Short Communication

MTHFR Polymorphisms and Risk of Chronic Lymphocytic Leukemia

Matthew F. Rudd,[1] Gabrielle S. Sellick,[1] Ruth Allinson,[1] Estella Matutes,[2] Daniel Catovsky,[2] and Richard S. Houlston[1]

[1]Section of Cancer Genetics, Institute of Cancer Research, Sutton, Surrey, United Kingdom and [2]Section of Haemato-Oncology, Institute of Cancer Research, London, United Kingdom

Abstract

Folate availability is critical for DNA integrity, required for the transfer of methyl groups in the biosynthesis of thymidilate. 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). Aberrant genomic DNA methylation, particularly hypomethylation, is associated with the genesis of various cancers (6, 7). Central to cellular DNA methylation is the enzyme methylenetetrahydrofolate reductase (*MTHFR*), which catalyses the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Reduced *MTHFR* activity has an inhibitory effect on the 5-methyltetrahydrofolate pathway, leading to increased 5-methylenetetrahydrofolate, which if not reduced results in the transfer of a methyl group converting dUMP to dTMP, required for DNA synthesis. Uracil, a normal RNA base, can become misincorporated into the DNA when methylation of dUMP to dTMP is deficient (8), and it has been proposed that defects in uracil excision repair results in DNA double-strand breaks and other anomalies which have carcinogenic effects (9, 10).

The *MTHFR* polymorphism C677T (Ala222Val) affects the activity and thermolability of the expressed protein, with heterozygotes having ~60% of wild-type activity and homozygous variants having ~30% of wild-type activity (11-13). A second *MTHFR* polymorphism, A1298C (Glu429Ala), has also been reported in some but not all studies, to affect *MTHFR* activity, with homozygous individuals having ~60% of wild-type *MTHFR* activity (12, 13).

Although not universal, some studies have shown that the *MTHFR* C677T and A1298C polymorphisms affect the risk of a number of malignancies including acute lymphoblastic leukemia (refs. 9, 14, 15). To determine whether the C677T and A1298C polymorphisms of *MTHFR* are associated with risk of CLL, we undertook

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

Requests for reprints: Richard Houlston, Section of Cancer Genetics, Institute of Cancer Research, Sutton, Surrey SM2 5NG United Kingdom. Phone: 44-208-722-4175; Fax: 44-208-722-4362. E-mail: richard.houlston@icr.ac.uk

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a large case-control study of 832 CLL patients and 886 controls. We included a high proportion of familial cases, thereby enriching for individuals with an inherited genetic risk, empowering the detection of an association (16).

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 7900HT 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′-gCACTTgAAggAgAAgg-TgTCT-3′), 900 nmol/L reverse primer (5′-CCTCAAA-gAAAAgCTgCgTgATg-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′-ggAggAgCTgCTgAAgATgTg-3′) and (5′-TggTTCTCCCgAgAggTAAAgA-3′), 200 nmol/L VIC-labeled MGB probe (5′-ACCAgTgAAgAAAg-TgT[NFQ]-3′) and 200 nmol/L FAM-labeled MGB probe (5′-CAgTgAAgCAAgTgT[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 χ^2 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 χ^2 statistic $2(\ln L_1 - \ln L_0)$, where L_1 is the likelihood of cases and controls analyzed together and L_0 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 (Epicenter 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 22% (22%); CC/CC, 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* 677T 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,

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

	Cases (*n*)	Controls (*n*)	OR	95% CI
MTHFR C677T				
CC	361	383	1.0*	—
CT	381	397	1.02	(0.83-1.24)
TT	90	106	0.90	(0.66-1.24)
MTHFR A1298C				
AA	397	412	1.0*	—
AC	363	389	0.97	(0.79-1.18)
CC	72	85	0.88	(0.62-1.24)
MTHFR C677T/A1298C				
CC/AA	118	107	1.0*,†,‡	—
CC/AC	171	191	0.81†	(0.58-1.13)
CC/CC	72	85	0.77†	(0.51-1.16)
CT/AA	189	199	0.86‡	(0.62-1.20)
CT/AC	192	198	0.88	(0.63-1.22)
TT/AA	90	106	0.77‡	(0.52-1.13)

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.
*Reference group.
†P_{trend} = 0.18; 1298 AA/AC/CC within 677CC.
‡P_{trend} = 0.18; 677 CC/CT/TT within 1298AA.

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, 0.63-1.10) for all individuals included in the study and 1.22 (95% CI, 0.65-2.30) when only familial cases are analyzed.

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 (21) and 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.

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