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Role of One-carbon Metabolizing Pathway Genes and Gene-Nutrient Interaction in the Risk of Non-Hodgkin Lymphoma

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Abstract

Purpose—Genetic polymorphisms in one-carbon metabolizing pathway genes have been associated with risk of malignant lymphoma. However, the results have been inconsistent. The objectives of this study were to examine the potential relationship between gene-nutrient interactions and the risk of non-Hodgkin lymphoma (NHL).

Methods—We examined 25 polymorphisms in 16 one-carbon metabolism genes for their main effect and gene-nutrient interactions in relation to NHL risk among 518 incident cases and 597 population-based controls of Connecticut women enrolled between 1996 and 2000.

Results—A significantly reduced risk of NHL was associated with the homozygous TT genotype in *CBS* (rs234706, Ex9+33C>T) (OR = 0.51, 95%CI, 0.31–0.84), the homozygous CC genotype in *MBD2* (rs603097, −2176C>T) (OR = 0.37, 95%CI, 0.17–0.79), the heterozygote AG genotype in *FTHFD* (rs1127717, Ex21+31A>G) (OR = 0.73, 95%CI, 0.55–0.98), and a borderline significantly reduced risk of NHL was observed for the homozygous CC genotype in *MTRR* (rs161870, Ex5+136T>C) (OR = 0.23, 95%CI, 0.05–1.04). The reduced risk of NHL associated with these genotypes was predominately in those with higher dietary vitamin B6 and methionine intakes, as well as with higher dietary folate intake although results were less stable. A borderline significantly increased risk of NHL was also observed for *CBS* (rs1801181, Ex13+41C>T), *FTHFD* (rs2305230, Ex10-40G>T), *SHMT1* (rs1979277, Ex12+138C>T), and *SHMT1* (rs1979276, Ex12+236T>C), and these associations appeared to be contingent on dietary nutrient intakes.

Conclusion—Our results suggest that variation in several one-carbon metabolizing pathway genes may influence the risk of NHL through gene-nutrient interactions involving dietary nutrient intakes.

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Keywords

dietary nutrients; folate; one-carbon metabolizing genes; non-Hodgkin lymphoma; cancer

Introduction

Nutrients involved in one-carbon metabolism include folate, vitamin B_2, B_6, B_{12} (as enzymatic cofactors), and methionine (as alternative suppliers of one-carbon units). Studies have showed that deficiencies in one-carbon metabolizing nutrients can lead to impaired immune responses (1), and various immune deficiencies are well-established risk factors for non-Hodgkin lymphoma (NHL) (2, 3). One-carbon metabolism is directly involved in DNA synthesis and methylation (4–6), and altered DNA synthesis and methylation have been linked to human cancer risk, including lymphoma (7, 8).

One-carbon metabolism is regulated by one-carbon metabolizing pathway genes. Thus, it is reasonable to speculate that genetic polymorphisms in this pathway may alter both the risk of NHL and the relationship between nutrients related to one-carbon metabolism and NHL risk (9). Indeed, genetic polymorphisms in these genes have been associated with risk of malignant lymphoma in recent studies, although the results are inconsistent (10–21). For example, a reduced risk of NHL has been reported in several studies for *MTHFR Ex5+79*C>T (commonly known as 677C>T) (19–21), *MTR Ex26-20*A>G (2756A>G) (11–13), *TYMS* variants of *VNTR* Ex1+52-28base (3R versus 2R) (13–16) and *SHMT1 Ex12+138*C>T and 1420C>T (14, 15). On the other hand, an increased risk of NHL was found to be associated with *CBS Ex13+41*C>T, *FPGS Ex15-263*T>C, *SHMT1 Ex12+138*C>T, and *Ex12+236*C>T genotypes (10).

In addition to these main effects, a population-based case-control study of 386 NHL cases and 319 controls conducted by the U.S. National Cancer Institute (NCI) reported a protective association between NHL and high dietary vitamin B_6 intake, but the association was limited to those with the *FPGS Ex15-263*T>C CC, *MTHFS IVS2-1411*T>G TT/TG, and *MTR Ex26-20*A>G AA genotypes (10). Similarly, the protective association for NHL associated with methionine intake was apparent only in people with the *FTHFD Ex10-40*G>T GG, *MTHFR Ex8–62*A>C CC, and *MTRR Ex5+136*T>C TT genotypes.

