Role of MTHFR genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia

Maja Krajnovic, Stéphanie Lamothe, Damian Labuda, Émilie Lemieux-Blanchard, Yves Théorêt, Albert Moghrabi, and Daniel Sinnett

The central role of methylenetetrahydrofolate reductase (MTHFR) in the folate metabolism renders MTHFR gene polymorphisms (C677T and A1298C) potential modulators of a variety of disorders whose development depends on folate/ homocysteine imbalance. Here, we provide additional evidence on the protective role of these polymorphisms in acute lymphoblastic leukemia (ALL), the most common pediatric cancer. A case-control study was conducted in 270 ALL patients and 300 healthy controls of French-Canadian origin. The TT677/AA1298 and CC677/CC1298 individuals were associated with reduced risk of ALL (crude odds ratio [OR] = 0.4; 95% confidence interval [CI], 0.2-0.9; and OR = 0.3; 95% CI, 0.1-0.6; respectively). Further stratification in patients born before and after January 1996 (approximate time of Health Canada recommendation for folic acid supplement in pregnancy) revealed that the protective effect of MTHFR variants is accentuated and present only in children born before 1996. Similar results were obtained when a transmission disequilibrium test was performed on a subset of children (n = 95) in a family-based study. This finding suggests gene-environment interaction and its role in the susceptibility to childhood ALL, which is consistent with previous findings associating either folate deficiency or MTHFR polymorphisms with risk of leukemia. (Blood. 2004;103:252-257)

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer. The development of this hematologic malignancy was suggested to arise by a combination of genetic susceptibility and the environmental exposure during early development in fetal life and infancy. Assuming that the effect of external factors is modulated by genes, the latter would influence the individual’s risk of cancer. Consistent with this paradigm, we have shown that variants of genes encoding xenobiotics-metabolizing enzymes could modify the risk of childhood ALL,1 but enzymes of other metabolic pathways are to be considered as well.

The 5,10-methylenetetrahydrofolate reductase (MTHFR) converts 5,10-methylenetetrahydrofolate (5,10-methylene-THF) into 5-methyltetrahydrofolate (5-methyl–THF), a major circulating form of folate. 5-Methyl–THF provides methyl group for homocysteine methylation, whereas 5,10-methylenetetrahydrofolate (5,10-methylene–THF) and its derivatives are essential cofactors for both thymidylate and de novo purine methylation. Homocysteine imbalance is associated with vascular disease due to an increase in homocysteine levels.12,13 The association of these variants with a variable risk of neoplasia was shown in several studies. Homozygotes TT at position 677 have a reduced incidence of colorectal cancer that can be further modified by dietary habits and lifestyle.14,15 In contrast, an increased frequency of TT677 homozgyotes was observed in cervical intraepithelial neoplasia16 and esophageal,17 endometrial,18 as well as certain forms of breast carcinoma.19

A relationship between plasma folate levels, the content of uracil, and DNA damage in dividing cells20-22 renders the MTHFR gene a suitable candidate for studies of leukemia susceptibility (eg, as reviewed by Robien and Ulrich23). Skibola et al24 reported that individuals with the MTHFR genotypes TT677, AC1298, and CC1298 had a lower risk of adult ALL but not that of acute myeloid leukemia25.
leukemia. Wiemels et al,25 found a protective role of T677 and C1298 variants in a subset of childhood leukemias, whereas Franco et al,26 observed the protective effect only for the T677 variant. Here, we report a case-control as well as a family-based study providing evidence for the protective role of both MTHFR variants T677 and C1298 in childhood ALL.

