MTHFR C677T Gene Polymorphism in Lymphoproliferative Diseases

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Methylenetetrahydrofolate reductase (*MTHFR*), a key enzyme in folate metabolism, has been implicated in cancer risk. In the present study we used a melting curve analysis to investigate the association of the common *MTHFR* C677T polymorphism with lymphoproliferative diseases. Patients ($n = 117$) were compared with age- and sex-matched control subjects ($n = 154$). Our results indicate that the 677T variant occurred less frequently in patients (26%) than in the control group (33.7%; $P = 0.05$). Investigation of the variant allele (677T) frequency in the subgroups with Hodgkin's lymphoma (HL) and B-cell neoplasms (BCNs) revealed that this difference was a

result of the significantly lower distribution of the variant allele in patients with HL (20.5%; $P = 0.01$). This was accompanied by a significantly higher frequency of the homozygote normal genotype (677CC) among the patients with HL. In patients with BCNs the distribution of the variant allele (30.3%) was comparable to that in the control group ($P = 0.47$). However, the difference between HL (20.5%) and BCNs (30.3%) did not reach statistical significance ($P = 0.09$). Our results suggest that the distribution of the C677T polymorphism may vary among lymphoproliferative diseases. *J. Clin. Lab. Anal.* 20:37–41, 2006. © 2006 Wiley-Liss, Inc.

Key words: lymphoproliferative diseases; Hodgkin's lymphoma; B-cell neoplasms; *MTHFR* polymorphism; melting curve analysis

INTRODUCTION

Lymphoproliferative diseases include a heterogeneous group of lymphoid neoplasms characterized by typical morphological, immunophenotypical, genotypic, and clinical features. These are broadly classified as Hodgkin's lymphoma (HL) or B-cell neoplasms (BCNs), which include non-Hodgkin's lymphomas (NHLs), myeloma, and lymphocytic leukemia/lymphoma. Although some risk factors, such as immunodeficiency, viral infections (e.g., Epstein-Barr virus, human T-cell leukemia virus, and HIV), or exposure to environmental and chemical factors (e.g., radiation and pesticides) have been defined, the etiology of most cases of lymphoproliferative disease is unknown (1–3). Many chromosomal aberrations involving critical oncogenes have also been defined; however, the mechanism of lymphoid transformation remains to be elucidated.

Folate deficiency has been associated with cardiovascular diseases and anemia, and more recently with a rapidly growing number of cancers (4–6). The associa-

tion between the *MTHFR* gene polymorphisms and cancer risk is the subject of current investigations, since *MTHFR* plays an important role in folate metabolism, contributing to DNA synthesis, methylation, and eventually to cancer susceptibility (7,8). This makes *MTHFR* an attractive cancer-predisposing gene. The *MTHFR* enzyme catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate (5,10-methylene THF) to 5-methyltetrahydrofolate (5-methyl THF), the methyl donor for the conversion of homocysteine to methionine, which is further converted to S-adenosylmethionine (SAM). SAM methylates specific cytosine residues in DNA. The consequence of the lack of 5-methyl THF

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is hypomethylation of critical genes, which may activate cell growth and promote malignant transformation (9). Folate in the form of 5,10-methylene THF is required for DNA synthesis. It donates a methyl group to uracil and converts it to thymine, which is normally used for DNA synthesis.

A common polymorphism at nucleotide 677 of the *MTHFR* gene, C677T (ala→val), affects the activity of the enzyme, resulting in a more labile enzyme with decreased activity, which leads to lower folate and elevated homocysteine levels (10–12). This polymorphism has been defined as a susceptibility factor for increased cancer risk in many cancer types, including endometrial, cervical, gastric, lung, and breast cancer (13–17). In colon cancer, the effect of the *MTHFR* polymorphism depends on the folate status (18,19).

Studies of the association between the *MTHFR* C677T polymorphism and lymphoproliferative diseases have provided inconsistent results. A primary study failed to observe an association between the *MTHFR* polymorphism and lymphoma (20), but found a protective effect conferred by the homozygote normal genotype (677CC) against myeloma. However, this effect was not confirmed by a subsequent study (21). In two independent studies both the homozygote normal (677CC) or the homozygote variant genotypes (677TT) were described as susceptibility factors for increased risk of lymphoma (22,23), while another study found no association between the *MTHFR* C677T polymorphism and NHL (24). The aim of the present case-controlled study was to investigate the association between the *MTHFR* C677T polymorphism and lymphoproliferative diseases in an ethnically homogenous population.

