tr>
<td>G</td>
<td>31 (13.1)</td>
<td></td>
<td></td>
<td>77 (35.3)</td>
<td></td>
<td></td>
<td>109 (21.2)</td>
</tr>
<tr>
<td><strong>MTRR 66 (n = 109)</strong></td>
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<tr>
<td>AA</td>
<td>28 (25.7)</td>
<td>1.00 Reference</td>
<td></td>
<td>51 (25.5)</td>
<td>1.00 Reference</td>
<td></td>
<td>59 (22.9)</td>
</tr>
<tr>
<td>AG</td>
<td>58 (53.2)</td>
<td>1.13 0.63–2.02 0.68</td>
<td></td>
<td>106 (53.0)</td>
<td>1.13 0.65–1.61 0.64</td>
<td></td>
<td>122 (47.5)</td>
</tr>
<tr>
<td>GG</td>
<td>23 (21.1)</td>
<td>0.71 0.35–1.40 0.32</td>
<td></td>
<td>43 (21.5)</td>
<td>0.70 0.35–1.12 0.31</td>
<td></td>
<td>76 (29.6)</td>
</tr>
<tr>
<td>AG + GG</td>
<td>81 (73.4)</td>
<td>0.87 0.51–1.46 0.60</td>
<td></td>
<td>149 (74.5)</td>
<td>0.90 0.54–1.35 0.91</td>
<td></td>
<td>198 (77.0)</td>
</tr>
<tr>
<td>A</td>
<td>114 (52.3)</td>
<td>0.80 0.57–1.09 0.17</td>
<td></td>
<td>208 (52.0)</td>
<td>0.84 0.60–1.06 0.26</td>
<td></td>
<td>240 (46.7)</td>
</tr>
<tr>
<td>G</td>
<td>104 (47.7)</td>
<td></td>
<td></td>
<td>192 (48.0)</td>
<td></td>
<td></td>
<td>274 (53.3)</td>
</tr>
</tbody>
</table>

Note: OR for MTHFR 677 polymorphism has been adjusted for MTHFR 1298. In addition, MTHFR 677 polymorphism has been also adjusted for MS 2756 and vice versa. The trend test yielded a significant value (P < 0.001) only for MTHFR 677 and MS 2756 variants in ALL cases.

Six patients were excluded from the analysis because DNA was not amplifiable by PCR (see Methods).

Two patients were excluded from the analysis because DNA was not amplifiable by PCR (see Methods).

Eleven patients were excluded from the analysis because DNA was not amplifiable by PCR (see Methods).

**MTHFR A1298C** The frequency of the 1298C polymorphic allele was 34.6% in cases and 29.6% in controls (P = 0.16). The MTHFR A1298C polymorphism did not significantly affect the risk of ALL in our population in single analysis (OR 0.90, 95% CI 0.36–2.20 and OR 1.07, 95% CI 0.65–1.77 for MTHFR 1298CC and 1298AC + CC individuals, respectively). The test for trend did not reach a significant level (P = 0.20).

**MS A2756G** The frequency of the 2756G polymorphic allele was 13.1% in cases and 21.2% in controls (P = 0.008). When the MS 2756AA genotype was defined as the reference, individuals with MS 2756GG genotype showed a 5.0-fold reduction in ALL risk (OR 0.20, 95% CI 0.02–1.45), but it did not reach statistical significance (P = 0.14), because of the low representation of the MS 2756GG homozygous genotype found in both ALL cases and controls (0.8% and 3.9%, respectively). In fact, data from the more represented AG heterozygous MS 2756 genotype gave a significant difference (OR 0.56, 95% CI 0.32–0.90, P = 0.030), and computing together the MS 2756GG and AG types, a significant (P = 0.011) 1.9-fold risk reduction was yielded (OR 0.52, 95% CI 0.30–0.80). The test for trend yielded a significant P value of <0.001.

**MTRR A66G** The frequency of the 66G polymorphic allele was 47.7% in cases and 53.3% in controls (P = 0.17). The MTRR A66G polymorphism did not affect significantly the risk of ALL in our population in single analysis (OR 0.71, 95% CI 0.35–1.40 and OR 0.87, 95% CI 0.51–1.46 for MTRR 66GG and AG + GG individuals, respectively). The test for trend did not reach a significant level (P = 0.30).

