Short Communication

Risk of Non–Hodgkin Lymphoma Associated with Polymorphisms in Folate-Metabolizing Genes

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Abstract

Genetic instability, including chromosomal imbalance, is important in the pathogenesis of lymphoproliferative disorders such as non–Hodgkin lymphoma (NHL). DNA synthesis and methylation, which are closely linked to folate metabolism and transport, may be affected by polymorphisms in genes involved in these pathways. Folate metabolism polymorphisms have been linked to acute lymphoblastic leukemia and colorectal cancer. To evaluate whether genetic variation in folate metabolism and transport may have a role in determining the risk of developing NHL, we analyzed several polymorphisms using DNA obtained as part of a large U.K. population-based case-control study of lymphoma. Polymorphisms studied include methylenetetrahydrofolate reductase (*MTHFR*) 677 C>T and 1298 A>C, methionine synthase (*MTR*) 2756 A>G, serine hydroxymethyltransferase (*SHMT1*) 1420 C>T, thymidylate synthase (*TYMS*) 1494del6 and 28–bp repeat, and reduced folate carrier (*RFC*) 80 G>A. Increased risks for NHL [odds ratio (OR), 1.48; 95% confidence intervals (CI), 1.12-1.97], and marginal zone lymphoma (OR, 3.38; 95% CI, 1.30-8.82) were associated with the *TYMS* 2R/3R variant. Marginal increased risks were also observed for diffuse large B cell lymphoma with the *TYMS* homozygous 6 bp deletion (OR, 1.61; 95% CI, 0.99-2.60) and for follicular lymphoma with *RFC* 80AA (OR, 1.44; 95% CI, 0.94-2.22) and *TYMS* 28–bp repeat 2R/3R (OR, 1.45; 95% CI, 0.96-2.2). We observed no association between NHL and haplotypes for *MTHFR* or *TYMS*. These findings are somewhat inconsistent with those of others, but may reflect differences in circulating folate levels between study populations. Thus, further investigations are warranted in larger series with dietary information to determine the roles that genetics and folic acid status play in the etiology of lymphoma. (Cancer Epidemiol Biomarkers Prev 2005;14(12):2999–3003)

Introduction

Non–Hodgkin lymphoma (NHL) is a complex group of heterogeneous diseases. Although most B–cell lymphomas arise from cells that have passed through the germinal center, they are diverse with respect to their molecular pathogenesis (1). While the underlying biological mechanisms involved have not been fully elucidated, there is evidence that chromosomal and genetic alterations arising from flawed DNA synthesis or altered methylation of oncogenes and tumor suppressor genes may play a role (1-3). Therefore, genetic variability in the activity of enzymes involved in DNA synthesis and methylation may influence susceptibility to NHL including specific histologic subtypes.

Folate metabolism regulates nucleotide synthesis and DNA methylation via a complex pathway involving at least 30 different enzymes (4). A simplified version is shown in Fig. 1 (5). Genetic polymorphisms in several genes encoding these enzymes have been linked with cancer risk (4, 6, 7). Methylenetetrahydrofolate reductase (*MTHFR*) catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate (5,10-MeTHF) to 5-methyltetrahydrofolate (5-MeTHF); the major circulating form of folate which acts as a methyl donor for *S*-adenosylmethionine production (Fig. 1). Two common single nucleotide polymorphisms (SNPs) in *MTHFR* have been reported (677 C>T and 1298 A>C) which result in a 40% to 70% decrease in enzyme activity. Both of these variants cause increased availability of 5,10-MeTHF for DNA synthesis along with a reduction in methionine availability for DNA methylation (refs. 8-11; Fig. 1).

5-MeTHF, the product of the *MTHFR* reaction, is a substrate for methionine synthase (*MTR*). A functional polymorphism in *MTR* at position 2756 (A>G) causes an increase in homocysteine levels through decreased methionine metabolism and may be associated with DNA hypomethylation (12). The transport of 5-MeTHF into cells is facilitated by reduced folate carrier (*RFC*) and interactions between *MTHFR* 677 C>T and a polymorphism in *RFC* (80 G>A) resulting in higher folate plasma levels have been reported (13).