**Patients, materials, and methods**

**Study population**

The population under study and the inclusion criteria were described previously.27 Incident cases of childhood ALL (n = 270) were diagnosed in the Division of Hematology-Oncology of the Ste-Justine Hospital in Montreal (QC) between August 1988 and May 2001. They comprised 157 males and 113 females with a median age of 4.9 years, all of French-Canadian descent from the province of Quebec. The subtypes of ALL determined by immunophenotyping were as follows: 228 pre-B, 31 T-cell, and 11 of undetermined lineage. Ninety-five children from whose parents’ DNA was available were enrolled in the family-based study. The healthy controls (n = 300) consisted of French-Canadians recruited while using clinical departments other than Hematology-Oncology of the Sainte-Justine Hospital. The institutional review board approved the research protocol, and informed consent was obtained from all participating individuals and/or their parents.

**Genotyping of MTHFR variants**

Both polymorphic sites C677T and A1298C were screened by allele-specific oligonucleotide (ASO) hybridization. DNA segments containing the polymorphic sites were amplified by polymerase chain reaction (PCR) using 15 ng genomic DNA, 0.5 μM of each of the primers, 100 μM deoxyribonucleotide triphosphates (dNTPs), 10 mM Tris (tris(hydroxymethyl)aminomethane)–HCl (pH 8.3), 1.5 mM MgCl2, 50 mM KCl, and 0.5 units of Platinum Taq polymerase (Invitrogen, Burlington, ON, Canada) in a total volume of 50 μL for 35 cycles of 30 seconds at 94°C, 30 seconds at 62°C or 51°C (for polymorphic sites at positions 677 and 1298, respectively), and 45 seconds at 72°C. The resulting PCR products were dot-blotted in duplicate on a nylon membrane and assayed for the presence of either allele by hybridization with ASO in parallel dot-blotted in duplicate on a nylon membrane and assayed for the presence of either allele or the other variant by hybridization with ASO in parallel as described in Labuda et al.28 The amplimers and oligonucleotide probes used for ASO analysis are given in Table 1.

**Statistical analysis**

The χ2 test was used to examine differences in frequencies between MTHFR genotypes in cases and controls or between parents and controls. The level of significance was calculated by Fisher exact test (2-sided). Crude odd ratios (ORs) are given with 95% confidence intervals (CIs). The MTHFR haplotypes (677 and 1298 sites combined) showing a protective role in the univariate analysis were grouped together and tested against the remaining MTHFR haplotypes (dichotomous MTHFR variable) in multivariate models. These models, besides MTHFR variable, included age and sex or loci previously shown in the same group of patients to influence the risk of ALL.27,29-31 These included the genotypes carrying CYP1A1*2A, CYP2E1*5, NQO1*23, and GSTP1*B/C variants as well as the GSTM1 null and slow acetylator NAT2 genotypes. SPSS (statistical package for the social sciences, version 10.0) was used for analysis. Linkage disequilibrium between 677 and 1298 sites was tested with the estimating haplotype-frequencies (EH) linkage utility software,25 whereas disequilibrium in allele transmission from parents to children was assessed with the help of FBAT (family-based association test) software.

**Results**

The allele T677 (Val222) was observed in 35% of ALL patients (n = 270) as compared with 36.6% among the French-Canadian controls (n = 300). The frequency of C1298 (Ala429) was 24.3% in the patients and 30.2% in the controls. Both frequencies were similar to those reported in other populations of European descent.23,34 The corresponding genotypes are given in Table 2. The pre-B and T-cell ALLs were analyzed together because no significant differences were observed in the corresponding genotypes. No difference in MTHFR genotype distribution was observed between either hyperdiploid and nonhyperdiploid leukemia (as estimated by DNA index) or in relation to the age of patient at diagnosis (data not shown). CC1298 homozygotes were underrepresented among the patients when compared with controls (4.4% versus 10.3%), suggesting a protective effect of this variant (OR = 0.4; 95% CI, 0.2-0.8; P < .01). No difference was observed in the distribution of T677 between cases and controls (Table 2).