**Combined Analysis in ALL Cases.** Table 2 shows combined results for MTHFR 677 and MS 2756 polymorphisms. Considering subjects with MTHFR 677CC and MS 27566A genotype as the reference, every combination of alleles showed lower than unit OR values, and the statistical significance was reached for large part of them. Anyhow, the evaluation of the combined risks did not differ from the risk estimate obtained by the analysis of the MTHFR 677TT genotype alone (Table 1), with the exception of the MTHFR 677TT and MS 2756AA combination, which yielded a 5.0-fold risk reduction. This leads to the conclusion that also subjects wild-type for both MTHFR 677 and MS 2756 substitution had an increased susceptibility of 3.6-fold to develop ALL than subjects with at least one polymorphic allele for both variants. Nevertheless, to investigate potential gene-gene interactions between MTHFR 677 and MS 2756, we applied a logistic regression analysis. Although data could suggest possible interactions, the
analysis did not have sufficient power to detect a significant interaction. The likelihood ratio test for the existence of interaction resulted in a marginal $P$ value of 0.098. Combined results for MTHFR 677 and MTRR 66 polymorphism showed statistical significance only for subjects with MTHFR TT and MTRR AG genotype, which showed a 4.2-fold ALL risk reduction (OR 0.24, 95% CI 0.14–0.85; $P = 0.04$) when MTHFR 677CC and MTRR 66AA genotype was considered as the reference. Combined results for MS 2756 and MTRR 66 polymorphism showed statistical significance only for subjects carrying at least one polymorphic allele for both variants yielding a 2.2-fold ALL risk reduction (OR 0.45, 95% CI 0.10–0.85; $P = 0.045$). Combined results for MTHFR C677T and A1298C polymorphism showed statistical significance only for those subjects carrying the 677TT and 1298AA homozygous genotype yielding a 3.0-fold ALL risk reduction (OR 0.33, 95% CI 0.15–0.83; $P = 0.04$). This finding is mainly due to the strong protective effect of the 677TT genotype necessarily present in conjunction with the 1298AA genotype because of the strong negative disequilibrium between the two MTHFR polymorphic alleles.

### Discussion

In the present study, we analyzed the association between susceptibility to adult ALL or NHL and four polymorphisms in the genes coding for the MTHFR, MS, and MTRR enzymes. Specifically, we found that individuals carrying at least one MTHFR substitution at site 677 (C→T) or one MS substitution at site 2756 (A→G) showed appreciable reduction in ALL susceptibility. This was particularly evident for subjects carrying either the homozygous 677TT substitution with a decreased ALL risk of 3.6-fold or the homozygous 2756GG substitution with a decreased ALL risk of 5.0-fold. Subjects carrying the 2756GG genotype showed the highest protection because only 1 ALL case among 118 had this genotype. However, the wide 95% CI exceeded the unit value probably because of the relatively small number of GG genotype found in both cases and controls. Heterozygous 677CT or 2756AG genotypes also had a significant ALL risk reduction of about 2-fold. The MTHFR 1298 and MTRR 66 substitutions did not show significant protection against ALL in single analysis; therefore, we looked at the joint effects between each couple of polymorphisms. In combined analysis for MTHFR 677 and MS 2756, the risk reduction values were equal to or lower than that of the MTHFR TT genotype alone, with the exception of subjects with MTHFR 677TT and MS 2756AA where the effect was higher (5.0-fold risk reduction). For the other polymorphism combinations, the effects were less pronounced. In fact, when MTHFR 677 and MTRR 66 were computed together, only the subjects carrying the 677TT with the 66AG genotype showed a 4.2-fold significant risk reduction. Naturally, the protection was not restricted only to those particular classes of genotypes, because other subsets of genotypes might expect

### Table 2. Number of ALL patients and controls, sex- and age-adjusted OR and 95% CI, for MTHFR C677T and MS 2756GG polymorphisms

