Gene-nutrient interactions among determinants of folate and one-carbon metabolism on the risk of non-Hodgkin lymphoma: NCI-SEER Case-Control Study

Unhee Lim,[1] Sophia S. Wang,[1] Patricia Hartge,[1] Wendy Cozen,[2] Linda E. Kelemen,[3] Stephen Chanock,[4] Scott Davis,[5] Aaron Blair,[1] Maryjean Schenk,[6] Nathaniel Rothman,[1] and Qing Lan[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health and Human Services (DHHS), Rockville, MD; [2]Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles; [3]Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN; [4]Core Genotyping Facility, Advanced Technology Center, Division of Cancer Epidemiology and Genetics and Pediatric Oncology Branch, Center for Cancer Research, NCI, NIH, DHHS, Bethesda, MD; [5]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center and Department of Epidemiology, University of Washington, Seattle; [6]Department of Family Medicine, Wayne State University and Karmanos Cancer Institute, Detroit, MI

We previously reported a lower risk of non-Hodgkin lymphoma (NHL) associated with high consumption of vitamin B6 and methionine, dietary determinants of one-carbon metabolism. Evidence has linked genetic variants involved in one-carbon metabolism to NHL. We investigated 30 polymorphisms in 18 genes for their main effect on NHL among 1141 incident cases and 949 population-based controls and examined gene-nutrient interactions in a subgroup of 386 cases and 319 controls who provided detailed food-frequency information. Odds ratios (ORs) and 95% confidence intervals (CIs) were adjusted for age, sex, and race. We observed a decreased risk of NHL overall with *BHMT* Ex8+453A>T and increased risk with *CBS* Ex13+41C>T, *FPGS* Ex15-263T>C, and *SHMT1* Ex12+138C>T and Ex12+236C>T. Furthermore, significant gene-nutrient interactions limited the protective association comparing high versus low vitamin B6 to *FPGS* Ex15-263T>C CC (OR = 0.22; 95% CI = 0.10-0.52), *MTHFS* IVS2-1411T>G TT/TG (OR = 0.54; 95% CI = 0.36-0.81), and *MTR* Ex26-20A>G AA (OR = 0.55; 95% CI = 0.35-0.86) genotypes, and the protective association of methionine to *FTHFD* Ex10-40G>T GG (OR = 0.63; 95% CI = 0.44-0.91), *MTHFR* Ex8-62A>C CC (OR = 0.13; 95% CI = 0.04-0.39), and *MTRR* Ex5+136T>C TT (OR = 0.67; 95% CI = 0.47-0.97) genotypes. Warranting replication, our finding of gene-nutrient interactions in one-carbon metabolism supports their etiologic involvement in lymphomagenesis. (Blood. 2007;109:3050-3059)

Introduction

One-carbon metabolism in eukaryotic cells involves reactions to transfer single carbon units for DNA synthesis and for methylation of biologic compounds, including DNA (Figure 1).[1] One-carbon transfer reactions are mediated by numerous enzymes that require nutritional coenzymes, most notably folate—a B vitamin that serves as a one-carbon carrier/donor, and also vitamins B12, B6, and B2 and methionine. Disruptions in one-carbon metabolism due to deficiency of the nutrients or genetic polymorphisms of the enzymes involved have been linked to cancer etiology through insufficient DNA synthesis/repair and aberrant gene expression.[2]

Epidemiologic evidence linking genetic susceptibility in one-carbon metabolism to cancer risk is most extensive for colorectum[7] and growing for cancers of breast,[8] stomach,[9] and other sites. Non-Hodgkin lymphoma (NHL), like other cancers, exhibits genetic instability[10] and aberrant DNA methylation patterns,[11] suggesting a key role of one-carbon metabolism in lymphoid tissues with high turnover rates and supporting its involvement in lymphomagenesis. In fact, genetic polymorphisms of some one-carbon metabolism enzymes have been associated with an altered risk of adult lymphomas[12-20] and leukemias[15,21-23]: lower risk was associated with *MTHFR* Ex5+79C>T (commonly known as 677C>T)[15,17,21] and Ex8-62A>C (1298A>C)[21] (or the combination[15,17,21]), *MTR* Ex26-20A>G (2756A>G),[14,16,20] *MTRR* Ex2-64A>G (66A>G),[15] *SHMT1* Ex12+138C>T (1420C>T),[13,22] and *TYMS* variants of variable number tandem repeats (VNTRs) Ex1+52-28base (3R versus 2R),[13,19,22] 494 6-bp deletion,[18] IVS6-68 C>T,[18] 1053C>T,[18] and their haplotypes.[18] Also, potential gene-gene interactions have been suggested.[13,15,20,22]

However, studies to date have considered only a few of these enzymes out of many involved in the metabolic pathway (Figure 1). Also, findings on these genetic variants have not been consistent in all studies: some studies showed conflicting findings for *MTHFR* Ex8-62A>C or the combination of its 2 single nucleotide polymorphisms (SNPs),[20] *MTR* Ex26-20A>G[12,17] and *TYMS* VNTR Ex1+52-28base,[20] and some showed null results for *CBS*,[16] *MTHFR*,[12,14-16,18,19] *MTR*,[15,18,22] and *SHMT1*.[18,19] This inconsistency in past studies may be in part due to the small size of most studies or to unavailability of dietary information to examine potential gene-nutrient interactions that have been reported for other cancers.[24-26]

In a US population–based case-control study, we previously reported that the highest quartiles of vitamin B6 and methionine intake were associated with about 50% lower risk of NHL overall and that folate intake was inversely associated with diffuse large

Submitted July 10, 2006; accepted November 12, 2006. Prepublished online as *Blood* First Edition Paper, November 21, 2006; DOI 10.1182/blood-2006-07-034330.