These results linking one-carbon metabolizing gene-nutrient interactions to NHL risk, if replicated in studies from other populations, would provide further clarification regarding the relationship between dietary nutrient intakes, genes, and risk of NHL, and may explain at least in part the previous inconsistent findings concerning nutrient intakes and NHL risk. For this reason, we have used the same laboratory at the NCI Core Genotyping Facility for genotyping one-carbon metabolizing genes as in the study by Lim et al. (10), using the blood samples from a population-based case-control study of NHL in Connecticut, USA. We have previously reported in our population-based case-control study of Connecticut women that dietary intake of nutrients related to one-carbon metabolism, including folate, methionine, and vitamin B_6, may be related to the risk of NHL, and similar findings have been observed in other studies (22–25). The availability of data on both dietary nutrient intakes and one-carbon metabolizing genes provides a unique opportunity to further examine the potential relationship between gene-nutrient interactions and the risk of NHL.

Materials and methods

Study Population

The study population has been previously described elsewhere (26–28). Briefly, all histologically-confirmed incident cases of NHL (ICD-O, M-9590–9642, 9690–9701, 9740–9750) from Connecticut between 1996 and 2000 were identified through the Yale Cancer Center's Rapid Case Ascertainment Shared Resource (RCA). Cases were Connecticut female residents, aged 21–84 years, alive at the time of interview, and without a previous diagnosis of cancer except for nonmelanoma skin cancer. Of 832 eligible cases, 601 (72%) completed in-person interviews. In order to reduce the misclassification of the disease, pathology slides (or tissue blocks) from all patients were obtained from the original pathology departments. The disease was classified using the World Health Organization NHL classification system through central review by study pathologists. Female population-based controls from Connecticut were recruited by random digit dialing methods for those less than 65 years of age or by random selection from Health Care Financing Administration files for those aged 65 years or older. The participation rate was 69% for those contacted by random digit dialing and 47% for those contacted through health care records. Cases and controls were frequency matched on age (5 years) by adjusting the number of controls randomly selected in each age stratum once every several months during the period of recruitment.

Data Collection

Written informed consent was obtained from all voluntary participants. Those who agreed to participate were interviewed by trained study nurses either at the subject's home or at a convenient location. Subjects were administered a questionnaire requesting information on demographic characteristics, family history of cancer, past medical conditions and medication use, diet, occupation, smoking, and drinking.

Following the interview, subjects provided a peripheral blood sample of about 10 ml. Subjects for whom the blood draw was contraindicated, or who refused to participate in the blood-draw, were offered the option to provide buccal-cell cotton swab samples instead. DNA samples were available from 518 cases (461 from blood and 57 from buccal cells) and from 597 of the population-based controls (535 from blood and 62 from buccal cells) for the gene analyses. The study was approved by Institutional Review Boards at Yale University, the Connecticut Department of Public Health, and the National Cancer Institute.

Dietary Assessment

A semiquantitative food frequency questionnaire developed by the Fred Hutchinson Cancer Research Center (Seattle, Washington) was completed by each subject. Subjects were asked to characterize their usual diets in the year prior to being interviewed. This type of assessment is highly correlated with diet in the more distant past (29). The food frequency questionnaire collected data on consumption frequency and portion size for approximately 120 foods and beverages. After completion, the food frequency questionnaire was sent to the Fred Hutchinson Cancer Research Center for analysis. Average daily nutrient intakes were calculated by using the University of Minnesota Nutrition Coordinating Center's Nutrition Data System for Research database (30). Intake of all nutrients was examined from food alone. Folate values represent pre-enrichment levels as pre-fortification folate levels are most representative of usual adult diets for the study subjects. Information about supplemental vitamin intake was not assessed on the food frequency questionnaire.