<table>
<thead>
<tr>
<th>MTHFR 677</th>
<th>MS 2756</th>
<th>Cases [$n$ (%); $n = 111$]</th>
<th>Controls [$n$ (%); $n = 257$]</th>
<th>OR</th>
<th>95% CI</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>AA</td>
<td>39 (35.1)</td>
<td>48 (18.7)</td>
<td>1.00</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>CT/TT*</td>
<td>AG/GG*</td>
<td>16 (14.4)</td>
<td>69 (26.8)</td>
<td>0.28</td>
<td>0.14–0.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CC</td>
<td>AG</td>
<td>12 (10.8)</td>
<td>26 (10.1)</td>
<td>0.57</td>
<td>0.25–1.29</td>
<td>0.18</td>
</tr>
<tr>
<td>CC</td>
<td>GG</td>
<td>0</td>
<td>4 (1.5)</td>
<td>NE</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CT</td>
<td>AA</td>
<td>39 (35.1)</td>
<td>80 (31.1)</td>
<td>0.61</td>
<td>0.34–1.08</td>
<td>0.09</td>
</tr>
<tr>
<td>CT</td>
<td>AG</td>
<td>11 (9.9)</td>
<td>44 (17.1)</td>
<td>0.31</td>
<td>0.14–0.67</td>
<td>0.003</td>
</tr>
<tr>
<td>CT</td>
<td>GG</td>
<td>1 (0.9)</td>
<td>4 (1.5)</td>
<td>0.31</td>
<td>0.03–2.87</td>
<td>0.53</td>
</tr>
<tr>
<td>TT</td>
<td>AA</td>
<td>5 (4.5)</td>
<td>30 (11.7)</td>
<td>0.20</td>
<td>0.07–0.57</td>
<td>0.003</td>
</tr>
<tr>
<td>TT</td>
<td>AG</td>
<td>4 (3.6)</td>
<td>19 (7.4)</td>
<td>0.27</td>
<td>0.07–0.57</td>
<td>0.030</td>
</tr>
<tr>
<td>TT</td>
<td>GG</td>
<td>0</td>
<td>2 (0.8)</td>
<td>NE</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CT + TT</td>
<td>AG + GG*</td>
<td>72 (64.9)</td>
<td>209 (81.3)</td>
<td>0.42</td>
<td>0.26–0.70</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Note: Test for interaction: $P = 0.098$. NE, not estimated.

*All the subjects carrying at least one variant allele for both polymorphisms in combined way.

*All the subjects carrying at least one variant allele for either polymorphisms in combined way.
to have greater protection as the double homozygotes (677TT/2756GG or 677TT/66GG), but for both the combinations, no subjects with the double variant were found among ALL cases. Similarly, when MS 2756 and MTRR 66 were analyzed in combination, only those with at least one variant allele in both genes had approximately a significant 2.2-fold lower susceptibility to develop ALL. In combined analyses, the loss of statistical significance is also due to the small number of patients with rare genotypes, but this observation supports the idea that polymorphic alleles are underrepresented in cases.

Our results on ALL are conceptually in accordance with those recently reported by other groups (16, 29–32). However, Skibola et al. (32) did not reach significant association in single analysis for MS 2756 but found a potential protective interaction with the serine hydroxymethyltransferase polymorphism against ALL. MTHFR 1298 does not seem to significantly affect the risk in our study, either isolated or combined with MTHFR 677. This finding contrasts with the hypothesized protective role of the 1298 polymorphic allele found by Skibola et al. (29) and Wiemels et al. (31) in adult ALL and in molecularly defined childhood leukemia, respectively, and with data from Franco et al. (30) in studies on childhood ALL. This could be partially explained by the strong negative disequilibrium found between 677T and 1298C alleles. To our knowledge, no study has been published on the association between MTRR 66 polymorphism and hematological malignancies, although data on this polymorphism and other diseases have been reported (24, 35, 36, 38). Results from our analyses on MTRR 66 in combination with MTHFR 677 and MS 2756 suggest a possible involvement of the MTRR polymorphism in decreasing ALL risk in the same direction as for the other polymorphisms investigated. In addition, in the subgroup of low-grade NHL, the MTRR homozygous 66GG condition was the only one to exhibit a significant 2.0-fold risk reduction, and when joined with the MS 2756AA, the risk reduction was higher suggesting a different role of these polymorphisms in ALL and NHL. As in ALL cases, where the combined analysis yielded the highest risk reduction for subjects carrying the MTHFR TT genotype with the MS AA wild-type, also in NHL, the absence or the very low percentage of combinations for two variants did not allow a complete computing and appreciable risk evaluation.

Very few data are present on lymphoma and folate metabolizing enzymes. Our data on NHL, although nonsignificant or with borderline P values, are in contrast with an interesting report of Matsuo et al. (16), which observed significant risk reduction for both MTHFR polymorphisms but a clearly increased susceptibility for MS 2756 in similar subsets of lymphoma. It is noteworthy that the MS 2756 genotype distribution in our control population (3.9% GG, 34.6% AG, and 61.5% AA) is comparable with that of Matsuo et al. (2.5% GG, 33.3% AG, and 64.2% AA). Therefore, the diverse outcomes should necessarily be ascribable to the lower frequency of the variant allele found in our cases (e.g., 0.8% versus 7.2% for the GG genotype, respectively, for our study and Matsuo et al.). The reasons for these opposite findings are unclear, although discrepancies may be in part related to differences in the genetic background and/or in features related to the diverse population investigated. Gene-environment interactions, such as diet, chemical exposure, or nutritional intake of folate and related vitamins should also be taken into account. In addition, another important point to consider is that both reports are composed of small numbers of cases and controls warranting the need for larger epidemiological studies.

