Short Communication

MTHFR Polymorphisms and Risk of Chronic Lymphocytic Leukemia

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Abstract

Folate availability is critical for DNA integrity, required for the transfer of methyl groups in the biosynthesis of thymidylate. Reduction of 5,10-methylenetetrahydrofolate, a donor for methylating dUMP to dTMP in DNA synthesis, to 5-methyltetrahydrofolate, the primary methyl donor for methionine synthesis, is catalyzed by 5,10-methylenetetrahydrofolate reductase (MTHFR). The MTHFR polymorphisms C677T and A1298C have been shown in some studies to alter the risk of a range of different malignancies. We evaluated the role of the C677T and A1298C polymorphisms on chronic lymphocytic leukemia (CLL) risk by genotyping 832 patients and 886 healthy controls. The odds ratio of CLL associated with 677CT and 677TT genotypes were 1.02 [95% confidence interval (95% CI), 0.83-1.24] and 0.90 [95% CI, 0.66-1.24], respectively. The odds ratio of CLL associated with 1298AC and 1298CC genotypes were 0.97 [95% CI, 0.79-1.18] and 0.88 [95% CI, 0.62-1.24], respectively. This data indicate that the MTHFR polymorphisms C677T and A1298C do not significantly contribute to an inherited genetic susceptibility to CLL. (Cancer Epidemiol Biomarkers Prev 2004;13(12):2268–70)

Introduction

Chronic lymphocytic leukemia (CLL) is the most common form of leukemia accounting for around 30% of all cases. Inherited predisposition to CLL is now recognized, with epidemiologic studies showing 3- to 7-fold elevations of risk in first-degree relatives of CLL cases (1, 2). To date, no gene, which when mutated, has been shown to unambiguously confer susceptibility to the disease (2). Whereas part of the familial risk could be due to high-penetrance mutations in as yet unidentified genes, a polygenic mechanism provides a plausible alternative explanation. Alleles conferring relative risks of ~2.0 will rarely cause multiple-case families and are difficult or impossible to identify through linkage (3). The search for low-penetrance alleles has therefore centered on association studies based on comparing the frequency of polymorphic genotypes in cases and controls.

Folate metabolism plays an important role in carcinogenesis due to its involvement in DNA methylation and nucleotide synthesis. DNA methylation is essential for gene regulation (4) and cellular differentiation (5).

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Note: M.F. Rudd and G.S. Sellick contributed equally to this work.

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Materials and Methods

Patients. Eight hundred and thirty-two patients with adult CLL (577 males, 255 females; mean age at diagnosis 61 years; range 21-94; SD ± 12) referred to the Royal Marsden Hospital NHS Trust were studied. One hundred and nineteen of the cases (76 males, 43 females; mean age at diagnosis 59 years; range 26-93; SD ± 12) had one or more first-degree relatives affected with CLL. Eight hundred and eighty-six healthy individuals were recruited as part of either the National Cancer Research Network Trial (1999-2002), the Royal Marsden Hospital Trust/Institute of Cancer Research Family History and DNA Registry (1999-2004), or the National Study of Colorectal Cancer Genetics Trial (2004), all established within the United Kingdom. Controls (523 males, 363 females; mean age 60 years; range 21-91; SD ± 12) were the spouses of patients with nonhematologic malignancies and were selected to closely match the age and sex of the cases. None of the controls had a personal history of CLL or other malignancy. All cases and controls were British Caucasians, and there were no obvious differences in the demography of cases and controls in terms of place of residence within the United Kingdom. Samples were obtained with informed consent and ethical review board approval in accordance with the tenets of the Declaration of Helsinki.