Genotyping and Quality Control

As detailed elsewhere (27, 28), genotyping was performed at the National Cancer Institute Core Genotyping Facility (http://cgf.nci.nih.gov). All TaqMan® assays (Applied Biosystems Inc., Foster City, CA) for this study were optimized on the ABI 7900HT detection system with 100% concordance with sequence analysis of 102 individuals as listed on the SNP500Cancer website (http://snp500cancer.nci.nih.gov). We selected 25 SNPs in 16 one-carbon metabolizing pathway genes based on the following criteria: minor allele frequencies greater than 5%, laboratory evidence of functional data from previous reports (15, 18, 31–34), or expected functional consequences in that the polymorphisms result in amino acid changes or are located within the 3' untranslated region, which contains regulatory sequences and binding sites for other molecules that could alter the stability of the mRNA transcript of the gene (10). Duplicate samples from 100 study subjects and 40 replicate samples from each of two blood donors were interspersed throughout each batch for all genotyping assays. The concordance rates for QC samples were 99%–100% for all assays. Quality control data were re-checked and the accuracy of each assay not in Hardy-Weinberg equilibrium (HWE) among controls was confirmed. Evaluation of all SNPs analyzed to date in the study showed that approximately 5% were not consistent with HWE, as expected.

Statistical Analysis

Unconditional logistic regression was used to estimate the gene-NHL associations using odds ratios (ORs) and 95% confidence intervals (CIs), adjusting for age (continuous) and family history of NHL in first degree relatives. Adjustment for alcohol consumption was also evaluated but this variable did not result in material changes in the observed associations, and therefore it was not included in the final model. The most prevalent homozygous genotype was used as the reference group. We assigned ordinal scores (0, 1, 2) to the homozygous wild-type, heterozygote, and homozygous variant genotypes, respectively, to obtain the *P* value for linear trend in the regression analyses. Gene-nutrient interactions were assessed by including ordinal score variables of each genotype and vitamin B6, methionine, and folate intakes (dichotomized at the median based on the nutrient distribution among control individuals: 1.47mg for vitamin B6, 1.36g for methionine, and 215.48 μg for folate) along with their product term in the regression model. The nutrient-NHL association comparing above versus below the median of the nutrient is presented stratified by genotypes.

To account for multiple comparisons, we used the Benjamini-Hochberg method to control for the False Discovery Rate (FDR). The FDR is defined as the expected ratio of erroneous rejections of the null hypothesis to the total number of rejected hypotheses. We applied the FDR method to the *P*-values of the risk for homozygous carriers of the rare vs. common allele, as this provides the greatest potential contrast in effects across genotypes. We considered FDR less than 0.2 as noteworthy. All analyses were conducted using Statistical Analysis Software, version 8.02 (SAS Institute Inc, 1996).

Results

Table 1 presents the descriptive characteristics of the cases and controls. The cases and controls were similar in terms of age, racial distribution, and DNA source for genotyping, while cases had a higher proportion of first degree relatives with NHL or other cancers. Results for analyses which included all study subjects were similar to those limited to non-Hispanic Caucasians (representing 93.2% and 91.6% of all cases and controls, respectively). Therefore, the results presented here include only the 483 cases and 547 controls of non-Hispanic Caucasian ancestry (Supplemental Table 1 presents the results among all subjects and non-Hispanic Caucasians).

Table 2 shows the results for SNPs that were significantly or borderline significantly associated with the risk of NHL (Supplemental Table 1 presents genotype-NHL associations for all analyzed SNPs). The homozygous variant TT genotype in *CBS* (rs234706, Ex9+33C>T) was associated with a significantly reduced risk of NHL (OR=0.51, 95% CI, 0.31–0.84) compared to the CC genotype. A significantly reduced risk of NHL was also observed for the heterozygote (AG versus AA) in *FTHFD* (rs1127717, Ex21+31A>G) (OR = 0.73, 95% CI, 0.54–0.98), and for the homozygous variant genotype (CC versus TT) in *MBD2* (rs603097, −2176C>T) (OR = 0.37, 95% CI, 0.17–0.79).

On the other hand, a significantly increased risk of NHL was observed for the homozygous variant genotype (TT versus CC) in *CBS* (rs1801181, Ex13+41C>T) (OR = 1.50, 95% CI, 1.01–2.23) and the homozygous variant genotype (TT versus GG) in *FTHFD* (rs2305230, Ex10-40G>T) (OR = 2.34, 95% CI, 1.03–5.33; Table 2). Moreover, a borderline significantly increased risk of NHL was seen for the homozygous genotype (TT versus CC) in *SHMT1* (rs1979277, Ex12+138C>T) (OR=1.57, 95% CI, 0.95–2.60) and the homozygous genotype (TT versus CC) in *SHMT1* (rs1979276, Ex12+236T>C) (OR=1.51, 95% CI, 0.95–2.41). FDR estimates for all our findings were above the cut-point value 0.2.