Cytosolic serine hydroxymethyltransferase (*SHMT1*) regulates the availability of 5,10-MeTHF to act as substrate for *MTHFR*. The 1420 C>T polymorphism in *SHMT1* leads to a reduction in circulating folate levels and may mimic folate deficiency, consequently shunting 5,10-MeTHF towards DNA synthesis (ref. 14; Fig. 1). The flux of deoxynucleotides for DNA synthesis is directly controlled by thymidylate synthase (*TYMS*), which has a polymorphic tandem repeat sequence within the promoter enhancer region containing a double (2R) or triple (3R) 28–bp repeat. The presence of the triple repeat leads to increased levels of gene expression and a reduction in DNA damage (15). A number of other polymorphisms in

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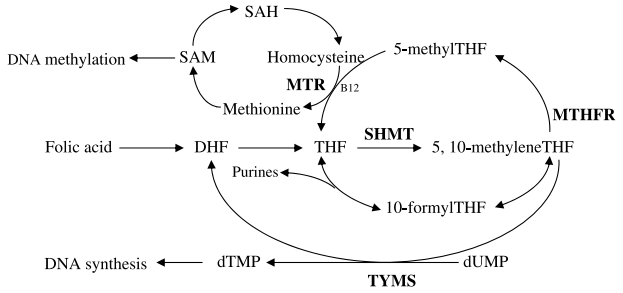

Figure 1. Simplified overview of the human folate metabolic pathway adapted from Skibola et al. (5). Metabolites: 5-methylTHF, 5-methyltetrahydrofolate; 10-formylTHF, 10-formyltetrahydrofolate; SAM-adenosylmethionine; SAH, *S*-adenosylhomocysteine; DHF, dihydrofolate; THF, tetrahydrofolate; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate. Enzymes: *MTR*, methionine synthase; *SHMT*, serine hydroxymethyltransferase; *MTHFR*, 5,10-methylenetetrahydrofolate reductase; *TYMS*, thymidylate synthase.

TYMS have been described, including a 6 bp deletion (1494del6) in the 3′ untranslated region that may also influence RNA levels (16).

Due to the complexity of the folate metabolic pathway, several possible mechanisms exist by which variation in the genes involved may influence risk of NHL subtypes. These include the reduction of uracil misincorporation into DNA by the promotion of thymidine synthesis, and the regulation of gene expression by maintaining adequate *S*-adenosylmethionine levels to control genomic CpG methylation patterns. However, few studies have examined genetic variation in folate transport and metabolism and risk of NHL and the findings are inconsistent (5, 17-21). Using DNA obtained as part of a large U.K. study of lymphoma, we analyzed polymorphisms in genes involved in folate metabolism and transport that may alter the risk of chromosomal translocations and DNA methylation patterns, and therefore NHL.

Study Population. Full study details have been published (22). Briefly, cases were between 18 and 64 years old and recently diagnosed with NHL between 1998 and 2001, while resident in parts of north and southwest England. Diagnoses were pathologically reviewed and coded according to the WHO classification (23). Controls matched on sex, ethnicity, and date of birth were randomly selected from the same general practice list as the case. Of the 700 Caucasian cases with a confirmed diagnosis of NHL, and of the 915 Caucasian controls who were interviewed, DNA samples were available from 589 cases and 755 controls. The study was approved by the United Kingdom Multi-Regional Ethics Committee and carried out in compliance with the principles of the Declaration of Helsinki.

DNA Extraction and Genotyping. DNA was isolated from peripheral blood samples collected in EDTA tubes using phenol-chloroform extraction and quantified using PicoGreen dsDNA Quantitation kits (Molecular Probes, Eugene, OR). Genotyping was carried out using TaqMan Assays-by-Design™ supplied by Applied Biosystems (ABI; Foster City, CA). Amplification reactions were performed with the following protocol on either a 9700 GeneAmp PCR System or 7700 ABI Sequence Detection System: 95°C for 10 minutes, then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. A post-PCR plate read on the 7700 was used to determine genotype. Probes and primer sets used for the *MTHFR* 677 C>T, *MTHFR* 1298 A>C, *SHMT1* 1420 C>T, *MTR* 2756 A>G, *RFC* 80 G>A and *TYMS* 1494del6 polymorphisms and the protocol for the *TYMS* 28–bp repeat are identical to those previously described (5, 24). TaqMan genotyping assays for *MTHFR* were verified by running 96 Coriell samples of known genotypes (http://snp500cancer.nci.nih.gov). All other TaqMan assays were verified by direct sequencing or using standard RFLP analysis. For added quality control, 5% of the samples were selected at random for repeat analysis and four independent control samples were included and analyzed on each 96-well plate.