Detection of MTHFR C677T and A1298C Genotypes. MTHFR C677T and A1298C genotypes were generated using Taqman technology implemented on an Applied Biosystems 7900 HT sequence detection system. PCR reactions for detection of the C677T polymorphism contained 6.25 µL ABI Taqman PCR Master Mix, 0.16 µL ABI SNP assay-by-design master mix containing 900 mol/L forward primer (5'-gCACTTGAAGGTAGGGTGCTCT-3'), 900 nmol/L reverse primer (5'-CCTCAAGAATGCTGTTGGCTG-3'), 200 nmol/L VIC-labeled MGB probe (5'-gCgggAgCCgATTTCAT[NFQ]-3') and 200 nmol/L FAM-labeled MGB probe (5'-gCgggAgTC-gATTTCAT[NFQ]-3'), 4 µL (10 ng) of template DNA, and double-distilled water to a final volume of 12.5 µL. PCR reactions for detection of the A1298C polymorphism contained 6.25 µL ABI Taqman PCR Master Mix, 0.16 µL ABI SNP assay-by-design master mix containing 900 nmol/L of primers (5'-ggAGTGCTGTTGGCTGTTG-3') and (5'-TgTTCTCCGAAATGTAAGA-3'), 200 nmol/L VIC-labeled MGB probe (5'-ACCAGTGAAAGAAAGTGTT[NFQ]-3') and 200 nmol/L FAM-labeled MGB probe (5'-CAAGTGAAACAAAGTGTT[NFQ]-3'), and DNA and water as above. All amplifications consisted of an initial denaturation at 95°C for 10 minutes followed by 40 cycles of denaturation (92°C/15 s) and extension (60°C/1 min).

Statistical Analyses. The relationship between MTHFR genotypes and risk of CLL was assessed by means of the odds ratio (OR) with 95% confidence interval (95% CI) calculated using both conditional and unconditional logistic regression (adjusting for age and sex). Because both models yielded very similar results, only those for conditional logistic regression are presented. A test for trend (P trend) in increasing CLL risk by having more than one putative high-risk allele or genotype was evaluated by means of the χ² test. To test for population stratification, the distribution of genotypes in controls was tested for a departure from Hardy-Weinberg equilibrium. An unequal distribution of haplotype frequencies between cases and controls was tested for by means of the χ² statistic 2(lnL1−lnL0), where L1 is the likelihood of cases and controls analyzed together and L0 the sum of the individual likelihoods. All computations were undertaken using the statistical software package STATA version 7.0 (Stata Corporation, College Station, TX). Power calculations were undertaken using the method published by Fleiss et al. (17) as implemented in the statistical program POWER version 1.30 (Epincic Software, http://icarus2.hsc.usc.edu/epicenter). P = 0.05 was considered statistically significant in all analyses.

Results and Discussion

The observed frequencies in the controls for both polymorphisms of MTHFR were in accordance with Hardy-Weinberg laws of equilibrium, providing no evidence of population stratification within the data set. The frequency of the MTHFR C677T and A1298C polymorphisms in the general population has been recently reported in a meta-analysis of 16 studies providing data on 5,389 individuals (18). The frequencies of both polymorphisms detected in our study are in close agreement with this report. Specifically, the frequencies in our study (and in the meta-analysis) are CC/AA, 12% (15%); CC/AC, 10% (8.5%); CT/AA, 22% (22%); CT/AC, 22% (20%); CT/CC, 0% (0.25%); TT/AA, 12% (11%); TT/AC, 0% (0.46%); and TT/CC, 0% (0.03%). As was the case with the meta-analysis (18), no individuals with the MTHFR 677TT/1298AC, 677CT/1298CC, and 677TT/1298CC genotypes were identified. Based on the number of cases and controls analyzed and the population frequencies of the rare alleles of each polymorphism, our study has 90% power to show an OR of 0.7 associated with each variant stipulating a P = 0.05.

Table 1 details the ORs of CLL and associated 95% CIs associated with each genotype. Adjustment for age and sex made no significant difference to findings hence only unadjusted ORs are presented. Individually, there is no evidence that either polymorphism affects the risk of CLL. Odds ratios for possession of the MTHFR 677TT and 1298C alleles were 0.97 (95% CI, 0.84-1.12) and 0.95 (95% CI, 0.82-1.10), respectively. Restricting the analysis to familial cases provided, no evidence of a relationship between MTHFR polymorphisms and risk of CLL [ORs for possession of 677T and 1298C alleles were 1.18 (95% CI, 0.89-1.56) and 0.87 (95% CI, 0.64-1.17), respectively].