Further analysis of the genotypes (Table 3) has shown, however, that CC677/AA1298 individuals were at a higher risk for developing ALL as compared with those with other genotypes (OR = 1.8; 95% CI, 1.1-2.8; P = .02). This suggests that children with normal activity level of MTHFR are more susceptible to ALL than those having reduced enzyme activity associated with the presence of T677 or C1298 allele. In other words, the homozygotes for either one or another of these variants appears to have a decreased susceptibility to ALL: TT677/AA1298 (OR = 0.4; 95% CI, 0.2-0.9; P = .02) or CC677/CC1298 (OR = 0.3; 95% CI, 0.1-0.6; P < .001). The age, sex, or other ALL risk-modifying factors (“Patients, materials, and methods”) did not affect these results. No homozygous carriers of both T677 and C1298 alleles were observed. T677 homozygotes were always AA1298, and CC1298

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**Table 1. Characteristics of PCR primers and allele-specific-oligonucleotides (ASO) used for genotyping MTHFR polymorphisms**

<table>
<thead>
<tr>
<th>Amino acid change</th>
<th>DNA variant</th>
<th>PCR primers, 5’ to 3’</th>
<th>Product size</th>
<th>Allele</th>
<th>ASO probe, 5’ to 3’</th>
<th>Washing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala222Val</td>
<td>C677T</td>
<td>F: tggaggaagcgctctccgg</td>
<td>198 bp</td>
<td>C677T</td>
<td>gctggagcgatttc</td>
<td>50°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: aagactgctgctgtaaggqtg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu428Ala</td>
<td>A1298C</td>
<td>F: cttgggagctgaaagacta</td>
<td>163 bp</td>
<td>A1298C</td>
<td>ccaagtgaagaaaggtg</td>
<td>48°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: tccattgctgaccattccg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Upper-case character indicates the polymorphic position.

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**Table 2. Distribution of MTHFR genotypes in childhood ALL patients and controls**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>ALL cases, no. (%)</th>
<th>Controls, no. (%)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>C677T</td>
<td>112 (41.3)</td>
<td>128 (42.0)</td>
<td>1 (referent)</td>
<td>—</td>
</tr>
<tr>
<td>CT</td>
<td>127 (47.0)</td>
<td>128 (42.7)</td>
<td>1.1 (0.8-1.6)</td>
<td>.6</td>
</tr>
<tr>
<td>TT</td>
<td>31 (11.5)</td>
<td>46 (15.3)</td>
<td>0.6 (0.4-1.3)</td>
<td>.4</td>
</tr>
<tr>
<td>A1298T</td>
<td>151 (55.9)</td>
<td>150 (50.0)</td>
<td>1 (referent)</td>
<td>—</td>
</tr>
<tr>
<td>AC</td>
<td>107 (39.6)</td>
<td>119 (39.7)</td>
<td>0.9 (0.6-1.3)</td>
<td>.5</td>
</tr>
<tr>
<td>CC</td>
<td>12 (4.4)</td>
<td>31 (10.3)</td>
<td>0.4 (0.2-0.8)</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>
Therefore, we analyzed the impact of MTHFR variants on the ALL risk in children born before and after January 1996 (Table 4). We assumed that choosing January 1996 allowed enough time to implement this recommendation. Indeed, the profound protective effect in TT677/AA1298, CC677/CC1298, and CC677/AC1298 individuals was only observed in the group of children that were born before 1996 (Table 4, OR estimates). On the contrary, MTHFR genotypes were not associated with any decrease in ALL risk in the group of children born after January 1996, even in certain instances in which OR estimates attained the value above 1.

Taking into account this striking difference, we subsequently carried the analysis in case-parental trios only for children who were born before January 1996 (n = 95) (Table 5). The genotype distribution in this patient subgroup did not differ from the rest of the patients; however, due to the smaller sample size only CC677/CC1298 individuals were significantly overrepresented compared with the controls (OR = 0.2; 95% CI, 0.05-0.7; P < .01). Such finding allows us to address if the protective effect of C1298 variant is that of the child itself or merely reflects the effect of either maternal or paternal MTHFR genotypes. We observed a deviation from the random transmission from parents to children (P = .05). In addition, when mothers were substituted for cases and compared with (female) controls, no effect was observed. Similar results were obtained for fathers and, finally, for both parents compared with controls (data not shown). This analysis suggests that the paternal effect of MTHFR is negligible and that it is the genotype of an eventual patient that counts.