MATERIALS AND METHODS

Study Group

This study included 117 newly diagnosed patients with lymphoproliferative diseases at the Oncology Institute and the Istanbul Medical Faculty who had not received previous treatment.

Diseases were classified according to the REAL/WHO classification system and included HL (n = 51; 22 cases with mixed cellularity, 20 with nodular sclerosis, and nine with lymphocyte rich-classic type) and BCNs including plasma cell myeloma/plasmacytoma (n = 28), diffuse large B-cell lymphoma (n = 26), follicular lymphoma (n = 5), B-cell chronic lymphocytic leukemia (n = 3), Burkitt's lymphoma (n = 2), extranodal marginal zone B-cell of MALT type (n = 1), and mantle cell lymphoma (n = 1). The patient group was compared with a control group of sex- and age-matched healthy individuals (n = 154) of similar ethnic background. The control subjects were randomly selected from partici-

TABLE 1. Characteristics of study participants

	N	Gender		Age	
		F	M	Median	Range
Controls	154	81	73	44	19–72
Patients	105	55	62	44	19–72
HL	51	24	27	35	19–70
BCNs	66	31	35	53	25–72

HL, Hodgkin's lymphoma; BCNs, B-cell neoplasms; N, number of the cases.

pants without any sign of a malignant disease who met the matching criteria for gender and age. All participants were informed about the study, and their characteristics are given in Table 1.

Polymorphism Analysis by LightCycler PCR[®] (Roche, Mannheim, Germany) and Melting Curve Analysis

Peripheral blood was placed into EDTA-containing tubes, and lymphocytes were immediately separated by Ficoll-gradient centrifugation. Genomic DNA was isolated from peripheral lymphocytes by the standard Proteinase K/isopropanol method. Primers and fluorescence-labeled hybridization probes designed by Nakamura et al. (25) were used for the LightCycler PCR[®]. The primer sequences were as follows: 5'-TGGCAGGT TACCCCAAAGG-3' (forward) and 5'-TGATGCC CATGTCCGTGC-3' (reverse) (IDT, Coralville, Iowa). Hybridization probes (5'-TGAGGCTGACCTGAAG CACTTGAAGGAGAAGGTGTCT-3'-Flu and 5'-LC-640-CGGGAGCCGATTTCATCAT-3'-PHO) were obtained from TIB Molbiol (Berlin, Germany). The conditions for PCR and melting curve analysis were as previously described (26). Positive controls were obtained by digesting the 163bp PCR product using the *Hinf*I restriction enzyme.

Statistical Analysis

The Chi-square test was used to determine the association between the allele and genotype frequencies using the SPSS statistical package. A two-sided alpha level of 0.05 was considered statistically significant. The alpha level was not adjusted for multiple comparisons. Unconditional regression analysis was used to calculate odds ratios (ORs) and confidence intervals (CIs).

RESULTS

The *MTHFR* genotypes were investigated by melting curve analysis (Fig. 1), and the allele and genotype frequencies were calculated (Table 2). The genotype

Fig. 1. Analysis of the *MTHFR* C677T polymorphism by LightCycler PCR. Following amplification, melting curves were obtained by increasing the temperature gradually from 40°C to 85°C with continuous measurement of fluorescence. The curves were then converted to melting peaks by plotting the negative derivative of the fluorescence with respect to temperature ($-dF/dT$) against temperature. The homozygote wild-type (677CC) and homozygote variant (677TT) genotypes display single peaks at approximately 63°C and 55°C, respectively. The heterozygote samples display both peaks. The negative control includes water instead of DNA and displays no peak.