Table 3 presents the results which showed a significant or borderline significant association between one-carbon metabolizing genes and NHL risk stratified by the three nutrients (vitamin B_6, methionine, and folate) that were linked to a reduced risk of NHL in our previous study. Supplemental Table 2 presents the results for gene-nutrient interaction for all SNPs examined. As shown in Table 3, the observed significantly or borderline significantly reduced risk of NHL associated with the *CBS* (*rs*234706, *Ex9+33C>T)*, *FTHFD* (*rs*1127717, *Ex21+31A>G)*, and *MBD2* (*rs*603097, *−2176*C>T) genotypes was primarily apparent for those with dietary vitamin B6 intakes ≥ the median. Conversely, the significantly or borderline significantly increased risk of NHL associated with the *CBS* (rs1801181, Ex13+41C>T), *FTHFD* (rs2305230, Ex10-40G>T), *MBD2* (rs7614, Ex8+438A>G), *MLH1* (rs1799977, Ex8-23A>G), *MLH1* (rs2286940, IVS12-169C>T), *SHMT1* (rs1979277, Ex12+138C>T) and *SHMT1* (rs1979276, Ex12+236T>C) genotypes was primarily limited to those with lower dietary vitamin B_6 intakes. There were significant interactions for two *MLH1* SNPs and vitamin B6 (*P* interaction for rs1799977, Ex8-23A>G: 0.01; *P* interaction for rs2286940, IVS12-169C>T: 0.001).

Table 3 also shows that the observed gene-NHL associations vary by the intake levels of dietary methionine and folate. The reduced risk of NHL associated with the heterozygote genotype in *FTHFD* (rs1127717, Ex21+31A>G), the rarer homozygous (CC) genotype in *MBD2* (rs603097, −2176C>T), and the rarer homozygous (GG) genotype in *MLH1* (rs1799977, Ex8-23A>G) was apparent only in those with a higher dietary intake of methionine. Conversely, the increased risk of NHL associated with the rarer homozygous (TT) and heterozygote (CT) genotypes in *CBS* (*rs*1801181, *Ex13+41*C>T) and *MLH1* (*rs*2286940, IVS12-169C>T), and the rarer homozygous genotype (GG) in *MLH1* (*rs*1799977, *Ex8-23*A>G) and in *MTHFS* (*rs*622506, *IVS2-1411*T>G), was apparent only in those with methionine intakes below the median level. There were significant interactions for the two *MLH1* SNPs and methionine intake (*P* interaction for rs1799977, Ex8-23A>G: 0.01; *P* interaction for rs2286940, IVS12-169C>T: 0.01). While the results for the folate-gene interactions were less stable compared to the results for vitamin B_6 and methionine, the observed patterns were similar (Table 3).

Discussion

The results from this population-based case-control study show that genetic polymorphisms in certain one-carbon metabolizing pathway genes may be associated with the risk of NHL,

and that variation in one-carbon metabolizing pathway genesmay modify the relationship between dietary nutrients related to one-carbon metabolism and NHL risk.

Specifically, in this study we found a significantly reduced risk of NHL for the *CBS* Ex9+33C>T (TT versus CC), the *MBD2* −2176C>T (CC versus TT), and the *FTHFD* Ex21+31A>G (AG versus AA) genotypes. The findings also showed that the reduced risk of NHL associated with these genotypes or their variants were mainly apparent among those who had higher dietary intakes of vitamin B_6, methionine, and folate. One-carbon metabolism related nutrients provide a more stable environment for DNA synthesis and methylation and thereby may reduce the risk of cancer (35). The proteins encoded by these genes and their variants are involved in one-carbon metabolism and DNA synthesis, which plays a role in lymphomagenesis (10). For example, the CBS enzyme has been shown to induce irreversible catalysis of homocysteine to cystathionine and, as a result, overexpression of *CBS* decreases levels of homocysteine inducing functional folate deficiency (36). Thus, genetic variability in the activity of enzymes involved in DNA synthesis and methylation could influence susceptibility to NHL.