### Discussion

The importance of MTHFR in cancer susceptibility arises from its involvement in 2 paths of folate metabolism. One leads to numerous methylation processes that are dependent on S-adenosyl–methionine (SAM), while the other, via thymidylate synthesis, contributes to DNA replication and cell division. Reduced activity of MTHFR may decrease the methylation of homocysteine to methionine and in turn the level of SAM, resulting in DNA hypomethylation. On the other hand, the reduced level of MTHFR substrate, 5,10-methylene–THF, required for thymidylate synthesis could lead to uracil misincorporation into DNA, diminished DNA repair, and increased frequency of chromosomal breaks and damage. Malignancies that are derived from rapidly proliferating tissues, thus having greatest requirement for DNA synthesis should be more susceptible to folate deficiency and resultant DNA damage. Indeed, the DNA variants causing the reduced MTHFR activity were associated with the reduced risk of leukemia, lymphoma, and colorectal carcinoma. The mechanism proposed to explain these associations was the shunt of folate metabolism versus thymidine and purine synthesis, which would slow the incorporation of uracil into DNA and protect against carcinogenesis.

Here we showed that MTHFR variants play a protective role in a group of nonselected ALL patients (in regard to cell type or cytogenetic features) supporting the idea that the enhanced availability of 5,10-methylene–THF in association with the reduced MTHFR activity may play a protective role in the onset of childhood leukemia. The reduction of risk of 2.5- and 3.3-fold was observed for TT677/AA1298 and CC677/CC1298 individuals, respectively. This confirms and extends previous findings of Skibola et al, who reported reduced frequency of the same MTHFR haplotypes in adult leukemia, as well as results of Wiemels et al, who found the protective effect of these variants in infant leukemia with MLL rearrangements and hyperdiploid pediatric leukemia. However, in this study we failed to detect any difference of MTHFR frequency between hyperdiploid and nonhyperdiploid leukemia, suggesting that the protective MTHFR effect equally applies to both cell types.

All studies addressing MTHFR’s role in leukemia susceptibility, with the sole exception of the study by Franco et al, found that both variants play a protective role. Variant T677 encodes a thermolabile enzyme with 40% activity of that of the wild type.

### Table 3. Analysis of MTHFR genotypes in children with ALL and controls

<table>
<thead>
<tr>
<th>MTHFR genotypes</th>
<th>ALL patients, no. (%)</th>
<th>Controls, no. (%)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>C677T A1298C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC AA</td>
<td>51 (18.9)</td>
<td>35 (11.7)</td>
<td>1.0 (referent)</td>
<td>—</td>
</tr>
<tr>
<td>CT AA</td>
<td>69 (25.6)</td>
<td>69 (23.0)</td>
<td>0.7 (0.4-1.2)</td>
<td>.2</td>
</tr>
<tr>
<td>TT AA</td>
<td>31 (11.5)</td>
<td>46 (15.3)</td>
<td>0.4 (0.2-0.9)</td>
<td>.02</td>
</tr>
<tr>
<td>CC AC</td>
<td>49 (18.1)</td>
<td>60 (20.0)</td>
<td>0.6 (0.3-1.0)</td>
<td>.06</td>
</tr>
<tr>
<td>CT AC</td>
<td>58 (21.5)</td>
<td>59 (19.7)</td>
<td>0.7 (0.4-1.2)</td>
<td>.2</td>
</tr>
<tr>
<td>CC CC</td>
<td>12 (4.4)</td>
<td>31 (10.3)</td>
<td>0.3 (0.1-0.6)</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

The risk of ALL for the CC677/AA1298 individuals compared with all other genotypes is 1.8 (95% CI, 1.1-2.8; P = .02). No individual with the following genotypes was observed: TT677/AC1298, TT677/CC1298, and CT677/CC1298. OR indicates crude odds ratio; CI, confidence interval; and —, not applicable.