Statistical Analyses. Odds ratios (OR) and 95% confidence intervals (CI), adjusted for age, sex, and region and were estimated using unconditional logistic regression for each SNP. The likelihood ratio test was used to test for interaction between pairs of SNPs by comparing the model with a multiplicative term combining the two SNPs to a model with single effects for each SNP. Haplotypes for *TYMS* and *MTHFR* were assigned using the log-linear modeling embedded within an expectation maximization algorithm. All analyses were conducted using Stata V.8 (College Station, TX).

Results and Discussion

Demographic characteristics of the study population have been described (22). Among subjects genotyped for folate polymorphisms, 52% of cases and 54% of controls were male, and mean ages of cases and controls were 53.6 and 52.0 years, respectively. Of the 589 cases genotyped, there were 270 diffuse large B cell lymphomas (DLBCL), 207 follicular lymphomas (FL), 21 mantle cell lymphomas, 51 marginal zone lymphomas, 26 T cell lymphomas, and 14 that were unclassified. The cases and controls with genotyping data were no different with respect to age, sex, or diagnostic subgroup to study participants without (data not shown).

Genotype distributions are shown in Table 1. The control frequencies for *MTHFR* 677 C>T, *MTHFR* 1298 A>C, *SHMT1* 1420 C>T, *MTR* 2756 A>G, *RFC* 80 G>A, *TYMS* 1494del6, and *TYMS* 28–bp repeat were all in Hardy-Weinberg equilibrium (data not shown) and are similar to those reported in other Caucasian populations (5-19, 20, 24). There were no statistically significant case-control differences in the distribution of folate polymorphisms, except for the *TYMS* 28–bp repeat polymorphism where we found that the 2R/3R genotype was associated with an increased risk of NHL (OR, 1.48; 95% CI, 1.12-1.97; Table 1) and marginal zone lymphomas (OR, 3.38; 95% CI, 1.30-8.82; data not shown). The ORs were similar when adjusted for SNPs within the same gene (data not shown).

Furthermore, we observed modest increased risks for DLBCL associated with the *TYMS* homozygous 6 bp deletion (6bp−/6bp−; OR, 1.61; 95% CI, 0.99-2.60) and for FL with *RFC* 80AA (OR, 1.44; 95% CI, 0.94-2.22) and *TYMS* 28–bp repeat 2R/3R (OR, 1.45; 95% CI, 0.96-2.21; Table 1). ORs were also computed for the 2R/3R, 2R/4R, 3R/3R, and 3R/4R genotypes combined, and a significant association was found for NHL (OR, 1.42; 95% CI, 1.08-1.86) and marginal zone lymphoma (OR, 2.89; 95% CI, 1.12-7.42) and approaching significance for FL (OR, 1.45; 95% CI, 0.97-2.15). Due to the small number of marginal zone lymphoma cases (n = 51), this finding will need to be reproduced in a larger case series. We also examined gene-gene interactions but found no significant interactions between any of the genes with respect to risk of total NHL (data not shown). No differences were observed when data were stratified by age and sex (data not shown).

The two *MTHFR* polymorphisms were in linkage disequilibrium (D′ = 1.00). Three haplotypes (HapA, HapB, and HapC) accounted for the majority of estimated haplotypes (Table 2). The rare haplotype (HapD) was not seen in controls but was observed in three NHL cases, two of which were diagnosed with FL. With respect to *TYMS*, four

Table 1. Number (%) of cases and controls, adjusted OR, and 95% CI by subtype of NHL for *MTHFR*677 C>T, *MTHFR*1298 A>C, *MTR*2756 A>G, *SHMT1*1420 C>T, *RFC*80 G>A, *TYMS*1494del6, and *TYMS*28 bp repeat