The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

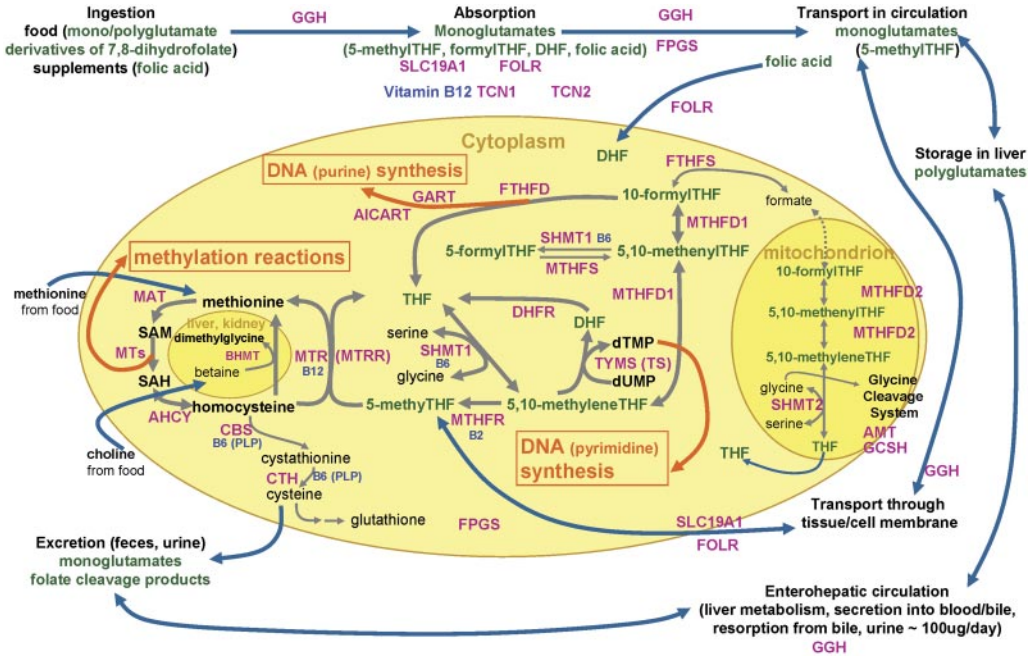

Figure 1. Diagram of folate and one-carbon metabolism in mammalian organisms. Intracellular one-carbon transfer reactions are essential for nucleotide (thymidylate and purine) synthesis and methylation of numerous compounds, including DNA, RNA, proteins, and phospholipids. These one-carbon transfer reactions are mainly supported by folate, a B vitamin that serves as a one-carbon carrier/donor. This diagram depicts absorption, transport, and metabolism of folate around the intracellular one-carbon metabolism as well as enzymes/proteins and other nutritional factors involved.[1-5] Folates in food, mostly polyglutamates, are hydrolyzed to monoglutamates by GGH in the gut and are absorbed across the intestinal mucosa with folic acid from fortified foods and supplements mostly by a saturable pH-dependent process, via reduced folate carrier (encoded by *SLC19A1*), and by passive diffusion at high concentrations. Once absorbed into the portal circulation, folates are taken up by the liver, where they are metabolized to polyglutamates by FPGS and retained or released into blood or bile as 5-methylTHF. Folate released in bile is reabsorbed in the small intestine. About two thirds of 5-methylTHF, the predominant form of folate in circulation, is bound to low-affinity proteins, mostly albumin: low levels of high-affinity folate binders are also found in blood. Blood 5-methylTHF is transported into the cell by carrier-mediated or receptor-mediated mechanisms. Reduced folate carrier has a higher affinity for reduced folate than oxidized folic acid and accounts for the transport of most folate and methotrexate. Membrane-bound folate receptors, including folate receptor 1 encoded by *FOLR1,* with high affinity for folic acid are expressed in epithelial tissues, and its expression is elevated in malignant epithelial tumors.[6] The predominant cytoplasmic folate, 5-methylTHF, donates its one-carbon moiety to methylate homocysteine to methionine, yielding THF. THF is a much preferred substrate to FPGS that lengthens the glutamate chain of the monoglutamate folate so folates can be retained in the cell. This polyglutamylation also enables folates to be used by one-carbon metabolizing enzymes that have much higher affinities for polyglutamates than monoglutamates. In deficiency of vitamin B12, which is a coenzyme for methionine synthase (MTR) that converts 5-methylTHF to THF, or with insufficient transcobalamins (TCN1, TCN2) for vitamin B12 absorption, deficiency of functional folate (THF) occurs despite sufficient folate in circulation ("methyl-trap"). MTR loses its activity when its vitamin B12–derived coenzyme, cobalamin, gets oxidized: MTRR reactivates MTR using the methyl supply from SAM. Homocysteine can be remethylated via an alternative mechanism of BHMT using betaine, supplied from dietary choline, in kidney and liver. Methionine, from homocysteine and also supplied from diet, is converted to SAM, a universal donor of one-carbon unit to numerous methylation reactions via MTs in part for DNA methylation. Resulting SAH is hydrolyzed to homocysteine, which then gets remethylated or catabolyzed via the transsulfuration pathway initiated by CBS. The active coenzyme THF obtains one-carbon moiety from amino acid serine via SHMT1 catalysis, yielding 5,10-methyleneTHF, which is an important common substrate to methylation pathway described (remethylation of homocysteine to methionine) via MTHFR or to nucleic acid synthesis pathways via TYMS (uridylate to thymidylate conversion; pyrimidine synthesis) or MTHFD1/FTHFD (purine synthesis). DHF, the remnant of TYMS reaction on THF, is also supplied from folic acid that is reported to be found in blood in higher proportion than usual when a large dose is consumed from fortified foods or supplements. 5,10-MethenylTHF can be interconverted with 5-formylTHF (also known as folinic acid or leucovorin; thought to be the storage form of folate) via SHMT1/MTHFS. Although less understood, mitochondrial one-carbon metabolism is proposed to be in equilibrium with cytoplasmic metabolism and contains glycine cleavage system. AHCY indicates S-adenosylhomocysteine hydrolase; AICART, phosphoribosylaminoimidazolecarboxamide formyltransferase; AMT, aminomethyltransferase; B2, vitamin B2; B6, vitamin B6; B12, vitamin B12; BHMT, betaine-homocysteine methyltransferase; CBS, cystathionine-beta-synthase; CTH, cystathionase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; FPGS, folylpolyglutamate synthase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; FOLR, folate receptor; FTHFD, 10-formyltetrahydrofolate dehydrogenase; FTHFS, 10-formyltetrahydrofolate synthase; FTHFSDC1, 10-formyltetrahydrofolate synthetase domain containing 1; GART, glycinamide ribonucleotide formyltransferase; GCPII, glutamate carboxypeptidase II; GCSH, glycine cleavage system protein H; GGH, gamma-glutamylhydrolase; MAT, methionine S-adenosyltransferase; MTs, a group of methyltransferases; MTHFD1, cytoplasmic 5,10-methylenetetrahydrofolate dehydrogenase; MTHFD2, mitochondrial 5,10-methylenetetrahydrofolate dehydrogenase; MTHFR, 5,10-methylenetetrahydrofolate reductase; MTHFS, 5,10-methenyltetrahydrofolate synthetase; MTR, methionine synthase; MTRR, methionine synthase reductase; PLP, pyridoxal 5′-phosphate; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SHMT1, cytoplasmic serine hydroxymethyltransferase; SHMT2, mitochondrial serine hydroxymethyltransferase; SLC19A1, reduced folate carrier; TCN1, transcobalamin 1; TCN2, transcobalamin 2; THF, tetrahydrofolate; and TYMS, thymidylate synthetase.

B-cell lymphoma (DLBCL).[27] In this report, we investigated genetic susceptibility of selected one-carbon metabolism enzymes and their interaction with diet using a comprehensive assessment of the metabolic pathways (Figure 1).

Materials and methods

Study population

As described in detail previously,[28] newly-diagnosed and human immunodeficiency virus (HIV)–negative NHL cases were identified from 4 Surveillance, Epidemiology, and End Results (SEER) registries among women and men aged 20 to 74 years during the period of July 1998 through June 2000. On all NHL cases from local SEER registries, we obtained pathology and subtype information that was based on abstracted reports of the diagnosing pathologists. We then classified the histologically confirmed cases into subtypes of DLBCL, follicular lymphoma, and small lymphocytic lymphoma (SLL) according to the International Classification of Diseases-Oncology (ICD-O-2; codes for NHL: 9590-9595, 9670-9717).[29] Although pathology samples were not reviewed centrally, we consider pathological diagnosis of these main NHL subtypes to be reliable and comparable across SEER study centers.[30,31] Population controls who were aged 20 to 74 years, HIV negative, and with no history of NHL were identified among the study area residents via random-digit-dialing and Health Care Financing Administration (Medicare) files. The study was

approved by the human subjects review boards at all participating institutions (NCI and SEER centers of Detroit, Iowa, Los Angeles, and Seattle), and we obtained written informed consent from all participants, in accordance with the Declaration of Helsinki.

We identified 2248 potentially eligible cases and interviewed 1321 cases (participation rate, 76%; response rate, 59%)[28]: we did not contact 520 cases (death, inability to locate, physician refusal, or relocation outside of the study area) and could not acquire participation of 407 cases. Of 2409 potentially eligible controls frequency matched to cases on sex, age, race, and SEER centers, 2046 were contacted, and 1057 were interviewed (participation rate, 52%; response rate, 44%). Among the interviewed participants, 1172 cases and 982 controls provided biologic samples for genotyping[32]: 773 cases and 668 controls provided blood samples, and 399 cases and 314 controls provided mouthwash buccal cell samples. Demographic characteristics (age, sex, education) were comparable among individuals who provided blood, buccal samples, or neither.[32] Among the 1141 cases and 949 controls who were genotyped successfully, 517 cases and 434 controls (about 50% of non–African American participants) were queried for diet/lifestyle history using a split-sample design described earlier,[27] of which 386 cases (75%) and 319 controls (74%) returned the questionnaire.

Genotyping

We considered enzymes and proteins involved in folate absorption and transport or intracellular one-carbon metabolism (Figure 1) and chose 14 genes that have been previously studied or are otherwise believed to play an important role in one-carbon metabolism.[3] In addition, we included 4 DNA repair genes *(MBD2, MGMT, MLH1,* and *MSH2)* that in part depend on one-carbon supplies, and therefore, may have a synergistic effect with nutritional or genetic factors of one-carbon metabolism on carcinogenesis. Twenty-nine SNPs and one insertion polymorphism in these genes were selected (Table 1), based on prior functional data from previous reports[18,22,34-37] or expected functional consequences in that the polymorphisms result in amino acid change or they are located within the 3′ untranslated region (UTR), which contains regulatory sequences and binding sites for other molecules that could alter the stability of the mRNA transcript of the gene.