TABLE 2. Allele and genotype frequencies in the patient and control groups and differences between the T frequencies

	n	677CC n (%)	677CT n (%)	677TT n (%)	CT+TT n (%)	T frequency (%)	P
Controls	154	66 (42.8)	72 (46.8)	16 (10.4)	88 (57.2)	33.7	Reference
Patients	117	63 (53.8)	47 (40.2)	7 (6)	54 (46.2)	26	0.05
HL	51	32 (63)	17 (33)	2 (4)	19 (37)	20.5	0.01
BCNs	66	31 (47)	30 (45.5)	5 (7.5)	35 (53)	30.3	0.47

frequencies in healthy individuals did not differ from those predicted by Hardy-Weinberg equilibrium. The frequency of the variant allele was 26% in the patient group and 33.7% in the control group, indicating that the variant allele occurred less frequently in the patients. The difference was weakly significant ($P = 0.05$) and resulted from the higher frequency of the homozygote normal genotype (677CC) in the patients (53.8% vs. 43%; $P = 0.07$; OR = 1.55, 95%CI = 0.96–2.5).

Given the fact that the variant allele frequency may vary in subgroups of lymphoproliferative diseases, we investigated the allelic distributions in patients with HL ($n = 51$) and BCNs ($n = 66$). We found that the frequency of the variant allele among the patients with HL (20.5%) was significantly lower than that in the whole control group ($P = 0.01$) and a subgroup of the controls with matched individuals ($P = 0.02$). This was associated with a significant higher frequency of the 677CC genotype in HL patients ($P = 0.01$; OR = 2.2, 95% CI = 1.17–4.3). In contrast, among the patients with BCNs the allele frequency was similar to that of the control group ($P = 0.47$). Our data indicate that there is

considerable difference in the prevalence of the *MTHFR* C677T polymorphism between HL and BCNs (20.5% vs. 30.3%, respectively). However, when the patient groups were compared with each other the difference in the distribution of the variant allele between HL and BCNs was not statistically significant ($P = 0.09$).

DISCUSSION

This study was performed to investigate the role of the common *MTHFR* C677T gene polymorphism as a risk factor for lymphoproliferative diseases. We analyzed an ethnically homogenous population to reduce the effect of ethnic variances. The polymorphism was investigated in patients and matched controls by melting curve analysis. In healthy individuals the genotype frequencies were in accordance with the Hardy-Weinberg law of equilibrium, indicating that no evolutionary change has occurred for the advantage of a given allele. Our findings show that the *MTHFR* C677T polymorphism occurs less frequently in patients with lymphoproliferative diseases. Accordingly, a higher frequency of the

homozygote normal genotype (677CC) suggests that this genotype may represent a risk factor for developing lymphoproliferative diseases. However, the data should be interpreted with caution.

Lymphoproliferative diseases include a heterogeneous group of malignancies in which the distribution of the *MTHFR* C677T polymorphism may vary. Investigation of the *MTHFR* C677T polymorphism in HL and BCNs revealed that the difference in the allele frequency was mainly caused by a differential distribution of the variant allele in patients with HL. In this group the variant allele displayed the lowest frequency when compared to BCNs and the control group. The difference in the variant allele frequency between HL and the normal population was statistically significant. The difference between HL and BCNs did not reach statistical significance, although there was a considerable difference (20.5% vs. 30.3%, respectively). The lower frequency of the 677T variant allele in HL was also associated with a significantly higher frequency of the homozygote normal genotype (677CC). Similar results (i.e., an increased risk conferred by the homozygote normal genotype) were obtained in a group of Japanese patients with lymphoma, the majority of whom had NHL (22).

Although they were not significant, differences in the distribution of the polymorphic allele in HL and BCNs suggest that the effect of the *MTHFR* C677T polymorphism may vary in lymphoproliferative diseases. In line with this, conflicting results regarding the role of the *MTHFR* C677T polymorphism in lymphoproliferative diseases have been reported. While Matsuo et al. (22) reported an elevated risk conferred by the 677CC genotype in patients with NHL, other studies could not confirm this effect (20,24). On the other hand, in a recent work by Skibola et al. (23) the homozygote variant genotype (677TT) was described as a susceptibility factor for NHL. The results concerning the role of the *MTHFR* polymorphism in myeloma pathogenesis are also inconsistent. Two studies (20,27) reported a protective effect for the 677CC genotype, while another study (21) failed to observe such a difference in myeloma. The reason for these discrepancies is not clear, but they may be attributable to ethnic and individual differences in the genetic background and varying sample sizes. Such heterogeneity could affect the mechanisms of transformation and result in inconsistencies. In accordance with this notion, recent immunophenotypic, tissue microarray, and molecular studies underlined the extreme heterogeneity of diffuse large B-cell lymphomas and suggested a subclassification of the tumor (28). The HL cases in our study were of classic histology. The classic type of HL is distinguished from the relatively rare variant lymphocyte-predominant HL

by the pattern of somatic mutations, inability of the Reed-Sternberg cells to express a B cell receptor, and defective apoptosis (29). By having a lower distribution, the *MTHFR* 677T variant may play a role in the development of classic type HL.