	Controls	Total NHL*		DLBCL*		FL*	
	n (%)	*n* (%)	OR (CI)	*n* (%)	OR (CI)	*n* (%)	OR (CI)
Total	755 (100)	589 (100)		270 (100)		207 (100)	
MTHFR (677 C>T)							
CC	356 (47.2)	247 (41.9)	1	119 (44.1)	1	82 (39.6)	1
CT	316 (41.8)	270 (45.8)	1.20 (0.95-1.51)	118 (43.7)	1.10 (0.82-1.49)	101 (48.8)	1.29 (0.93-1.81)
TT	83 (11.0)	72 (12.3)	1.27 (0.89-1.81)	33 (12.2)	1.20 (0.76-1.89)	24 (11.6)	1.27 (0.76-2.13)
MTHFR (1298 A>C)							
AA	347 (46.0)	288 (48.9)	1	128 (47.4)	1	103 (49.8)	1
AC	331 (43.8)	250 (42.4)	0.90 (0.72-1.14)	120 (44.4)	0.98 (0.74-1.32)	84 (40.6)	0.84 (0.61-1.17)
CC	77 (10.2)	51 (8.7)	0.76 (0.51-1.12)	22 (8.2)	0.76 (0.45-1.27)	20 (9.6)	0.81 (0.47-1.40)
MTR (2756 A>G)							
AA	507 (67.2)	382 (64.9)	1	181 (67.1)	1	130 (62.8)	1
AG	222 (29.4)	190 (32.2)	1.14 (0.90-1.45)	80 (29.6)	1.01 (0.75-1.38)	69 (33.3)	1.24 (0.88-1.73)
GG	26 (3.4)	17 (2.9)	0.87 (0.47-1.64)	9 (3.3)	0.98 (0.45-2.13)	8 (3.9)	1.17 (0.51-2.67)
RFC (80 G>A)							
GG	263 (34.8)	199 (33.8)	1	89 (33.0)	1	70 (33.8)	1
GA	369 (48.9)	277 (47.0)	0.98 (0.77-1.25)	133 (49.3)	1.05 (0.77-1.44)	90 (43.5)	0.89 (0.63-1.27)
AA	123 (16.3)	113 (19.2)	1.21 (0.88-1.66)	48 (17.7)	1.15 (0.76-1.74)	47 (22.7)	1.44 (0.94-2.22)
SHMT1 (1420 C>T)							
CC	349 (46.2)	279 (47.4)	1	136 (50.4)	1	92 (44.4)	1
CT	316 (41.9)	257 (43.6)	1.02 (0.81-1.28)	109 (40.9)	0.89 (0.66-1.19)	99 (47.8)	1.19 (0.86-1.66)
TT	89 (11.8)	53 (9.0)	0.75 (0.51-1.09)	25 (8.7)	0.72 (0.44-1.17)	16 (7.8)	0.68 (0.38-1.22)
TYMS (1494 del6)							
6bp+/6bp+	372 (49.3)	271 (46.0)	1	122 (45.2)	1	96 (46.4)	1
6bp+/6bp−	325 (43.0)	263 (44.7)	1.13 (0.90-1.42)	115 (42.6)	1.09 (0.81-1.46)	95 (45.9)	1.15 (0.83-1.59)
6bp−/6bp−	58 (7.7)	53 (9.0)	1.21 (0.81-1.82)	31 (11.5)	1.61 (0.99-2.60)	16 (7.7)	0.96 (0.52-1.75)
TYMS 28−bp repeat							
2R/2R	181 (24.0)	107 (18.2)	1	59 (21.9)	1	37 (17.9)	1
2R/3R	364 (48.2)	321 (54.5)	1.48 (1.12-1.97)	140 (51.8)	1.18 (0.83-1.67)	109 (52.6)	1.45 (0.96-2.21)
2R/4R	0	1 (0.2)	—	1 (0.4)	—	—	—
3R/3R	205 (27.1)	155 (26.3)	1.29 (0.94-1.78)	67 (24.8)	1.01 (0.67-1.51)	60 (29.0)	1.44 (0.91-2.29)
3R/4R	2 (0.3)	2 (0.3)	1.50 (0.21-10.89)	2 (0.7)	2.92 (0.40-21.25)	—	—
Others vs. 2R/2R			1.42 (1.08-1.86)		1.12 (0.81-1.57)		1.45 (0.97-2.15)