The protein encoded by *MBD2* is capable of binding specifically to methylated DNA, and it can also repress transcription from methylated gene promoters (37). Studies suggest that *MBD2* may also function as a mediator of the biological consequences of the methylation signal or function as a demethylase to activate transcription, as DNA methylation causes gene silencing (37, 38). The protein encoded by *FTHFD* catalyzes the conversion of 10-formyltetrahydrofolate to tetrahydrofolate in DNA synthesis (17). The *FTHFD* Ex21+31A>G variant is located in the catalytic carboxyl-terminal domain (39), and might affect enzyme activity through the amino acid change from aspartic acid to glycine. It has been suggested that down-regulation of FTHFD in tumors may increase proliferation of tumor cells (40).

We also found significantly or borderline significantly increased risks of NHL for the *CBS* Ex13+41C>T (TT versus CC), the *FTHFD* Ex10-40G>T (TT versus GG), the *SHMT1* Ex12+138C>T (TT versus CC), and the *SHMT1* Ex12+236T>C (TT versus CC) genotypes. Interestingly, the NCI population-based case-control study (10) reported an elevated risk of NHL associated with *CBS* Ex13+41 (TT versus CC) and *SHMT1* Ex12+138 and Ex12+236 (CT versus CC for both). The study by Lightfoot et al. (16) also reported an increased risk of NHL for the *TYMS* 2R/3R variant, a marginal increased risk for diffuse large B cell lymphoma with the *TYMS* homozygous 6 bp deletion, and for follicular lymphoma with *RFC* 80AA and *TYMS* 28–bp repeat 2R/3R.

Our study suggests that the increased risk of NHL associated with specific genotypes is mainly among those who had lower dietary intakes of vitamin B_6, methionine, and folate. We controlled for the FDR using different significance levels (significant value = 0.05, 0.10, 0.15, and 0.20) as a sensitivity analysis and the conclusions did not change. Although significant associations found in this study did not remain noteworthy after applying the FDR method ($P > 0.2$), this is the largest study to date that has examined gene-environment interactions involving one-carbon metabolizing pathway genes, nutrient intakes, and risk of NHL, and we also note that these SNPs were strong *a priori* hypotheses based on their functional relevance. Interestingly, our observations indicating a potential etiologic role of these gene-environment interactions are consistent with previous epidemiologic findings for overall NHL and childhood acute lymphoblastic leukemia (10, 41). In particular, the NCI study by Lim et al. (10) also showed that the risk of NHL associated with specific genotypes or their variants appears to be contingent on dietary intakes of one-carbon metabolizing nutrients. For example, the inverse association of *MTHFR* and *MTR* variants with NHL in the NCI study appeared contingent on methionine and vitamin B_6 intake, respectively. The

gene-nutrient interactions observed in this study and the NCI study (10) could be used to at least partially explain the discrepancies from earlier epidemiological studies which have inconsistently linked one-carbon metabolizing pathway genes or nutrients to the risk of NHL (7–9, 11–18, 20, 21).

Some strengths and limitations of our study must be considered when interpreting our findings. First, we used the same laboratory for genotyping one-carbon metabolizing genes as in the NCI study in order to increase the comparability of the study results. Second, in-person interviews were conducted to collect information on dietary intakes by trained interviewers using a semiquantitative food frequency questionnaire developed by the Fred Hutchinson Cancer Research Center. Third, the population-based nature of the study reduced the potential for selection bias by avoiding recruiting controls whose diseases may be associated with the exposure of interest as in a hospital setting. Finally, systematic pathological review of the diagnosis for the cases was conducted and therefore misclassification of disease status is less likely. However, non-differential misclassification of the exposure was likely since the precise measurement of exposure to dietary factors is often difficult because of issues involving recall or limited awareness of previous exposures, and the complex pattern of many nutrient intakes (42). It is also possible that cases may recall dietary habits differently compared to controls, given the influence of their disease status, and it has previously been shown that under certain conditions differential misclassification of the exposure may bias a multiplicative interaction effect towards the null in studies of gene-environment interactions(43, 44). As pointed out by others (10), our results are based on retrospective assessment of diet and therefore our findings need to be replicated using prospective data, though this type of assessment is considered to be highly correlated with diet in the more distant past (29). Another limitation of our study is its relatively limited statistical power to examine the gene-nutrient interactions which is reflected by the wide confidence intervals after the stratification by nutrient intake to assess the gene-NHL association. Moreover, we were unable to evaluate the potential associations with specific NHL subtypes due to the small sample size. Further studies should focus on the associations with NHL subtypes particularly since etiologic heterogeneity for different NHL subtypes is now recognized (45).