Genotyping details are available elsewhere.[32] We extracted DNA using Puregene Autopure DNA extraction kits (Gentra Systems, Minneapolis, MN) from buffy coats and using phenol chloroform extraction methods for buccal cell samples.[38] All genotyping was conducted at the NCI Core Genotyping Facility (CGF, Advanced Technology Corporation, Gaithersburg, MD), using the Taqman platform (http://snp500cancer.nci.nih.gov; National Cancer Institute).[39] In order to conserve DNA, we genotyped the blood-based DNA samples first (~ 75%) and expanded to buccal cell samples if the blood-based DNA data yielded significant ($P < .05$) or suggestive (ie, borderline significance or significant linear trend) associations with NHL. As a result, 16 polymorphisms from 11 genes were not genotyped in buccal cell–based DNA due to null results in blood-based DNA (noted in Table 1). Genotype frequencies in general were similar for individuals who provided blood versus buccal cells and also similar by participation status.[40]

Genotyping was successful in 96% to 100% of DNA samples, similarly in blood- and buccal cell–based samples. Only 3 SNPs were not in Hardy-Weinberg equilibrium in white controls (*CBS* Ex9+33C>T, *MSH2* Ex6+23G>A, and *MTR* Ex26-20A>G; Table 1). To assure quality control, 40 replicate samples from 2 blood donors and duplicate samples from 100 study subjects were interspersed blindly among study samples for all genotyping, which yielded an agreement of 99% or more.[32]

Dietary assessment

Dietary intake was assessed using a modified version of the self-administered Block food frequency questionnaire.[27] Participants were queried on 107 food and beverage items for their "usual eating habits (as an

Table 1. Genes involved in one-carbon metabolism and their polymorphic variants investigated in relation to non-Hodgkin lymphoma, the NCI-SEER Case-Control Study, 1998-2000

Gene	Enzyme/protein	Location	dbSNP ID [amino acid change]
BHMT	Betaine-homocysteine methyltransferase	5q13.1-q15	rs585800 (Ex8+453A>T)†
CBS	Cystathionine-beta-synthase	21q22.3	rs234706 (Ex9+33C>T)‡§ [Y233Y]; rs12613 (Ex18-39IG>A)‡; rs1801181 (Ex13+41C>T)‡ [A360A]
FPGS	Folylpolyglutamate synthase	9q34.1	rs10106 (Ex15-263T>C)‡
FTHFD	10-Formyltetrahydrofolate dehydrogenase	3q21.3	rs2305230 (Ex10-40G>T)‡ [L395L]; rs1127717 (Ex21+31A>G)† [D793G]
GGH	Gamma-glutamyl hydrolase	8q12.1	rs719236 (−423G>T)‡; rs1031552 (IVS7-3001C>T)‡
MBD2	Methyl-CpG binding domain protein 2	18q21	rs7614 (Ex8+438A>G)†; rs603097 (−2176T>C)‡
*MGMT**	O-6-Methylguanine-DNA methyltransferase	10q26	rs2308321 (Ex4+13A>G)‡ [I143V]; rs2308327 (Ex4+119A>G)† [K178R]; rs12917 (Ex2-25C>T)† [L84F]
MLH1	MutL homolog 1	3p21.3	rs1799977 (Ex8-23A>G)† [I219V]; rs2286940 (IVS12-169C>T)†
MSH2	MutS homolog 2	2p22-p21	rs4987188 (Ex6+23G>A)‡§ [G322D]
MTHFD2	5,10-Methylenetetrahydrofolate dehydrogenase 2	2p13.1	rs1667627 (IVS1+3323T>C)†
MTHFR	5,10-Methylenetetrahydrofolate reductase	1p36.3	rs1801131 (Ex8-62A>C or 1298A>C)‡ [E429A]; rs1801133 (Ex5+79C>T or 677C>T)‡ [A222V]
MTHFS	5,10-Methenyltetrahydrofolate synthetase (or 5-formyltetrahydrofolate cyclo-ligase)	15q24.3	rs622506 (IVS2-1411T>G)†
MTR	5-Methyltetrahydrofolate-homocysteine methyltransferase (or methionine synthase)	1q43	rs1805087 (Ex26-20A>G or 2756A>G)†§ [D919G]
MTRR	5-Methyltetrahydrofolate-homocysteine methyltransferase reductase (or methionine synthase reductase)	5p15.3-15.2	rs161870 (Ex5+136T>C)‡ [L206L]
SHMT1	Cytoplasmic serine hydroxymethyltransferase 1	17p11.2	rs1979277 (Ex12+138C>T or 1420C>T)† [L435F]; rs1979276 (Ex12+236C>T)†
SLC19A1	Solute carrier family 19 (folate transporter), member 1 (or reduced folate carrier 1)	21q22.3	rs12659 (Ex4-254C>T)† [P232P]; rs1051296 (Ex8-233T>G)†
TCN1	Transcobalamin I	11q11-q12	rs526934 (IVS1+372T>C)†
TYMS	Thymidylate synthetase	18p11.32	rs699517 (Ex8+157C>T)‡; VNTR (Ex1+52-28base 3R>2R)‡

*The main effect of *MGMT* has been reported previously.[33]

†Genotyping was done in subjects with DNA from blood samples only.

‡Genotyping was done in all of the subjects with DNA from blood or buccal tissue samples.

§$P < .05$ for deviation from Hardy-Weinberg equilibrium among controls.

adult and before one year ago, not including any recent dietary changes)" by giving responses for 9 frequencies and 3 portion sizes. The instrument was validated against multiple diet records (correlations were 0.5-0.6 for most nutrients).[41,42]

Statistical analyses

We used the Wilcoxon nonparametric test for continuous variables and chi-squared tests for categoric variables to compare the descriptive characteristics of (1) cases and controls with genetic information, separately by the availability of diet information (Table 2); and (2) controls with and without diet information.

For the main effect of the genotype on NHL, we estimated odds ratios (ORs) and 95% confidence intervals (CIs) comparing heterozygote and homozygous variant (or less prevalent genotype) to homozygous wild-type (or more prevalent genotype) in unconditional logistic regression models (Table 3). Based on the risk estimates, heterozygotes were combined with either homozygous variants or homozygous wild types to explore a dominant or recessive model, respectively. We assigned ordinal scores (0, 1, 2) to homozygous wild-type, heterozygote, and homozygous variant, respectively, to obtain the *P* value for linear trend in regression. Heterogeneity among NHL subtypes for the main effect of each polymorphism was assessed by contrasting one subtype as "cases" and another as "controls" in the logistic regression model (eg, SLL versus DLBCL or SLL versus follicular lymphoma for the main effect of *CBS* Ex13+41C>T or *FPGS* Ex15-263T>C). We adjusted for the matching factors of age (continuous), sex, and race, but not study centers, to have the most parsimonious estimates.

We examined gene-nutrient and gene-gene interactions regardless of significance of the main effects of genetic polymorphisms. Gene-nutrient interactions were assessed by including ordinal score variables of each

Table 2. Descriptive characteristics of non-Hodgkin lymphoma (NHL) cases and controls with genotype and diet information, the NCI-SEER Case-Control Study, 1998-2000