NOTE: ORs adjusted for sex, age, and region estimated using unconditional logistic regression.
*Samples were not amplifiable for one control (0.1%) when testing for *SHMT* 1420 C>T; two cases (0.3%) for *TYMS* 1494del6; and three cases (0.5%) and three controls (0.4%) for *TYMS* 28 bp repeat.

haplotypes (Hap1, Hap2, Hap4, and Hap5) constituted almost 100% of the estimated haplotypes. Neither haplotypes in *MTHFR* or *TYMS* were associated with risk of NHL, DLBCL, or FL.

Our findings for *MTHFR* 677 C>T and 1298 A>C are comparable to those previously published in Caucasian (5, 19, 20) and Japanese populations (17, 21), where no statistically significant associations with risk of total NHL were reported. Skibola et al. (5), found a significantly increased risk of FL associated with *MTHFR* 677 TT (OR, 1.8; 95% CI, 1.0-3.1), although elevated statistical significance was not reached in the current study (OR, 1.27; 95% CI, 0.76-2.13). With respect to *MTR* 2756 A>G, some studies, including our own, found no association with the G allele (19, 20), whereas others have reported increased risks (5, 17, 21). Although Gemmati et al. (20) reported no association with the *MTR* polymorphism, they found an increased risk of NHL when the *MTR* 2756 G allele was inherited in combination with the methionine synthase reductase variant allele (*MTRR* 66G; OR, 0.37; 95% CI, 0.14-0.85). Of the three studies reporting an association with *MTR* 2756 GG, two were based on Japanese populations and the third, based in the U.S., reported a

Table 2. Estimated haplotype frequencies for *MTHFR* and *TYMS*, adjusted OR and 95% CI by subtype of NHL

			Controls (%)	Total NHL		DLBCL		FL	
				(%)	OR (CI)	(%)	OR (CI)	(%)	OR (CI)
MTHFR									
	677 C>T	1298 A>C							
Hap A	C	A	543 (36)	415 (35)	1	192 (36)	1	143 (34)	1
Hap B	C	C	485 (32)	349 (30)	0.94 (0.78-1.13)	164 (30)	0.96 (0.75-1.22)	122 (30)	0.96 (0.73-1.26)
Hap C	T	A	482 (32)	411 (35)	1.11 (0.93-1.34)	184 (34)	1.08 (0.85-1.37)	147 (36)	1.17 (0.90-1.51)
Hap D	T	C	0	3	—	0	—	2	—
TYMS									
	1494del6	28−bp repeat							
Hap 1	6bp+	2R	619 (41)	451 (39)	1	215 (40)	1	154 (37)	1
Hap 2	6bp+	3R	444 (30)	348 (30)	1.08 (0.90-1.30)	141 (26)	0.91 (0.71-1.17)	132 (32)	1.19 (0.91-1.58)
Hap 3	6bp+	4R	0	1	—	1	—	0	—
Hap 4	6bp−	2R	107 (7)	82 (7)	1.05 (0.77-1.43)	41 (8)	1.09 (0.74-1.61)	29 (7)	1.09 (0.67-1.73)
Hap 5	6bp−	3R	332 (22)	284 (24)	1.17 (0.96-1.43)	134 (25)	1.16 (0.90-1.49)	97 (23)	1.17 (0.87-1.58)
Hap 6	6bp−	4R	2	2	1.08 (0.13-8.75)	2	2.18 (0.26-18.01)	0	—

NOTE: ORs adjusted for sex, age, and region estimated using unconditional logistic regression.

marginally significant association (OR, 1.3; 95% CI, 0.99-1.7) when heterozygote and homozygote variants were combined (5). Interestingly, the *MTR* homozygote variant genotype has been shown to confer protection against colon cancer (6).