In summary, the results from this population-based case-control study suggest that genetic polymorphisms in certain one-carbon metabolizing pathway genes may be associated with the risk of NHL, and the risk of NHL associated with one-carbon metabolizing pathway genes appears to be contingent upon the dietary intakes of nutrients related to one-carbon metabolism. Both the observed main effects for specific genotypes and the gene-nutrient interactions and risk of NHL, while intriguing, require replication in epidemiological studies with larger sample size and in different populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Characteristics of study participants (n=1,115)

Characteristics	Cases (n=518) n (%)	Controls (n=597) n (%)	*P*-Value
Age (years)			
<40	43 (8.30%)	51 (8.54%)	0.6
40–49	59 (11.39%)	66 (11.06%)	
50–59	109 (21.04%)	109 (18.26%)	
60–69	132 (25.48%)	144 (24.12%)	
70+	175 (33.78%)	227 (38.02%)	
Race			
Caucasian	497 (95.95%)	561 (93.97%)	0.14
Non-Hispanic	483 (93.24%)	547 (91.62%)	
Hispanic	12 (2.32%)	14 (2.34%)	
Unknown	2 (0.39)	0 (0%)	
African-American	16 (3.09%)	17 (2.85%)	
Other	5 (0.97%)	19 (3.18%)	
Family history*			
No	110 (21.24%)	147 (24.62%)	0.06†
NHL‡	9 (1.74%)	3 (0.50%)	
Other cancer	399 (77.03%)	447 (74.87%)	
DNA source			
Blood	461 (89.00%)	535 (89.61%)	0.74
Buccal cells	57 (11.00%)	62 (10.39%)	
Case pathology§			
All B cell NHL	411 (79.34%)	-	
DLBCL	161 (31.08%)	-	
Follicular	119 (22.97%)	-	
SLL/CLL	59 (11.39%)	-	
MZBL	35 (6.76%)	-	
Other	37 (7.14%)	-	
All T cell	39 (7.53%)	-	
NOS	68 (13.13%)	-	

*Family history of cancer in first degree relatives.

†Exact test.

‡Non-Hodgkin Lymphoma.

§DLBL=Diffuse large B-cell lymphoma; CLL/SLL= B-cell chronic lymphocytic leukemia/prolymphocytic leukemia/small lymphocytic lymphoma; MZBL=Marginal zone B-cell lymphoma.

Table 2

The associations between selected genetic polymorphisms in one-carbon metabolism pathway genes and risk of NHL

SNPs	Controls (n=547)	Cases (n=483)	OR[a] (95%CI)
CBS (*rs*234706, *Ex9+33*C>T)			
CC	255	253	1
CT	200	159	0.80 (0.61–1.05)
TT	53	27	0.51 (0.31–0.84)
P trend			0.01
CBS (*rs*1801181, *Ex13+41*C>T)			
CC	225	164	1
CT	243	223	1.26 (0.96–1.66)
TT	64	68	1.50 (1.01–2.23)
P trend			0.03
FTHFD (*rs*2305230, *Ex10-40*G>T)			
GG	381	313	1
GT	122	115	1.15 (0.86–1.55)
TT	9	17	2.34(1.03–5.33)
P trend			0.06
FTHFD (*rs*1127717, *Ex21+31*A>G)			
AA	292	286	1
AG	155	111	0.73 (0.54–0.98)
GG	21	25	1.21 (0.66–2.22)
P trend			0.30
MBD2 (*rs*603097, −2176C>T)			
TT	297	279	
CT	181	154	0.91 (0.69–1.19)
CC	27	9	0.37 (0.17–0.79)
P trend			0.05
SHMT1 (*rs*1979277, *Ex12+138*C>T)			
CC	233	195	1
CT	212	184	1.04 (0.79–1.37)
TT	31	41	1.57 (0.95–2.60)
P trend			0.17
SHMT1 (*rs*1979276, *Ex12+236*T>C)			
CC	234	194	1
CT	217	188	1.05 (0.80–1.38)
TT	39	49	1.51 (0.95–2.41)
P trend			0.15

[a] Adjusted for age and family history of NHL in first degree relatives.