### Table 4. MTHFR genotypes in ALL patients stratified by the year of birth

<table>
<thead>
<tr>
<th>MTHFR genotypes</th>
<th>Born before January 1996, no. (%)</th>
<th>Born after January 1996, no. (%)</th>
<th>Controls, no. (%)</th>
<th>OR before 1996 (95% CI)</th>
<th>OR after 1996 (95% CI)</th>
<th>P before 1996</th>
<th>P after 1996</th>
</tr>
</thead>
<tbody>
<tr>
<td>C677T A1298C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC AA</td>
<td>48 (21.6)</td>
<td>3 (6.3)</td>
<td>35 (11.7)</td>
<td>1 (referent)</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>CT AA</td>
<td>51 (23.0)</td>
<td>18 (37.5)</td>
<td>69 (23.0)</td>
<td>0.5 (0.3-0.9)</td>
<td>.03</td>
<td>3.0 (0.8-11)</td>
<td>.1</td>
</tr>
<tr>
<td>TT AA</td>
<td>26 (11.7)</td>
<td>5 (10.4)</td>
<td>46 (15.3)</td>
<td>0.4 (0.2-0.8)</td>
<td>.01</td>
<td>1.3 (0.3-5.7)</td>
<td>NS</td>
</tr>
<tr>
<td>CC AC</td>
<td>36 (16.2)</td>
<td>13 (27.1)</td>
<td>60 (20.0)</td>
<td>0.4 (0.2-0.8)</td>
<td>&lt; .01</td>
<td>2.5 (0.7-9.5)</td>
<td>.3</td>
</tr>
<tr>
<td>CT AC</td>
<td>49 (22.1)</td>
<td>9 (18.6)</td>
<td>59 (19.7)</td>
<td>0.6 (0.3-1.1)</td>
<td>.1</td>
<td>1.8 (0.5-7.0)</td>
<td>.5</td>
</tr>
<tr>
<td>CC CC</td>
<td>12 (5.4)</td>
<td>0 (0)</td>
<td>31 (10.3)</td>
<td>0.3 (0.1-0.6)</td>
<td>&lt; .01</td>
<td>ND</td>
<td>.3</td>
</tr>
</tbody>
</table>

The frequency of MTHFR genotype combinations of each group of ALL patients, born before (n = 222) and after January 1996 (n = 48), was compared with the frequency in controls using CC677/AA1298 individuals as the referent group (OR = 1). When CC677/AA1298 individuals are compared with others, the ALL risk (OR) in the group born before 1996 was 2.2 (95% CI, 1.3-3.5; P = .001) and for those born after 1996, OR = 0.5 (95% CI, 0.1-1.8; P = .4).

ND indicates not determined due to zero observations; NS, not significant; and —, not applicable.
usually associated with the accumulation of homocysteine.\textsuperscript{6,11} Homozygotes for C1298, on the other hand, do not have the enzymatic properties distinguishable from the wild-type \textit{MTHFR}.\textsuperscript{10} Decrease in enzyme activity in these individuals is lesser than that of T677 variant (65\% of the wild type) and does not appear to be sufficient to affect plasma homocysteine level.\textsuperscript{7,40} Therefore, it is surprising that the protective effect of this variant overpasses that of T677, suggesting that C1298 variant may act through a different mechanism. The Ala222Val replacement is located in the region encoding the N-terminal catalytic domain, whereas Glu429Ala substitution resides within C-terminal SAM regulatory domain of the enzyme. It has been shown that SAM inhibits \textit{MTHFR}\textsuperscript{41,42} and that this feedback loop is essential for methyl group biogenesis and prevention of 5,10-methylene–THF depletion. SAM-insensitive \textit{MTHFR}, on the other hand, is expected to direct more one-carbon unit to 5-methyl–THF and hence to SAM synthesis.\textsuperscript{41} It is thus possible that C1298 variant might affect this regulation and, as suggested by Wiemels et al.,\textsuperscript{45} act through a different pathway than T677, leading to a prevention of aberrant methylation pattern. The possibility that the Ala429 form is less dependent on SAM inhibition may also explain the absence of hyperhomocysteinemia in individuals with CC1298 genotype.