Characteristic	Among subjects with genotype data, no. (%)†		Among subjects with both genotype and diet data, no. (%)†	
	NHL cases	Controls	NHL cases	Controls
No. of cases	1141	949	386	319
Men	629 (55)	506 (53)	215 (56)	166 (52)
Women	512 (45)	443 (47)	171 (44)	153 (48)
Age at enrollment				
Younger than 45 y	203 (18)	150 (16)	67 (17)	40 (13)
45 y to younger than 55 y	252 (22)	178 (19)	90 (23)	54 (17)
55 y to younger than 65 y	311 (27)	228 (24)	105 (27)	79 (25)
65 y to 74 y	375 (33)*	393 (41)	124 (32)*	146 (46)
Race‡				
White	981 (86)*	760 (80)	366 (95)	305 (96)
African American	80 (7)	127 (13)	0 (0)	NA
Others	80 (7)	62 (7)	20 (5)	14 (4)
Study center				
Detroit	236 (21)	170 (18)	38 (10)	26 (8)
Iowa	333 (29)	272 (29)	151 (39)	118 (37)
Los Angeles	283 (25)	238 (25)	87 (23)	69 (22)
Seattle	289 (25)	269 (28)	110 (29)	106 (33)
Education				
Less than 12 y	109 (10)	89 (10)	26 (7)	28 (9)
12 to less than 16 y	693 (61)	543 (58)	237 (61)	178 (56)
16 y or more	334 (29)	297 (32)	123 (32)	113 (35)
Family history of NHL	48 (4)	30 (3)	20 (5)	16 (5)
Body mass index				
Normal, less than 25 kg/m^2	459 (40)	389 (41)	142 (37)	112 (35)
Overweight, 25 kg/m^2 or greater to less than 30 kg/m^2	408 (36)	336 (35)	160 (41)	132 (41)
Obese, 30 kg/m^2 or greater	274 (24)	224 (24)	84 (22)	75 (24)
DNA source				
Blood	735 (64)	631 (66)	271 (70)	242 (76)
Buccal tissue	395 (35)	313 (33)	112 (29)	75 (24)
Both	11 (1)	5 (1)	3 (1)	2 (1)
Patient pathology				
All B-cell	927 (81)	NA	327 (85)	NA
Diffuse large B-cell	360 (32)	NA	142 (37)	NA
Follicular	271 (24)	NA	98 (25)	NA
SLL	147 (13)	NA	48 (12)	NA
Marginal zone	92 (8)	NA	26 (7)	NA
Other	57 (5)	NA	13 (3)	NA
All T-cell	73 (6)	NA	21 (5)	NA
NOS	141 (12)	NA	38 (10)	NA

NA indicates not applicable.

*$P < .05$ for comparison of cases versus controls, within the group of genetic and genetic + diet information availability, using Wilcoxon nonparametric test for continuous variables and using Pearson chi-squared test for categoric variables.

†Number and percent for each category of descriptive characteristic variables.

‡By study design, study participants were divided into 2 groups for detailed questionnaires, and all African Americans and a random half of the rest were assigned to one group for information on self/family medical history, while the other participants were assigned to fill out diet information.

genotype and nutrient (dichotomized at the median based on the nutrient distribution among control individuals) along with their product term in the regression model and were concluded significant by the *P* value less than .10 of the cross-product term. For significant gene-nutrient interactions, the nutrient-NHL association comparing above versus below median of the nutrient is presented stratified by genotypes (Table 4). All nutrients except alcohol were adjusted for total energy intake by the nutrient-density method.[43] Regression models of folate, vitamin B6, and methionine were adjusted for each other for potential confounding. Additional adjustments for energy intake and other nutrients did not materially change the risk estimates (ie, change < 10%).

We used SAS/Genetics (version 9.1.3; SAS Institute, Cary, NC) to assess Hardy-Weinberg equilibrium and linkage disequilibrium among SNPs from the same gene. We explored haplotype analyses for genes with 2 or more polymorphisms but did not detect any stronger associations, and therefore, present the findings from individual polymorphism analyses. To evaluate the probability of false-positive associations, we computed the false discovery rate (FDR),[44] which controls the proportion of false positives out of all significant findings using the *P* values from the regression of score variables (ie, the trend test, also referred to as the additive model) using SAS software: we considered FDR less than 0.2 as noteworthy. We also computed the false-positive report probabilities (FPRPs)[45] using prior probabilities ranging from 0.1 to 0.001 based on gene selection criteria described in "Genotyping" and considered values below a criterion of 0.2 noteworthy as recommended in the initial description of the method.[45]

Results

Descriptive characteristics and histologic subgroup distributions were comparable between all participants who were genotyped and the subgroup who had both genotype and diet information (Table 2): controls with and without diet information were similar in the characteristics compared, except the racial distribution due to study design. In the subgroup with diet data, cases tended to be younger, but were otherwise similar to controls with respect to sex, race, study center, education, family history of NHL, body mass index (weight in kilograms/height in meters squared), and the availability of biospecimens.

Table 3 shows the genotype-NHL association of polymorphisms that exhibited a significant main effect, linear trend, or interaction with a nutrient among people with both genotype and diet data. Associations for all polymorphisms are available on the *Blood* website; see the Supplemental Materials link at the top of the online article (Table S1, for participants with diet data; Table S2, for all participants with genotype data). The rarer homozygous genotype for *BHMT* Ex8+453A>T (TT versus AA) was associated with a lower risk of NHL overall, whereas the rarer homozygotes for *CBS* Ex13+41C>T (TT versus CC) and *FPGS* Ex15-263T>C (CC versus TT) were associated with an elevated risk. Heterozygotes for *SHMT1* Ex12+138C>T and Ex12+236C>T (CT versus CC for both) were associated with a higher risk of NHL overall. Findings were similar in pattern for main subtypes: although the results reached statistical significance for the positive association of *CBS* Ex13+41C>T and *FPGS* Ex15-263T>C with the risk of SLL and of *SHMT1* Ex12+138C>T with follicular lymphoma (Table 3), the difference in risk estimates among subtypes was not significant in case-patient comparisons. Main effects among all genotyped data (Table S2) showed similar association patterns and identified additional variants in *FTHFD, MTHFS,* and *MTR* for significant associations or trends with NHL overall (ie, *P* value < .05). FDR and FPRP estimates for all our findings were above the cut-point value .2.

Based on similar main effects across main NHL subtypes, we assessed gene-nutrient interactions on combined NHL. We examined interactions between all genetic variants and nutritional determinants, specifically vitamin B6 and methionine that were previously found protective against NHL.[27] We detected a number of significant interactions (by *P* value < .1). The main nutrient-NHL association in these data was attenuated from the previous diet analyses[27] due to loss of participants without genotype information and also less detailed categories applied. The inverse association between vitamin B6 intake and NHL was apparent only in the *FPGS* Ex15-263T>C CC (*P* interaction = .002), *MTHFS* IVS2-1411T>G TT/TG (*P* interaction = .06), and *MTR* Ex26-20A>G AA (*P* interaction = .05) genotypes. Also, the inverse association between dietary methionine and overall NHL appeared to be limited to *FTHFD* Ex10-40G>T GG (*P* interaction = .04), *MTHFR* Ex8-62A>C CC (*P* interaction = .007), and *MTRR* Ex5+136T>C TT genotypes (*P* interaction = .05). There were no meaningful interactions for other nutrients, including folate. The main associations and gene-nutrient interactions were not confounded by other risk factors in combined and individual subtype analyses.

Among all participants with genotype information, we detected an interaction between *CBS* and *FPGS* (*P* interaction = .007), but without synergistic effects (all other genotypes compared with *CBS* Ex13+41C>T CC/*FPGS* Ex15-263T>C TT showed similarly elevated risk for NHL by about 50%) and without a further interaction with vitamin B6 (data not shown).

Discussion

We observed that the protective association of high dietary intake of one-carbon nutrients with NHL varied by genetic polymorphisms of the one-carbon metabolizing enzymes: especially, vitamin B6 by polymorphisms in *FPGS, MTHFS,* and *MTR* and methionine by polymorphisms in *FTHFD, MTHFR,* and *MTRR.* To our knowledge, this is the first investigation of NHL that examined potential gene-nutrient interactions involving one-carbon metabolism.

Our findings were especially strong for polymorphisms in *FPGS* and *MTHFR:* their interactions with vitamin B6 and methionine intake, respectively, determined by the logistic regression methods were also evident using the case-only analysis (data not shown), but other borderline findings, which are more likely due to chance variation in controls, went away in the case-only analysis. The enzyme FPGS catalyzes an essential and rate-limiting polyglutamylation step in the intracellular one-carbon metabolism[46,47]: it adds several glutamyl residues to all reduced folates supplied from the circulation. The polyglutamylation by FPGS improves both retention of folates within the cell and their affinity to one-carbon metabolizing enzymes. Expression of *FPGS* was found to be low in acute lymphoblastic leukemias,[48] and the low expression in normal-appearing mucosa of colorectal carcinoma cases has been associated with poor survival.[49] Common genetic variants of FPGS, although not studied to date, may convey functional attributes: our data suggest a detrimental effect associated with its Ex15-263T>C variant located in the 3′ UTR, which may reflect the functional significance of the variant (eg, stability of mRNA[50]) or its linkage with a true functional variant.[51] The risk associated with the variant was moderate in the group consuming high vitamin B6, yielding the protective association of vitamin B6 only among the homozygous variant genotype. We currently do not have explanations for the mechanism.