In most cancer types the increased cancer risk conferred by the *MTHFR* polymorphism has been associated with the homozygote variant genotype (677TT) (15,16,24,30). In some cancers this association depends on the folate status (18,19). Association of the homozygote normal genotype (677CC) with increased cancer risk in patients with HL indicates that the cancer risk associated with the *MTHFR* polymorphism is not only determined by the homozygote variant genotype. The higher risk conferred by the normal genotype is in accord with the fact that cells of individuals carrying the 677CC genotype contain mainly 5-methyl THF, the carrier of methyl groups for DNA methylation (31). Since this condition is detrimental to DNA synthesis, it may lead to increased cancer susceptibility by increasing the uracil misincorporation rate, leading to a higher number of double-strand breaks and chromosomal instability. On the other hand, the significantly lower frequency of the combined variant genotypes (677CT+677TT) in the patients with HL suggests that carrying the variant allele may confer a protective effect against HL. Such an effect can be explained by the fact that in the presence of 5,10-methylene THF, lower levels of the uracil residues are incorporated into DNA, thereby reducing the mutation rate and possibly the cancer risk (7).

To our knowledge, this is the first study to provide evidence that the *MTHFR* 677T allele is observed less frequently in HL than in BCNs, and thus may be a susceptibility factor for HL. These results need to be confirmed in further studies with a larger sample size.

REFERENCES

1. Chiu BC, Weisenburger DD. An update of the epidemiology of non-Hodgkin's lymphomas. *Clin Lymphoma* 2003;4:161-168.
2. Fisher SG, Fisher RI. The epidemiology of non-Hodgkin's lymphoma. *Oncogene* 2004;23:6524-6534.
3. Bukowski JA, Huebner WW, Schnatter AR, Wojcik NC. An analysis of the risk of B-lymphocyte malignancies in industrial cohorts. *J Toxicol Environ Health A* 2003;66:581-597.
4. Verhaar MC, Stroes E, Rabelink TJ. Folate and cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2002;22:6-13.
5. Kwasniewska A, Tukendorf A, Semczuk M. Folate deficiency and cervical intraepithelial neoplasia. *Eur J Gynaecol Oncol* 1997;18:526-530.
6. Eichholzer M, Luthy J, Moser U, Fowler B. Folate and the risk of colorectal, breast and cervix cancer: the epidemiological evidence. *Swiss Med Wkly* 2001;131:539-549.
7. Robien K, Ulrich CM. 5,10-Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: a HuGE minireview. *Am J Epidemiol* 2003;157:571-582.