Altogether, the results of our study are in accordance with the hypothesis that an increased availability of methylene-THF for DNA synthesis and a reduced availability of methionine for DNA methylation may confer protection against ALL (29, 32). The causative mechanisms remain partially unclear. Because the different forms of folate participate in distinct pathways of single-carbon metabolism, several mechanisms may be involved. MTHFR C677T determines a reduction in the enzyme activity increasing the pool of methylene-THF and reducing the availability of methyl-THF required for methionine synthesis. Hence, both DNA synthesis and DNA methylation processes might be influenced. The protective effect given by MTHFR C677T may be ascribable to a more efficient DNA synthesis due to a reduced rate of uracil misincorporation. The removal of this abnormal base with excision repair processes could provoke DNA double-strand breaks and cause chromosomal instability, translocations, or deletions probably sufficient to originate malignant transformation and clonal expansion (39–41). Alternatively, the lower MTHFR activity, reducing the availability of one-carbon donor methyl-THF, could also affect the remethylation of homocysteine to methionine via MS enzyme, which is maintained in the activated form by MTRR. Polymorphisms within these genes, like MS 2756 and MTRR 66, affecting the respective enzymatic activities, might act in concert with MTHFR polymorphism causing DNA hypomethylation (13) via reduction of S-adenosylmethionine levels, the major cellular methyl donor for DNA and RNA methylation. Hypermethylation and

### Table 3. Number of NHL patients (low grade) and controls, sex- and age-adjusted OR and 95% CI, for MTRR polymorphism

<table>
<thead>
<tr>
<th>MTRR A66G</th>
<th>Cases [n (%); n = 102]</th>
<th>Controls [n (%); n = 257]</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>33 (32.3)</td>
<td>59 (22.9)</td>
<td>1.00</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>48 (47.0)</td>
<td>122 (47.5)</td>
<td>0.70</td>
<td>0.41–1.21</td>
<td>0.26</td>
</tr>
<tr>
<td>GG</td>
<td>21 (20.6)</td>
<td>76 (29.6)</td>
<td>0.50</td>
<td>0.25–0.99</td>
<td>0.06</td>
</tr>
<tr>
<td>AG + GG</td>
<td>69 (67.6)</td>
<td>198 (77.0)</td>
<td>0.62</td>
<td>0.37–1.03</td>
<td>0.08</td>
</tr>
<tr>
<td>A</td>
<td>114 (55.9)</td>
<td>240 (47.5)</td>
<td>0.69</td>
<td>0.50–0.96</td>
<td>0.03</td>
</tr>
<tr>
<td>G</td>
<td>90 (44.1)</td>
<td>274 (52.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
consequent silencing of tumor suppressor genes such as p15, p16, and p53 have been established to be important epigenetic mechanisms affecting the genesis of cancer as well as of lymphoid malignancies (42–45). The underrepresentation of the polymorphic variants in our ALL cases suggests that defects in these enzymes and a limited DNA methylation in specific genes might play important roles in ALL risk reduction and in accordance with a reported growth reduction of human tumor cell lines due to DNA hypomethylation (46).

Methylation status and DNA genetic damage are critical also in lymphomas, although the observed protective effect of the polymorphisms investigated was more strongly evident in leukemia. Apparently, the polymorphisms investigated have different effects in distinct types of cancer, analogous to reports by Skibola et al. (29) describing protection against ALL and not for acute myeloid leukemia by MTHFR substitutions. Moreover, causative events involved in lymphomagenesis may overcome the protective effects of these polymorphisms as described previously for high alcohol intake in colorectal cancer (25, 28).

In conclusion, our results show that MTHFR 677 and MS 2756 variants alleles strongly reduce the risk of ALL and suggest a smaller role for MTRR and MS variants in the risk reduction of both ALL and NHL. These results suggest that these polymorphisms may play a more important role in leukemogenesis than in lymphomagenesis. The means by which they can modulate cancer risk is not yet clearly established. Indeed, we must consider that unfavorable environmental situations such as inadequate folate or micronutrient intakes may act in concert with peculiar genotypes modulating cancer risk by gene-environment interactions (47). Folate assessment is strongly recommended in future studies, because its levels could mask/influence the protective effect of genetic variants. Definitive conclusions should nevertheless be drawn with extreme caution, and further larger epidemiological studies or meta-analyses are required to confirm the present findings.

References


Common Gene Polymorphisms in the Metabolic Folate and Methylation Pathway and the Risk of Acute Lymphoblastic Leukemia and non-Hodgkin’s Lymphoma in Adults

Donato Gemmati, Alessia Ongaro, Gian L. Scapoli, et al.


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