Table 3. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the association between selected genetic polymorphisms involved in one-carbon metabolism and overall and subtype non-Hodgkin lymphoma (NHL) among participants with diet information, the NCI-SEER Case-Control Study, 1998-2000

		NHL cases							
		Overall		Diffuse large B-cell lymphoma		Follicular lymphoma		Small lymphocytic lymphoma	
Gene, nucleotide change	No. of controls*; n = 319	No. of cases*; n = 386	OR (95% CI)	No. of cases*; n = 142	OR (95% CI)	No. of cases*; n = 98	OR (95% CI)	No. of cases*; n = 48	OR (95% CI)
***BHMT* Ex8+453A>T**									
AA	122	160	1.0 (reference)	46	1.0 (reference)	46	1.0 (reference)	23	1.0 (reference)
AT	99	98	0.76 (0.53-1.10)	30	0.83 (0.49-1.43)	28	0.76 (0.44-1.33)	13	0.65 (0.31-1.35)
TT	19	13	0.54 (0.25-1.14)	5	0.74 (0.26-2.13)	2	—	3	—
AT/TT	118	111	0.73 (0.51-1.04)	35	0.82 (0.49-1.37)	30	0.68 (0.40-1.17)	16	0.67 (0.34-1.33)
P trend			.05		.43		.08		.34
***CBS* Ex13+41C>T**									
CC	142	149	1.0 (reference)	56	1.0 (reference)	36	1.0 (reference)	16	1.0 (reference)
CT	136	165	1.15 (0.83-1.59)	58	1.08 (0.70-1.68)	51	1.57 (0.96-2.58)	17	1.14 (0.55-2.35)
TT	33	59	1.68 (1.03-2.73)	22	1.59 (0.85-2.98)	8	1.01 (0.42-2.40)	12	3.32 (1.43-7.71)
CT/TT	169	224	1.25 (0.92-1.70)	80	1.18 (0.78-1.79)	59	1.46 (0.91-2.36)	29	1.56 (0.81-3.01)
P trend			.05		.21		.35		.02
***FPGS* Ex15-263T>C**									
TT	108	126	1.0 (reference)	47	1.0 (reference)	30	1.0 (reference)	12	1.0 (reference)
CT	160	171	0.91 (0.65-1.28)	57	0.81 (0.51-1.28)	45	1.04 (0.61-1.77)	24	1.35 (0.65-2.83)
CC	44	79	1.58 (1.00-2.49)	31	1.64 (0.91-2.94)	21	1.93 (0.98-3.80)	11	2.47 (1.00-6.15)
CT/CC	204	250	1.06 (0.77-1.45)	88	0.98 (0.64-1.50)	66	1.22 (0.74-2.01)	35	1.57 (0.78-3.16)
P trend			.12		.23		.10		.06
***FTHFD* Ex10-40T>G**									
GG	224	284	1.0 (reference)	101	1.0 (reference)	72	1.0 (reference)	34	1.0 (reference)
GT	76	85	0.90 (0.63-1.29)	31	0.92 (0.57-1.50)	23	0.93 (0.54-1.60)	11	0.95 (0.46-1.97)
TT	10	9	0.67 (0.26-1.68)	5	1.08 (0.36-3.26)	2	—	2	—
GT/TT	86	94	0.87 (0.62-1.23)	36	0.94 (0.59-1.49)	25	0.90 (0.53-1.52)	13	1.00 (0.50-1.99)
P trend			.35		.87		.60		.88
***MTHFR* Ex8-62A>C (1298A>C)**									
AA	135	183	1.0 (reference)	66	1.0 (reference)	48	1.0 (reference)	24	1.0 (reference)
AC	141	165	0.86 (0.63-1.19)	61	0.90 (0.59-1.39)	40	0.79 (0.48-1.29)	20	0.79 (0.42-1.51)
CC	38	35	0.67 (0.40-1.13)	12	0.63 (0.31-1.30)	10	0.72 (0.33-1.58)	4	—
AC/CC	179	200	0.82 (0.61-1.11)	73	0.85 (0.56-1.27)	50	0.78 (0.49-1.23)	24	0.75 (0.41-1.39)
P trend			.12		.25		.29		.31
***MTHFS* IVS2-1411T>G**									
TT	80	99	1.0 (reference)	35	1.0 (reference)	21	1.0 (reference)	16	1.0 (reference)
GT	108	135	0.99 (0.67-1.46)	37	0.80 (0.46-1.40)	43	1.55 (0.84-2.85)	17	0.79 (0.37-1.66)
GG	42	32	0.58 (0.34-1.02)	11	0.57 (0.26-1.24)	9	0.78 (0.33-1.88)	5	0.58 (0.20-1.71)
GT/GG	150	167	0.87 (0.60-1.27)	48	0.73 (0.44-1.24)	52	1.33 (0.74-2.38)	22	0.73 (0.36-1.47)
P trend			.11		.15		.95		.30
***MTR* Ex26-20A>G**									
AA	169	186	1.0 (reference)	58	1.0 (reference)	53	1.0 (reference)	26	1.0 (reference)
AG	62	79	1.15 (0.77-1.71)	23	1.09 (0.61-1.93)	21	1.01 (0.56-1.84)	12	1.18 (0.56-2.48)
GG	10	7	0.65 (0.24-1.76)	2	—	2	—	1	—
AG/GG	72	86	1.08 (0.74-1.58)	25	1.02 (0.59-1.77)	23	0.96 (0.54-1.71)	13	1.10 (0.53-2.27)
P trend			.98		.85		.76		.97
***MTRR* Ex5+136C>T**									
TT	230	278	1.0 (reference)	101	1.0 (reference)	69	1.0 (reference)	36	1.0 (reference)
CT	70	91	1.03 (0.72-1.48)	35	1.05 (0.65-1.69)	26	1.19 (0.69-2.03)	10	0.90 (0.42-1.93)
CC	8	11	1.19 (0.47-3.02)	2	—	2	—	2	—
CT/CC	78	102	1.04 (0.74-1.48)	37	0.99 (0.63-1.58)	28	1.15 (0.69-1.94)	12	0.99 (0.48-2.01)
P trend			.75		.79		.69		.83
***SHMT1* Ex12+138C>T**									
CC	127	125	1.0 (reference)	37	1.0 (reference)	30	1.0 (reference)	21	1.0 (reference)
CT	87	128	1.53 (1.05-2.22)	40	1.59 (0.94-2.71)	40	1.86 (1.07-3.24)	16	1.11 (0.54-2.26)
TT	26	17	0.66 (0.34-1.29)	5	0.70 (0.25-1.96)	3	—	3	—
CT/TT	113	145	1.33 (0.93-1.89)	45	1.39 (0.83-2.32)	43	1.54 (0.90-2.65)	19	0.99 (0.51-1.95)
P trend			.68		.65		.66		.71

Table 3. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the association between selected genetic polymorphisms involved in one-carbon metabolism and overall and subtype non-Hodgkin lymphoma (NHL) among participants with diet information, the NCI-SEER Case-Control Study, 1998-2000 (continued)

		NHL cases							
		Overall		Diffuse large B-cell lymphoma		Follicular lymphoma		Small lymphocytic lymphoma	
Gene, nucleotide change	No. of controls*; n = 319	No. of cases*; n = 386	OR (95% CI)	No. of cases*; n = 142	OR (95% CI)	No. of cases*; n = 98	OR (95% CI)	No. of cases*; n = 48	OR (95% CI)
***SHMT1* Ex12+236C>T**									
CC	121	122	1.0 (reference)	37	1.0 (reference)	31	1.0 (reference)	20	1.0 (reference)
CT	93	134	1.46 (1.01-2.12)	41	1.46 (0.86-2.47)	42	1.68 (0.97-2.90)	17	1.10 (0.54-2.25)
TT	26	17	0.64 (0.33-1.25)	5	0.66 (0.23-1.86)	3	—	3	—
CT/TT	119	151	1.28 (0.90-1.82)	46	1.28 (0.77-2.13)	45	1.40 (0.82-2.39)	20	0.99 (0.51-1.95)
P trend			.81		.83		.89		.70

We omitted estimates based on case counts less than 5 (indicated by —).