8. Shellnutt KD, Kauwell GP, Gregory 3rd JF, et al. Methylenetetrahydrofolate reductase 677C→T polymorphism affects DNA methylation in response to controlled folate intake in young women. *J Nutr Biochem* 2004;15:554–560.
9. Zingg JM, Jones PA. Genetic and epigenetic aspects of DNA methylation on genome expression, evolution, mutation and carcinogenesis. *Carcinogenesis* 1997;18:869–882.
10. Frosst P, Blom HJ, Milos R, et al. Candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Gen* 1995;10:111–113.
11. Jacques PF, Bostom AG, Williams RR, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* 1996;93:7–9.
12. Kauwell GP, Wilsky CE, Cerda JJ, et al. Methylenetetrahydrofolate reductase mutation (677C→T) negatively influences plasma homocysteine response to marginal folate intake in elderly women. *Metabolism* 2000;49:1440–1443.
13. Esteller M, Garcia A, Martinez-Palones JM, Xercavins J, Reventos J. Germ line polymorphisms in cytochrome-P450 1A1 (C4887 CYP1A1) and methylenetetrahydrofolate reductase (MTHFR) genes and endometrial cancer susceptibility. *Carcinogenesis* 1997;18:2307–2311.
14. Piyathilake CJ, Macaluso M, Johanning GL, Whiteside M, Heimbürger DC, Giuliano A. Methylenetetrahydrofolate reductase (MTHFR) polymorphism increases the risk of cervical intraepithelial neoplasia. *Anticancer Res* 2000;20:1751–1757.
15. Siemianowicz K, Gminski J, Garczorz W, et al. Methylenetetrahydrofolate reductase gene C677T and A1298C polymorphisms in patients with small cell and non-small cell lung cancer. *Oncol Rep* 2003;10:1341–1344.
16. Shen H, Xu Y, Zheng Y, et al. Polymorphisms of 5,10-methylenetetrahydrofolate reductase and risk of gastric cancer in a Chinese population: a case-control study. *Int J Cancer* 2001;95:332–336.
17. Gershoni-Baruch R, Dagan E, Israeli D, Kasinetz L, Kadouri E, Friedman E. Association of the C677T polymorphism in the MTHFR gene with breast and/or ovarian cancer risk in Jewish women. *Eur J Cancer* 2000;36:2313–2316.
18. Chen J, Giovannucci E, Kelsey K, et al. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res* 1996;56:4862–4864.
19. Chen J, Giovannucci E, Hunter DJ. MTHFR polymorphism, methylene-replete diets and the risk of colorectal carcinoma and adenoma among U.S. men and women: an example of gene-environment interactions in colorectal tumorigenesis. *J Nutr* 1999;129(2S Suppl):560S–564S.
20. Gonzalez Ordóñez AJ, Fernandez Carreira JM, Fernandez Alvarez CR, et al. Normal frequencies of the C677T genotypes on the methylenetetrahydrofolate reductase (MTHFR) gene among lymphoproliferative disorders but not in multiple myeloma. *Leuk Lymphoma* 2000;39:607–612.
21. Gonzalez-Fraile MI, Garcia-Sanz R, Mateos MV, et al. Methylenetetrahydrofolate reductase genotype does not play a role in multiple myeloma pathogenesis. *Br J Haematol* 2002;117:890–892.
22. Matsuo K, Suzuki R, Hamajima N, et al. Association between polymorphisms of folate- and methionine-metabolizing enzymes and susceptibility to malignant lymphoma. *Blood* 2001;97:3205–3209.
23. Skibola CF, Forrest MS, Coppede F, et al. Polymorphisms and haplotypes in folate metabolizing genes and risk of non-Hodgkin lymphoma. *Blood* 2004;104:2155–2162.
24. Gemmati D, Ongaro A, Scapoli GL, et al. Common gene polymorphisms in the metabolic folate and methylation pathway and the risk of acute lymphoblastic leukemia and non-Hodgkin's lymphoma in adults. *Epidemiol Biomarkers Prev* 2004;13:787–794.
25. Nakamura S, Aoshima T, Ikeda M, Sekido Y, Shimokata K, Niwa T. Simultaneous detection of methylenetetrahydrofolate reductase gene polymorphisms, C677T and A1298C, by melting curve analysis with LightCycler. *Anal Biochem* 2002;306:40–43.
26. Deligezer U, Akisik E, Dalay N. Genotyping of the MTHFR gene polymorphism, C677T in patients with leukemia by melting curve analysis. *Mol Diagn* 2003;7:181–185.
27. Yanamandra K, Bocchini Jr JA, Thurmon TF. Methylenetetrahydrofolate reductase 677CC normal genotype may protect against multiple myeloma. *Br J Haematol* 2003;120:1094–1095.
28. Pileri SA, Dirnhofer S, Went P, et al. Diffuse large B-cell lymphoma: one or more entities? Present controversies and possible tools for its subclassification. *Histopathology* 2002;41:482–509.
29. Hansmann ML, Willenbrock K. WHO classification of Hodgkin's lymphoma and its molecular pathological relevance. *Pathologie* 2002;23:207–218.
30. Sharp L, Little J, Schofield AC, et al. Folate and breast cancer: the role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR). *Cancer Lett* 2002;181:65–71.
31. Bagley PJ, Selhub J. A common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of formylated tetrahydrofolates in red blood cells. *Proc Natl Acad Sci USA* 1998;95:13217–13220.