Table 3

One-carbon metabolizing genes and risk of non-Hodgkin lymphoma by levels of dietary nutrient intakes

SNPs	Vitamin B6				Methionine				Folate			
	Low, less than median		High, median or higher		Low, less than median		High, median or higher		Low, less than median		High, median or higher	
	Ca/Co	OR[a] (95%CI)	Ca/Co	OR[a] (95%CI)	Ca/Co	OR[a] (95%CI)	Ca/Co	OR[a] (95%CI)	Ca/Co	OR[a] (95%CI)	Ca/Co	OR[a] (95%CI)
CBS												
rs234706, Ex9+33C>T												
CC	128/121	1	121/132	1	128/139	1	121/114	1	126/120	1	123/133	1
CT	72/107	0.61 (0.42–0.91)	86/92	1.02 (0.69–1.50)	68/105	0.69 (0.46–1.01)	90/94	0.90 (0.61–1.32)	76/104	0.68 (0.46–1.01)	82/95	0.95 (0.65–1.40)
TT	14/23	0.57 (0.28–1.16)	13/29	0.50 (0.25–1.01)	10/25	0.43 (0.20–0.93)	17/27	0.58 (0.30–1.14)	13/27	0.45 (0.22–0.92)	14/25	0.60 (0.30–1.21)
$P_{interaction}$				0.39				0.35				0.28
rs1801181, Ex13+41C>T												
CC	77/111	1	85/111	1	68/122	1	94/100	1	79/106	1	83/116	1
CT	105/118	1.31 (0.88- 1.95)	116/123	1.21 (0.83–1.77)	110/122	1.70 (1.14–2.53)	111/119	0.98 (0.67–1.44)	106/123	1.18 (0.80–1.75)	115/118	1.37 (0.93–2.01)
TT	39/33	1.76 (1.02–3.05)	28/31	1.21 (0.67–2.18)	37/35	1.99 (1.14–3.45)	30/30	1.13 (0.63–2.04)	40/33	1.68 (0.97–2.91)	27/31	1.25 (0.69–2.26)
$P_{interaction}$				0.43				0.07				0.74
FTHFD rs1127717, Ex21+31A>G												
AA	139/147	1	145/145	1	131/154	1	153/138	1	142/145	1	142/147	1
AG	57/70	0.84 (0.55–1.28)	52/82	0.63 (0.41–0.96)	61/79	0.90 (0.60–1.35)	48/73	0.57 (0.37–0.89)	56/73	0.77 (0.51–1.17)	53/79	0.69 (0.45–1.05)
GG	10/12	0.92 (0.38–2.20)	15/9	1.58 (0.66–3.75)	9/13	0.78 (0.32–1.92)	16/8	1.91 (0.79–4.62)	12/10	1.27 (0.53–3.04)	13/11	1.16 (0.50–2.72)
$P_{interaction}$				0.98				0.90				0.79
MBD2 rs7614, Ex8+438A>G												
AA	46/75	1	70/71	1	57/70	1	59/76	1	50/70	1	66/76	1
AG	110/102	1.68 (1.07–2.66)	97/114	0.87 (0.57–1.35)	101/129	0.95 (0.62–1.48)	106/87	1.60 (1.02–2.51)	111/102	1.48 (0.94–2.34)	96/114	1.00 (0.65–1.54)
GG	37/44	1.36 (0.76–2.40)	32/39	0.80 (0.45–1.42)	32/40	0.98 (0.55–1.76)	37/43	1.09 (0.62–1.92)	38/46	1.31 (0.64–1.99)	31/37	0.98 (0.54–1.76)
$P_{interaction}$				0.15				0.59				0.58
rs603097, −2176T>C												
TT	139/147	1	138/148	1	135/161	1	142/134	1	136/143	1	141/152	1
CT	68/93	0.80 (0.54–1.18)	84/86	1.06 (0.72–1.56)	67/93	0.86 (0.59–1.28)	85/86	0.94 (0.64–1.38)	76/95	0.86 (0.59–1.27)	76/84	0.96 (0.65–1.42)
CC	5/9	0.60 (0.20–1.85)	4/18	0.25 (0.08–0.75)	4/12	0.41 (0.13–1.29)	5/15	0.40 (0.13–1.03)	5/10	0.56 (0.19–1.69)	4/17	0.26 (0.08–0.72)