Unconditional logistic regression model adjusted for age at study entry (continuous), sex, and race (white, non–African American others).

*Counts of controls and overall/subtype cases vary across polymorphisms due to (1) 2-step genotyping strategy (genotyping was not completed for certain polymorphisms [Table 1] in participants who provided only buccal cell samples [~25%] if genotype information from blood-based DNA samples did not yield significant or suggestive associations with overall NHL) and (2) error in genotyping or insufficient DNA (2%-4% in general).

MTHFR is the most studied enzyme in one-carbon metabolism that irreversibly "commits" 5,10-methylenetetrahydrofolate, a common substrate to both methylation pathways and nucleotide synthesis, toward the methylation cycle and away from nucleotide/DNA synthesis.[52] Its 2 common variants, Ex8-62A>C (also known as 1298A>C) and Ex5+79C>T (or 677C>T), are in linkage disequilibrium,[53] have shown diminished enzyme activity compared with the wild types,[34,35] and have been associated with a lower risk of leukemia.[21] The protective association may be due to preferential DNA synthesis with these *MTHFR* polymorphisms than with wild types, especially when one-carbon nutrients are sufficient, which was clearly demonstrated in a recent in vivo

Table 4. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the association between nutritional factors of one-carbon metabolism and non-Hodgkin lymphoma, stratified by selected genetic polymorphisms, the NCI-SEER Study, 1998-2000

	Dietary intake levels of nutrients			
	Low, less than median	High, median or higher		
Nutritional factor, genotype	No. of cases/controls*	No. of cases/controls*	OR (95% CI)	*P* interaction
Vitamin B6				
Combined genotypes	237/162	149/157	0.70 (0.51-0.95)	N/A
FPGS Ex15-263T>C				
TT	69/59	57/49	1.03 (0.61-1.74)	
CT	104/81	67/79	0.71 (0.46-1.12)	
CC	59/17	20/27	0.22 (0.10-0.52)	.002
MTHFS IVS2-1411T>G				
TT/TG	149/89	85/99	0.54 (0.36-0.81)	
GG	18/26	14/16	1.68 (0.61-4.65)	.06
MTR Ex26-20A>G				
AA	117/78	69/91	0.55 (0.35-0.86)	
AG/GG	50/44	36/28	1.20 (0.62-2.33)	.05
Methionine				
Combined genotypes	225/164	161/155	0.82 (0.60-1.11)	N/A
FTHFD Ex10-40G>T				
GG	179/112	105/112	0.63 (0.44-0.91)	
GT/TT	43/44	51/42	1.34 (0.74-2.46)	.04
MTHFR Ex8-62A>C (1298A>C)				
AA	104/77	79/58	1.04 (0.66-1.63)	
AC	92/72	73/69	0.93 (0.58-1.49)	
CC	27/11	8/27	0.13 (0.04-0.39)	.007
MTRR Ex5+136T>C				
TT	165/108	113/122	0.67 (0.47-0.97)	
CT/CC	57/48	45/30	1.28 (0.69-2.35)	.05

Unconditional logistic regression model adjusted for age at study entry (continuous), sex, race (white, non-African American others), and mutually for dietary intake of methionine and vitamin B6. Median dietary intake is defined as 0.97 mg/1000 kcal for vitamin B6 and 0.97 g/1000 kcal for methionine.

For low dietary intakes of both vitamin B6 and methionine, all ORs were 1.0 (reference).

*Counts of cases and controls vary across polymorphisms due to (1) two-step genotyping strategy (genotyping was not completed for certain polymorphisms [Table 1] in participants who provided only buccal cell samples [~25%] if genotype information from blood-based DNA samples did not yield significant or suggestive associations with overall NHL) and (2) error in genotyping or insufficient DNA (2%-4% in general).

experiment.[52] Such promotion of DNA synthesis over methylation with adequate one-carbon supply may be critical to prevent DNA aberrations and carcinogenesis in lymphoid cells with high rate of turnover. Our study is the first to show an interaction between a variant in *MTHFR* and one-carbon nutrients, specifically methionine, in relation to NHL. This finding emulates the previous epidemiologic reports of such interactions in colorectal cancer.[24,54-56]

Markedly different incidence patterns[57] as well as pathologic and clinical heterogeneity among NHL subtypes[58] support subtype-specific investigations of etiologic factors. At the same time, some risk factors, including family history and occupational/environmental factors, have shown fairly consistent positive or inverse associations across histologic subtypes.[58] For example, in large pooled analyses from the InterLymph, a genetic polymorphism in tumor necrosis factor was associated with DLBCL, but not follicular lymphoma.[30] Further, we previously reported a protective association of dietary folate with DLBCL, but not follicular lymphoma.[27] However, dietary methionine was inversely associated with main subtypes similarly.[27] In the current study, we explored the associations with all NHL as well as by main subtypes and found the main effect of one-carbon–related genetic polymorphisms to be mostly consistent across subtypes.

We did not detect a significant main effect of the variants in *MTHFR, MTRR,* or *TYMS*[12,14-16,18,22] and found approximately 50% lower risk associated with *MTR* Ex26-20A>G (Table S2),[14,16,20] which is consistent with previous findings. The moderate inverse association of the variants in *MTHFR* or *MTR* with NHL appeared contingent on methionine or vitamin B6 intake, respectively, in our data. Our investigation of additional enzymes that had not been explored led to a discovery of moderate associations regarding *BHMT, CBS,* and *FPGS* (and *FTHFD* and *MTHFS* in Table S2), of which *FPGS, FTHFD,* and *MTHFS* showed an interaction with nutritional status. These findings for novel main effects and interactions are intriguing and warrant replications, especially in pooled analyses in order to reduce the possibility of chance findings based on small numbers.

For a number of cancers that one-carbon metabolism has been linked to, gene-nutrient interactions are considered to be an integral part of the epidemiologic investigation. Folate has been reported to compensate the reduced enzymatic activity of MTHFR with the Ex5+79C>T (or 677C>T) variant by changing the conformation and structure of the enzyme.[59] However, we did not observe any independent interaction of folate when simultaneously adjusted for vitamin B6. The lack of an independent interaction may be due to the correlation between dietary folate and vitamin B6 from common food sources ($r = 0.65$ in our data) or due to improved folate status since fortification above the range where one-carbon metabolism relies on folate dose-dependently: the manifestation of genetic susceptibility may rather vary by the overall availability of one-carbon moieties/nutrients.[60] For example, the protective association between *MTHFR* variants and childhood acute lymphoblastic leukemia was apparent only among children who were born *before* the prenatal folate supplementation was widely put into practice in Canada.[61]

This study had a number of strengths. The population-based design of this case-control study might have reduced selection bias compared with hospital-based recruitment. The median consumption of vitamin B6 (1.9 mg/2000 kcal) and methionine (1.9 g/2000 kcal) among our control individuals was largely comparable with the Recommended Dietary Allowances and the median intake levels of the US population.[4,62] We used histologically confirmed cases, reducing misclassification bias. We incorporated a detailed paradigm of one-carbon metabolism compared with previous studies, which is an important consideration for studying a composite effect of a number of genes involved in complex metabolic pathways.

On the other hand, our findings of gene-nutrient interactions have similar limitations that were discussed in our previous study of dietary associations.[27] We had assessed whether the low response rates of our study could have driven the inverse associations between one-carbon nutrients and NHL and did not find a strong indication for such selection bias, based on similar associations across sex and study centers with different response rates or by education levels (a correlate of participation among controls), and also based on no confounding by these factors. Our findings based on postdiagnostic assessment of diet need to be replicated in prospective data with less influence of potential bias associated with recalled diet and selective participation of healthier controls. Although this is one of the largest polymorphism studies to date regarding one-carbon metabolism, it was still limited to examine gene-nutrient interactions.

Our strategy of targeting few SNPs per gene based on literature evidence and limited variation data available at the time of genotyping might have missed important variants for NHL risk that are not in linkage disequilibrium with the ones we studied. This strategy also limited our ability to construct and perform a meaningful haplotype analysis. Similarly, future work could expand to nutritional hypotheses in the context of other genes involved in one-carbon metabolism, including *DHFR,*[63] *FOLR2,*[6] and *MTHFD1*[64] (Figure 1).