Little has been reported regarding the potential relationship between NHL and *RFC* 80 A>G, *SHMT1* 1420 C>T, *TYMS* 28–bp and 1494del6 polymorphisms. With respect to *SHMT1,* our U.K. data are similar to those previously reported by Skibola et al. for Caucasians (5). In contrast, in a Japanese series the T–allele in *SHMT1* was associated with decreased susceptibility to NHL (OR, 0.46; 95% CI, 0.23-0.93); however, the authors commented that the frequency of the T–allele in their study was relatively low, which may account for this finding (18). In our data, the marginally increased risk observed for FL with *RFC* 80 AA (OR, 1.44; 95% CI, 0.94-2.22) was comparable with that previously published by Skibola et al. (5) (OR, 1.5; 95% CI, 0.89-2.6), and warrants further investigation in a larger case series.

Whereas the function of the 28–bp triple repeat allele in *TYMS* is associated with enhanced mRNA translation efficiency (15), the functional significance of the 1494del6 polymorphism remains unclear, although it may also affect expression (16). Although we found no significant association between the *TYMS* 1494del6 6bp−/6bp− genotype and risk of total NHL (OR, 1.21; 95% CI, 0.81-1.82), Skibola et al. (5) reported an almost 2-fold significantly decreased risk (OR, 0.57; 95% CI, 0.34-0.94). For DLBCL, we observed a borderline increase in risk associated with the 6bp−/6bp− genotype (OR, 1.61; 95% CI, 0.99-2.60), whereas Skibola and colleagues found a >3-fold decrease in risk (OR, 0.29; 95% CI, 0.10-0.82; ref. 5). Also, in the present study, the *TYMS* 2R/3R variant was associated with increased risks of NHL (OR, 1.48, 1.12-1.97), marginal zone lymphomas (OR, 3.38, 1.30-8.82), and FL (OR, 1.45; 95% CI, 0.96-2.21), but no significant associations were observed in the U.S. study (5). Despite the observed increased risks of NHL with polymorphisms in the *TYMS* gene, no association was observed when haplotypes were estimated. While this lack of association may indicate that the *TYMS* 1494del6 and *TYMS* 28–bp repeat polymorphisms are not associated with NHL, the polymorphisms may be in linkage disequilibrium with other SNPs outside the haplotype region that are related to lymphoma.

The reasons for the apparent differences between the U.K. and U.S. studies are unclear, but may reflect differences in circulating folate levels between populations. Based on a North American study, Ulrich et al. (25) previously reported a significant gene–exposure interaction between the *TYMS* 28–bp repeat polymorphism and folate intake; the 3R/3R genotype in combination with high folate intake was associated with a decreased risk of colorectal cancer. Although the relationship with folate intake was not as clear for the *TYMS* 1494del6 (25), it is likely that the effect of this polymorphism may also be modified by folate levels. Furthermore, the effect of the *MTHFR* 677 polymorphism on colorectal cancer risk is also predicted to be modified by differences in folate levels; and there is limited evidence that the effect of *MTR* 2756GG may also be modified by folate intake [reviewed in ref. (6)]. Folic acid fortification was introduced in the U.S. during the late 1990s, and individuals have higher circulating levels of folate as a consequence. In contrast, fortification of foods with folic acid is not mandatory in the U.K., and it is likely that circulating folate levels differ between the U.K. and the U.S. Therefore, it is possible that the observed interaction between *TYMS* and folate levels and its effect on colorectal cancer risk also may be important in determining NHL risk. Specifically, the functional effect of the polymorphisms may be influenced by folate availability, which, in turn, may have a bearing on the association of the polymorphism with lymphoma risk. This could account for the different findings between the two study populations.

In summary, data from previous studies that have examined polymorphisms in *MTHFR, MTR, TYMS, SHMT1*, and *RFC* in relation to NHL etiology are inconsistent. The data reported here, like elsewhere, are limited by problems of multiple testing leading to potential false-positive results; nevertheless, our observed association between NHL and *TYMS* 2R/3R remains significant at the 1% significance level. Although our U.K. study is the largest to date, more comprehensive international studies that address population substructure will be needed to identify potentially important gene-environment interactions involving folate fortification in different populations. Furthermore, whereas our study examined five critical genes that regulate DNA synthesis and methylation, there are >30 different genes involved in the folate metabolic pathway. Thus, the inclusion of additional folate-metabolizing genes in further investigations may help to clarify the role of this pathway in lymphomagenesis.

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