In the context of these different mechanisms it is interesting to discuss the possible genotype-phenotype effect of compound heterozygotes. Such individuals were found to have decreased enzyme activity and are associated with higher plasma levels of homocysteine.\textsuperscript{10} It is therefore surprising that we did not observe the protective effect against childhood leukemia for CT677/AC1298 individuals. Contrary to this, Skibola et al.\textsuperscript{45} observed protective effect against adult leukemia for such individuals. It is not clear if the power of the studies or different etiology of childhood versus adult leukemia accounts for the observed difference. However, based on the hypothesis that T677 and C1298 exert their action by different mechanisms, one may speculate that differential action of enzymatic variants—Ala429 tending to accumulate 5-methyl–THF and Val222 to accumulate 5,10-methylene–THF—might result in 5,10-methylene–THF/5-methyl–THF balance not necessarily significantly different from that of individuals with \textit{MTHFR} CC677/AA1298 (wild-type) genotypes. The observation that the loss of activity of Val222 \textit{MTHFR} is slowed in the presence of 5-methyl–THF or SAM\textsuperscript{43} may favor this notion. The effect of CT677/AC1298 genotypes in disease susceptibility certainly deserves further attention.

Similar to other studies\textsuperscript{34,44,46} we observed that the alleles T677 and C1298 were never present in \textit{cis}, suggesting that the mutation might have occurred on the background of A1298 and the second on the background of C677. Analysis of neonatal and fetal \textit{MTHFR} genotypes revealed the presence of T677-C1298 haplotype in fetus and its absence in neonatal group, suggesting the potential role of this haplotype in compromised fetal viability.\textsuperscript{8,46} On the other hand, other severe mutations have been already reported in \textit{cis} to T677 variant, suggesting that recombination creating haplotype T677-C1298 did not increase to significant frequencies.\textsuperscript{10}

The analysis of \textit{MTHFR} genotypes in the context of specific metabolic biomarkers such as folate or homocysteine levels provides means to estimate both the impact of genetic variants and gene-environmental interaction. The interaction between \textit{MTHFR} polymorphisms and folate status has been documented in several studies.\textsuperscript{14,15,47} This interaction attracts even more attention because a protective association between folate supplements in pregnancy and the risk of common childhood ALL was demonstrated.\textsuperscript{47} An increase in the proportion of women taking folate supplements resulted in a decreased incidence of neural tube defects as well as that of leukemia.\textsuperscript{47} For that reason we separated the patients whose mothers brought their pregnancy to term before and after the Health Canada recommendation for folate dietary supplementation. The protective ALL effect of \textit{MTHFR} variants was highly significant in the group born before 1996, presumably reflecting the maternal folate insufficiency during pregnancy, whose effect was mediated by the metabolism (genotype) of the child. In contrast, this effect was lost among children born after January 1996. This was particularly obvious for the haplotypes carrying the T677 allele. A similar genetic-nutrient interaction has been observed for neural tube defects, where \textit{MTHFR} variants and low folate status were associated with the greater risk than either variable alone.\textsuperscript{48} Homozygosity for T677 was associated with an increased plasma homocysteine level, particularly in the presence of folate insufficiency.\textsuperscript{34,49-51} All together, these findings suggest that the effect of \textit{MTHFR} polymorphisms is less apparent when the folate levels are adequate. In other words, the predictive importance of \textit{MTHFR} polymorphisms in leukemia susceptibility would be of less relevance in a folate-supplemented diet. However, we cannot exclude the possibility that results were obtained by chance due to a small sample size in the group of patients born after January 1996, which has 60\% power to detect the strength of associations observed in those born before 1996.