In conclusion, this study provides further evidence that the one-carbon pathway could contribute to lymphomagenesis, specifically through interactions between common genetic variation and dietary intake. However, these results require replication in further large studies as well as pooled analyses. As the understanding of gene-nutrient interactions in one-carbon metabolism advances in relation to lymphomagenesis, our findings could be incorporated into evaluation of nutritional intervention trials and development of suitable biomarkers for detection of high-risk individuals (eg, CpG island methylation phenotype[65]). Lastly, this line of investigation could also have implications for prognosis and survival of NHL.

Acknowledgment

This work was supported by the Intramural Research Program of the NIH at the NCI and by contracts with the NCI (N01-PC-67010, N01-PC-67008, N02-PC-71105, N01-PC-67009, and N01-PC-65064).

We thank our study participants for their involvement. We gratefully acknowledge the contributions of the SEER centers of Los Angeles, Iowa, Seattle, and Detroit for rapid identification of cases; the Centers for Medicare & Medicaid Services for selection of older controls; Drs Meredith Yeager and Robert Welch at the Core Genotyping Facility at the Advanced Technology Center for carrying out genotyping and providing bioinformatics support; Dr Nilanjan Chatterjee for input on statistical analyses; Drs Min Shen and Sonja Berndt for assistance with genotype and haplotype analyses; Carol Haines (Westat) for development of study materials and procedures, for selection of younger controls, and for study coordination; Steve Palladino and Peter Hui (IMS) for computer support; Carla Chorley (BBI

Biotech Research Laboratories) for specimen handling; and Geoffrey Tobias for research assistance.

Authorship

Contribution: The principal investigator of the NCI-SEER NHL Study is P.H.; questionnaire and cancer outcome data from the 4 NCI-SEER registries were obtained and provided by W.C., L.E.K., S.D., and M.S.; the one-carbon metabolism polymorphism project was conceived and led by Q.L., N.R., and U.L.; bioinformatics support and genotyping were supervised by S.C. at the NCI Core Genotyping Facility; the statistical analysis was performed by U.L. and Q.L. with input from S.S.W., P.H., and N.R.; the paper was drafted and revised by U.L., S.S.W., P.H., L.E.K., A.B., N.R., and Q.L.; and all authors reviewed and approved the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Unhee Lim, Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd, EPS 320, Rockville, MD 20852; e-mail: limu@mail.nih.gov.

Appendix

Abbreviations of enzymes or proteins (genes) are as follows: BHMT *(BHMT)* indicates betaine-homocysteine methyltransferase; CBS *(CBS),* cystathionine-beta-synthase; DHFR *(DHFR),* dihydrofolate reductase; FOLR2 *(FOLR2),* folate receptor 2 (or folate receptor beta); FPGS *(FPGS),* folylpolyglutamate synthase; FTHFD *(FTHFD),* 10-formyltetrahydrofolate dehydrogenase (or aldehyde dehydrogenase 1 family, member L1; ALDH1L1); GGH *(GGH),* gamma-glutamyl hydrolase; MBD2 *(MBD2),* methyl-CpG binding domain protein 2; MGMT *(MGMT),* O-6-methylguanine-DNA methyltransferase; MLH1 *(MLH1),* mutL homolog 1; MSH2 *(MSH2),* mutS homolog 2; MTHFD1 *(MTHFD1),* (cytoplasmic) 5,10-methylenetetrahydrofolate dehydrogenase 1; MTHFD2 *(MTHFD2),* (mitochondrial) 5,10-methylenetetrahydrofolate dehydrogenase 2; MTHFR *(MTHFR),* 5,10-methylenetetrahydrofolate reductase; MTHFS *(MTHFS),* 5,10-methenyltetrahydrofolate synthetase (or 5-formyltetrahydrofolate cycloligase); MTR *(MTR),* 5-methyltetrahydrofolate-homocysteine methyltransferase (or methionine synthase); MTRR *(MTRR),* 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (or methionine synthase reductase); SHMT1 *(SHMT1),* (cytoplasmic) serine hydroxymethyltransferase 1; SLC19A1 *(SLC19A1),* solute carrier family 19 (folate transporter), member 1 (or reduced folate carrier 1); TCN1 *(TCN1),* transcobalamin 1; and TYMS *(TYMS),* thymidylate synthetase.

References

1. Shane B. Folic Acid, Vitamin B12, and Vitamin B6. In: Stipanuk MH, ed. Biochemical and physiological aspects of human nutrition. Philadelphia, PA: W. B. Saunders Company; 2000:453-518.
2. Choi SW, Mason JB. Folate and carcinogenesis: an integrated scheme. J Nutr. 2000;130:129-132.
3. Folate in health and disease. New York, NY: Marcel Dekker; 1995.
4. Institute of Medicine. Dietary Reference Intakes: for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington, DC: National Academy Press; 2000.
5. Herbig K, Chiang EP, Lee LR, Hills J, Shane B, Stover PJ. Cytoplasmic serine hydroxymethyltransferase mediates competition between folate-dependent deoxyribonucleotide and S-adenosylmethionine biosyntheses. J Biol Chem. 2002;277:38381-38389.
6. Kelemen LE. The role of folate receptor alpha in cancer development, progression and treatment: cause, consequence or innocent bystander? Int J Cancer. 2006;119:243-250.
7. Kono S, Chen K. Genetic polymorphisms of methylenetetrahydrofolate reductase and colorectal cancer and adenoma. Cancer Sci. 2005;96:535-542.
8. Zintzaras E. Methylenetetrahydrofolate reductase gene and susceptibility to breast cancer: a meta-analysis. Clin Genet. 2006;69:327-336.
9. Zintzaras E. Association of methylenetetrahydrofolate reductase (MTHFR) polymorphisms with genetic susceptibility to gastric cancer: a meta-analysis. J Hum Genet. 2006;51:618-624.
10. Gaidano G, Pastore C, Volpe G. Molecular pathogenesis of non-Hodgkin lymphoma: a clinical perspective. Haematologica. 1995;80:454-472.
11. Esteller M. Profiling aberrant DNA methylation in hematologic neoplasms: a view from the tip of the iceberg. Clin Immunol. 2003;109:80-88.
12. 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.
13. Hishida A, Matsuo K, Hamajima N, et al. Associations between polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and susceptibility to malignant lymphoma. Haematologica. 2003;88:159-166.
14. Lincz LF, Scorgie FE, Kerridge I, Potts R, Spencer A, Enno A. Methionine synthase genetic polymorphism MS A2756G alters susceptibility to follicular but not diffuse large B-cell non-Hodgkin's lymphoma or multiple myeloma. Br J Haematol. 2003;120:1051-1054.
15. 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. Cancer Epidemiol Biomarkers Prev. 2004;13:787-794.
16. Linnebank M, Schmidt S, Kolsch H, et al. The methionine synthase polymorphism D919G alters susceptibility to primary central nervous system lymphoma. Br J Cancer. 2004;90:1969-1971.
17. Matsuo K, Hamajima N, Suzuki R, et al. Methylenetetrahydrofolate reductase gene (MTHFR) polymorphisms and reduced risk of malignant lymphoma. Am J Hematol. 2004;77:351-357.
18. 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.
19. Lightfoot TJ, Skibola CF, Willett EV, et al. Risk of non-Hodgkin lymphoma associated with polymorphisms in folate-metabolizing genes. Cancer Epidemiol Biomarkers Prev. 2005;14:2999-3003.
20. Niclot S, Pruvot Q, Besson C, et al. Implication of the folate-methionine metabolism pathways in susceptibility to follicular lymphomas. Prepublished on January 12, 2006, as DOI 10.1182/blood-2005-04-1567. (Now available as Blood. 2006;108:278-285.)
21. Skibola CF, Smith MT, Kane E, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc Natl Acad Sci U S A. 1999;96:12810-12815.
22. Skibola CF, Smith MT, Hubbard A, et al. Polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and risk of adult acute lymphocytic leukemia. Blood. 2002;99:3786-3791.
23. Robien K, Ulrich CM. 5,10-Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: a HuGE minireview. Am J Epidemiol. 2003;157:571-582.
24. Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. Am J Epidemiol. 2004;159:423-443.
25. Powers HJ. Interaction among folate, riboflavin, genotype, and cancer, with reference to colorectal and cervical cancer. J Nutr. 2005;135:2960S-2966S.
26. Chen J, Gammon MD, Chan W, et al. One-carbon metabolism, MTHFR polymorphisms, and risk of breast cancer. Cancer Res. 2005;65:1606-1614.
27. Lim U, Schenk M, Kelemen LE, et al. Dietary determinants of one-carbon metabolism and the risk of Non-Hodgkin's lymphoma: NCI-SEER Case-Control Study, 1998-2000. Am J Epidemiol. 2005;162:953-964.
28. Hartge P, Colt JS, Severson RK, et al. Residential herbicide use and risk of non-Hodgkin lymphoma. Cancer Epidemiol Biomarkers Prev. 2005;14:934-937.
29. Percy C, Van Holten V, Muir C, eds. International Classification of Diseases-Oncology. 2nd ed. Geneva, Switzerland: World Health Organization; 1990.
30. Rothman N, Skibola CF, Wang SS, et al. Genetic variation in TNF and IL10 and risk of non-Hodgkin lymphoma: a report from the InterLymph Consortium. Lancet Oncol. 2006;7:27-38.
31. Clarke CA, Undurraga DM, Harasty PJ, Glaser SL, Morton LM, Holly EA. Changes in cancer registry coding for lymphoma subtypes: reliability over time and relevance for surveillance and study. Cancer Epidemiol Biomarkers Prev. 2006;15:630-638.
32. Wang SS, Davis S, Cerhan JR, et al. Polymorphisms in oxidative stress genes and risk for non-Hodgkin lymphoma. Carcinogenesis. 2006;27:1828-1834.
33. Hill DA, Wang SS, Cerhan JR, et al. Risk of Non-Hodgkin lymphoma (NHL) in relation to germline variation in DNA repair and related genes. Prepublished on July 20, 2006, as DOI 10.1182/blood-2005-01-026690. (Now available as Blood. 2006;108:3161-3167.)

34. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995;10:111-113.
35. van der Put NM, Gabreels F, Stevens EM, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet. 1998;62:1044-1051.
36. Kawakami K, Salonga D, Park JM, et al. Different lengths of a polymorphic repeat sequence in the thymidylate synthase gene affect translational efficiency but not its gene expression. Clin Cancer Res. 2001;7:4096-4101.
37. Lim U, Peng K, Shane B, et al. Polymorphisms in cytoplasmic serine hydroxymethyltransferase and methylenetetrahydrofolate reductase affect the risk of cardiovascular disease in men. J Nutr. 2005;135:1989-1994.
38. Garcia-Closas M, Egan KM, Abruzzo J, et al. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiol Biomarkers Prev. 2001; 10:687-696.
39. Packer BR, Yeager M, Burdett L, et al. SNP500-Cancer: a public resource for sequence validation, assay development, and frequency analysis for genetic variation in candidate genes. Nucleic Acids Res. 2006;34:D617-D621.
40. Bhatti P, Sigurdson AJ, Wang SS, et al. Genetic variation and willingness to participate in epidemiologic research: data from three studies. Cancer Epidemiol Biomarkers Prev. 2005;14:2449-2453.
41. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. Am J Epidemiol. 1986;124:453-469.
42. Block G, Woods M, Potosky A, Clifford C. Validation of a self-administered diet history questionnaire using multiple diet records. J Clin Epidemiol. 1990;43:1327-1335.
43. Willett WC. Nutritional Epidemiology. 2nd ed. New York, NY: Oxford University Press; 1998.
44. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. Behav Brain Res. 2001;125: 279-284.
45. Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. J Natl Cancer Inst. 2004;96:434-442.
46. Lowe KE, Osborne CB, Lin BF, Kim JS, Hsu JC, Shane B. Regulation of folate and one-carbon metabolism in mammalian cells, II: effect of folylpoly-gamma-glutamate synthetase substrate specificity and level on folate metabolism and folylpoly-gamma-glutamate specificity of metabolic cycles of one-carbon metabolism. J Biol Chem. 1993;268:21665-21673.
47. Osborne CB, Lowe KE, Shane B. Regulation of folate and one-carbon metabolism in mammalian cells, I: folate metabolism in Chinese hamster ovary cells expressing Escherichia coli or human folylpoly-gamma-glutamate synthetase activity. J Biol Chem. 1993;268:21657-21664.
48. Kager L, Cheok M, Yang W, et al. Folate pathway gene expression differs in subtypes of acute lymphoblastic leukemia and influences methotrexate pharmacodynamics. J Clin Invest. 2005;115:110-117.
49. Odin E, Wettergren Y, Nilsson S, et al. Altered gene expression of folate enzymes in adjacent mucosa is associated with outcome of colorectal cancer patients. Clin Cancer Res. 2003;9:6012-6019.
50. Wang J, Pitarque M, Ingelman-Sundberg M. 3'-UTR polymorphism in the human CYP2A6 gene affects mRNA stability and enzyme expression. Biochem Biophys Res Commun. 2006;340:491-497.
51. Smith CA, Cross JA, Bognar AL, Sun X. Mutation of Gly51 to serine in the P-loop of Lactobacillus casei folylpolyglutamate synthetase abolishes activity by altering the conformation of two adjacent loops. Acta Crystallogr D Biol Crystallogr. 2006;62:548-558.
52. Quinlivan EP, Davis SR, Shelnutt KP, et al. Methylenetetrahydrofolate reductase 677C-T polymorphism and folate status affect one-carbon incorporation into human DNA deoxynucleosides. J Nutr. 2005;135:389-396.
53. Chen J, Ma J, Stampfer MJ, Palomeque C, Selhub J, Hunter DJ. Linkage disequilibrium between the 677CT and 1298AC polymorphisms in human methylenetetrahydrofolate reductase gene and their contributions to risk of colorectal cancer. Pharmacogenetics. 2002;12:339-342.
54. Chen J, Giovannucci E, Kelsey K, et al. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. Cancer Res. 1996; 56:4862-4864.
55. Su LJ, Arab L. Nutritional status of folate and colon cancer risk: evidence from NHANES I epidemiologic follow-up study. Ann Epidemiol. 2001;11: 65-72.
56. Curtin K, Bigler J, Slattery ML, Caan B, Potter JD, Ulrich CM. MTHFR C677T and A1298C polymorphisms: diet, estrogen, and risk of colon cancer. Cancer Epidemiol Biomarkers Prev. 2004;13:285-292.
57. Morton LM, Wang SS, Devesa SS, Hartge P, Weisenburger DD, Linet MS. Lymphoma incidence patterns by WHO subtype in the United States, 1992-2001. Blood. 2006;107:265-276.
58. Hartge P, Wang SS, Bracci PM, Devesa SS, Holly EA. Non-Hodgkin lymphoma. In: Schottenfeld D, Fraumeni JF Jr, eds. Cancer Epidemiology and Prevention. New York, NY: Oxford University Press; 2006:898-918.
59. Guenther BD, Sheppard CA, Tran P, Rozen R, Matthews RG, Ludwig ML. The structure and properties of methylenetetrahydrofolate reductase from Escherichia coli suggest how folate ameliorates human hyperhomocysteinemia. Nat Struct Biol. 1999;6:359-365.
60. Bailey LB. Folate, methyl-related nutrients, alcohol, and the MTHFR 677C→T polymorphism affect cancer risk: intake recommendations. J Nutr. 2003;133:3748S-3753S.
61. Krajinovic M, Lamothe S, Labuda D, et al. Role of MTHFR genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia. Blood. 2004;103:252-257.
62. Institute of Medicine. Dietary Reference Intakes: for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients). Washington, DC: National Acedemy Press; 2005.
63. Goto Y, Yue L, Yokoi A, et al. A novel single-nucleotide polymorphism in the 3'-untranslated region of the human dihydrofolate reductase gene with enhanced expression. Clin Cancer Res. 2001;7: 1952-1956.
64. De Marco P, Merello E, Calevo MG, et al. Evaluation of a methylenetetrahydrofolate-dehydrogenase 1958G>A polymorphism for neural tube defect risk. J Hum Genet. 2006;51:98-103.
65. Chiusolo P, Farina G, Putzulu R, et al. Analysis of MTHFR polymorphisms and P16 methylation and their correlation with clinical-biological features of multiple myeloma. Ann Hematol. 2006;85:474-477.