SNPs	Vitamin B6				Methionine				Folate			
	Low, less than median		High, median or higher		Low, less than median		High, median or higher		Low, less than median		High, median or higher	
	Ca/Co	OR[a] (95%CI)	Ca/Co	OR[a] (95%CI)	Ca/Co	OR[a] (95%CI)	Ca/Co	OR[a] (95%CI)	Ca/Co	OR[a] (95%CI)	Ca/Co	OR[a] (95%CI)
$P_{interaction}$				1.00				0.93				0.73
MLH1 rs1799977, Ex8-23A>G												
AA	89/127	1	103/109	1	86/136	1	106/100	1	97/122	1	95/114	1
AG	83/88	1.33 (0.88–2.00)	82/92	0.93 (0.62–1.39)	79/93	1.39 (0.92–2.09)	86/87	0.93 (0.63–1.37)	83/89	1.17 (0.78–1.75)	82/91	1.07 (0.71–1.60)
GG	26/14	2.70 (1.33–5.47)	23/32	0.73 (0.40–1.33)	28/19	2.40 (1.25–4.58)	21/27	0.33 (0.12–0.94)	23/16	1.83 (0.91–3.66)	26/30	0.98 (0.54–1.78)
$P_{interaction}$				0.01				0.01				0.27
rs2286940, IVS12-169C>T												
CC	60/95	1	74/71	1	60/101	1	74/65	1	66/91	1	68/75	1
CT	103/112	1.47 (0.96–2.25)	105/122	0.83 (0.54–1.26)	100/118	1.51 (0.99–2.29)	108/116	0.81 (0.53–1.24)	107/109	1.38 (0.91–2.09)	101/125	0.87 (0.57–1.33)
TT	47/33	2.35 (1.34–4.11)	34/53	0.62 (0.36–1.08)	45/40	2.02 (1.17–3.48)	36/46	0.70 (0.40–1.22)	41/38	1.52 (0.88–2.63)	40/48	0.89 (0.52–1.54)
$P_{interaction}$				0.001				0.01				0.14
MTHFS rs622506, IVS2-1411T>G												
TT	94/104	1	87/114	1	88/125	1	93/93	1	94/104	1	87/114	1
GT	80/102	0.86 (0.57–1.29)	99/103	1.29 (0.87–1.92)	80/106	1.11 (0.74–1.66)	99/99	1.03 (0.68–1.54)	83/101	0.90 (0.60–1.34)	96/104	1.24 (0.83–1.84)
GG	34/32	1.17 (0.67–2.05)	24/28	1.15 (0.62–2.13)	33/26	1.90 (1.06–3.41)	25/34	0.71 (0.39–1.29)	35/31	1.24 (0.71–2.17)	23/29	1.05 (0.57–1.95)
$P_{interaction}$				0.63				0.06				0.89
SHMT1 rs1979277, Ex12+138C>T												
CC	96/124	1	96/108	1	101/125	1	91/107	1	102/120	1	90/112	1
CT	82/98	1.10 (0.74–1.64)	102/112	1.05 (0.71–1.55)	75/110	0.85 (0.57–1.26)	109/100	1.32 (0.89–1.97)	86/98	1.05 (0.71–1.55)	98/112	1.09 (0.74–1.62)
TT	24/16	1.99 (1.00–3.96)	16/15	1.19 (0.56–2.55)	21/16	1.64 (0.81–3.31)	19/15	1.44 (0.69–3.03)	21/15	1.65 (0.81–3.38)	19/16	1.46 (0.70–3.01)
$P_{interaction}$				0.40				0.50				0.93
rs1979276, Ex12+236C>T												
CC	97/124	1	95/109	1	103/125	1	89/108	1	101/122	1	91/111	1
CT	84/97	1.13 (0.76–1.68)	103/118	1.03 (0.70–1.51)	77/114	0.83 (0.56–1.22)	110/101	1.36 (0.92–2.03)	90/97	1.13 (0.76–1.67)	97/118	1.00 (0.68–1.48)
TT	28/19	1.95 (1.03–3.70)	20/20	1.13 (0.57–2.24)	24/20	1.47 (0.76–2.81)	24/19	1.48 (0.76–2.90)	24/19	1.53 (0.79–2.97)	24/20	1.45 (0.75–2.81)
$P_{interaction}$				0.30				0.36				0.77

Abbreviation: ca/co, numbers of cases and controls.

Adjusted for age and family history of NHL in first degree relatives. Median dietary intake of defined as 1.47mg for vitamin B6, 1.36g for methionine, and 215.48 μg for floate