There was evidence of significant linkage disequilibrium between the two polymorphisms (P < 0.001). In both cases and controls, we observed that subjects homozygous for either of the MTHFR mutations always possessed the wild-type genotype at the alternate site. For both MTHFR polymorphisms C677T and A1298C,
the wild-type amino acid residues Ala222 and Glu429 are evolutionarily conserved between human and mouse sequences, with Ala222Val and Glu429Ala substitutions representing moderately conservative and moderately radical changes, respectively (19). This suggests that each polymorphism is likely to have an independent effect on MTHFR function, consistent with in vitro measurements of MTHFR-specific activity associated with specific genotypes (13, 20).

The joint effects of the two polymorphisms on the risk of CLL are also shown in Table 1. ORs of CLL associated with possession of rare alleles of MTHFR polymorphisms were consistently less than 1.0 but were not statistically significant. Overall, the distribution of haplotypes was not significantly different between cases and controls (P = 0.97). The OR of CLL associated with possession of at least one allelic variant compared with the wild-type 677CC/1298AA genotype is 0.83 (95% CI, 0.66–1.24), representing moderately conservative and moderately evolutionarily conserved between human and mouse evolution. Science 1974;185:862–4.

Variation in MTHFR has been implicated in a range of cancer types, including acute leukemias (9, 15). There is some evidence that hypomethylation is a feature of CLL, which may contribute to the disease etiology. Furthermore, deletion breakpoints in CLL have been reported to colocalize at specific sites within chromosome 11q where folate-sensitive CCG repeats are located (22). Although our findings provide no statistically significant evidence that MTHFR plays a role in development of CLL, we cannot entirely exclude a small effect in risk of CLL associated with MTHFR variants. Moreover, as there is evidence of a gene-environment effect with respect to folate metabolism and cancer risk (23, 24), it is possible that polymorphisms of MTHFR may mediate CLL risk in the context of a low-folate diet. Unfortunately, we do not know the folate status of individuals in this study to permit this possibility to be examined.

Table 1. Association between MTHFR C677T and A1298C genotypes and chronic lymphocytic leukemia risk

<table>
<thead>
<tr>
<th>MTHFR C677T</th>
<th>Cases (n)</th>
<th>Controls (n)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>361</td>
<td>383</td>
<td>1.0*</td>
</tr>
<tr>
<td>CT</td>
<td>381</td>
<td>397</td>
<td>1.02 (0.83–1.24)</td>
</tr>
<tr>
<td>TT</td>
<td>90</td>
<td>106</td>
<td>0.90 (0.66–1.24)</td>
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<table>
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<tr>
<th>MTHFR A1298C</th>
<th>CC</th>
<th>363</th>
<th>389</th>
<th>0.97 (0.79–1.18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>397</td>
<td>412</td>
<td>1.0*</td>
<td>—</td>
</tr>
<tr>
<td>AC</td>
<td>72</td>
<td>85</td>
<td>0.88 (0.62–1.24)</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MTHFR C677T/A1298C</th>
<th>CC/AA</th>
<th>118</th>
<th>107</th>
<th>1.0*</th>
<th>—</th>
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<tbody>
<tr>
<td>CC/AC</td>
<td>171</td>
<td>191</td>
<td>0.81</td>
<td>(0.58–1.13)</td>
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<tr>
<td>CC/CC</td>
<td>72</td>
<td>85</td>
<td>0.77</td>
<td>(0.51–1.16)</td>
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<tr>
<td>CT/AA</td>
<td>189</td>
<td>199</td>
<td>0.86</td>
<td>(0.62–1.20)</td>
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<tr>
<td>CT/AC</td>
<td>192</td>
<td>198</td>
<td>0.88</td>
<td>(0.63–1.22)</td>
<td></td>
</tr>
<tr>
<td>TT/AA</td>
<td>90</td>
<td>106</td>
<td>0.77*</td>
<td>(0.52–1.13)</td>
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</tr>
</tbody>
</table>

NOTE: Unadjusted ORs and 95% CIs are shown for 832 cases and 886 controls; adjusting for age and sex was not significant. MTHFR 677/1298 genotypes CT/CC, TT/AC, and TT/CC were not observed in this study.

References

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