The modulation of genetic effect by dietary folate may include either maternal and/or child \textit{MTHFR} genotypes. We have recently shown that in the case of certain loci, the genotypes of parents might be more relevant to disease susceptibility than that of the child.\textsuperscript{52} The importance of maternal \textit{MTHFR} genotypes has been shown in several instances. The risk of neural tube defects was increased if both mother and child carried \textit{MTHFR} genotypes at risk.\textsuperscript{53} Maternal folate status was suggested to play a role in meiotic nondisjunction, and the risk of aneuploidy was further increased in the presence of the \textit{MTHFR} T677 allele.\textsuperscript{54} Here we observed that

### Table 5. \textit{MTHFR} genotypes in ALL patients and their mothers

<table>
<thead>
<tr>
<th>\textit{MTHFR} genotypes</th>
<th>ALL, no. (%)</th>
<th>Mothers, no. (%)</th>
<th>Controls, no. (%)</th>
<th>OR\textsubscript{ALL} (95% CI)</th>
<th>P\textsubscript{ALL}</th>
<th>OR\textsubscript{mothers} (95% CI)</th>
<th>P\textsubscript{mothers}</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC AA</td>
<td>18 (18.9)</td>
<td>14 (14.9)</td>
<td>35 (11.7)</td>
<td>1 (referent)</td>
<td>.1</td>
<td>1 (referent)</td>
<td>.1</td>
</tr>
<tr>
<td>CT AA</td>
<td>25 (26.3)</td>
<td>27 (28.7)</td>
<td>69 (23.0)</td>
<td>0.7 (0.3-1.5)</td>
<td>.4</td>
<td>1.0 (0.5-2.1)</td>
<td>NS</td>
</tr>
<tr>
<td>TT AA</td>
<td>13 (13.7)</td>
<td>17 (18.1)</td>
<td>46 (15.3)</td>
<td>0.5 (0.2-1.3)</td>
<td>.2</td>
<td>0.7 (0.3-1.6)</td>
<td>.5</td>
</tr>
<tr>
<td>CC AC</td>
<td>15 (15.8)</td>
<td>19 (20.2)</td>
<td>60 (20.0)</td>
<td>0.5 (0.2-1.1)</td>
<td>.1</td>
<td>0.7 (0.3-1.5)</td>
<td>.4</td>
</tr>
<tr>
<td>CT AC</td>
<td>21 (22.1)</td>
<td>12 (12.8)</td>
<td>59 (19.7)</td>
<td>0.7 (0.3-1.5)</td>
<td>.4</td>
<td>1.4 (0.6-3.7)</td>
<td>.5</td>
</tr>
<tr>
<td>CC CC</td>
<td>3 (3.2)</td>
<td>5 (5.3)</td>
<td>31 (10.3)</td>
<td>0.2 (0.05-0.7)</td>
<td>.01</td>
<td>0.4 (0.1-1.2)</td>
<td>.1</td>
</tr>
</tbody>
</table>

The frequency of \textit{MTHFR} genotypes in ALL patients (n = 95) and their mothers (n = 94) was compared with the frequency in controls. The referent group (OR = 1) comprised controls and either parents or mothers homozygous for C677T/AA1298 haplotype.

— indicates not applicable; NS, not significant.
maternal folate intake but not the genotype of MTHFR locus played a role in ALL susceptibility. Protective MTHFR effect was dependent of child MTHFR genotype, at least in the group of patients investigated. In the context of gene-environment interaction it is worth noting that MTHFR gene polymorphisms can also have impact on leukemia outcome by modulating the effect of antifolate treatment, more particularly methotrexate.53-55

In conclusion, our study provides further evidence for the protective role of MTHFR polymorphisms in childhood ALL. Both MTHFR polymorphisms may affect the risk of ALL among the carriers in the instances of folate deficiency such as insufficient maternal folate intake. Because birth year of the child was here used as a surrogate marker for folate levels, further studies including folate level measurements as well as larger sample size are required to confirm this gene-environment interaction in ALL patients.

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