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ORIGINAL INVESTIGATION

One-carbon metabolism gene polymorphisms and risk of non-Hodgkin lymphoma in Australia

Kyoung-Mu Lee · Qing Lan · Anne Kricker · Mark P. Purdue · Andrew E. Grulich · Claire M. Vajdic · Jennifer Turner · Denise Whitby · Daehee Kang · Stephen Chanock · Nathaniel Rothman · Bruce K. Armstrong

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Abstract Dysregulation of the one-carbon metabolic pathway, which controls nucleotide synthesis and DNA methylation, may promote lymphomagenesis. We evaluated the association between polymorphisms in one-carbon metabolism genes and risk of non-Hodgkin lymphoma (NHL) in a population-based case-control study in Australia. Cases ($n = 561$) and controls ($n = 506$) were genotyped for 14 selected single-nucleotide polymorphisms in 10 genes (*CBS*, *FPGS, FTHFD, MTHFR, MTHFS*, *MTR*, *SHMT1*, *SLC19A1*, *TCN1*, and *TYMS*). We also conducted a meta-analysis of all studies of Caucasian populations investigating the association between *MTHFR Ex5+79C > T* (a.k.a., *677C>T*) and NHL risk. A global test of 13 genotypes was statistically significant for diffuse large B-cell lymphoma (DLBCL; $P = 0.008$), but not for follicular lymphoma (FL; $P = 0.27$) or all NHL ($P = 0.17$). The *T* allele at *MTHFR Ex5+79* was marginally significantly associated with all NHL (OR = 1.25, 95% CI = 0.98–1.59) and DLBCL (1.36, 0.96–1.93). The *T* allele at *TYMS Ex8+157* was associated with a reduced risk of FL (0.64, 0.46–0.91). An elevated risk of NHL was also observed among carriers of the *G* allele at *FTHFD Ex21+31* (all NHL, 1.31, 1.02–1.69; DLBCL, 1.50, 1.05–2.14). A meta-analysis of 11 studies conducted in Caucasian populations of European origin (4,121 cases and 5,358 controls) supported an association between the *MTHFR Ex5+79 T* allele and increased NHL risk (additive model, $P = 0.01$). In conclusion, the results of this study suggest that genetic polymorphisms of one-carbon metabolism genes such as *MTHFR* and *TYMS* may influence susceptibility to NHL.

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K.-M. Lee (✉) · Q. Lan · M. P. Purdue · N. Rothman
Occupational and Environmental Epidemiology Branch,
Division of Cancer Epidemiology and Genetics,
National Cancer Institute, NIH, DHHS,
6120 Executive Blvd. EPS 8118,
Bethesda, MD 20892-7240, USA
e-mail: leekyou@mail.nih.gov

A. Kricker · B. K. Armstrong
School of Public Health, The University of Sydney,
Sydney, Australia

A. E. Grulich · C. M. Vajdic
National Centre for HIV Epidemiology and Clinical Research,
Sydney, Australia

J. Turner
St. Vincent's Hospital, Sydney, Australia

D. Whitby
NCI-Frederick, Frederick, MD, USA

D. Kang
Department of Preventive Medicine,
Seoul National University College of Medicine,
Seoul, South Korea

S. Chanock
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, USA

Introduction

Non-Hodgkin lymphoma (NHL) has been increasing in incidence in industrialized countries over the past 50 years (Hartge and Wang 2004). However, its etiology remains poorly understood. There is some evidence that chromosomal alterations arising from flawed DNA synthesis or altered methylation of oncogenes or tumor suppressor genes play a role in lymphomagenesis (Hanada et al. 1993; Rossi et al. 2004; Kuppers 2005). Thus, genetic variability in the activity of enzymes involved in DNA synthesis and methylation could influence susceptibility to NHL.

The one-carbon metabolic pathway regulates nucleotide synthesis and DNA methylation via a complex process involving at least 30 different enzymes (Kim 2005). A number of studies have investigated the relationship between polymorphisms in one-carbon metabolism genes and NHL risk with inconsistent results (Gonzalez Ordonez et al. 2000; Matsuo et al. 2001; Lincz et al. 2003; Toffoli et al. 2003; Hishida et al. 2003; Linnebank et al. 2004; Gemmati et al. 2004; Skibola et al. 2004; Matsuo et al. 2004; Lightfoot et al. 2005; Habib et al. 2005; Deligezer et al. 2006; Niclot et al. 2006; Timuragaoglu et al. 2006; Lim et al. 2007). The most widely investigated variant, the T allele of *MTHFR Ex5+79C>T* (a.k.a., *677C>T*: rs1801133), was significantly more frequent among NHL cases than controls in one study (Skibola et al. 2004), and significantly less frequent in another (Matsuo et al. 2004). Other studies found no significant association between *MTHFR* genotype and NHL as a whole (Gonzalez Ordonez et al. 2000; Lincz et al. 2003; Toffoli et al. 2003; Linnebank et al. 2004; Gemmati et al. 2004; Lightfoot et al. 2005; Habib et al. 2005; Deligezer et al. 2006; Niclot et al. 2006; Timuragaoglu et al. 2006; Lim et al. 2007). This inconsistency could be due to small sample sizes, ethnic diversity, and possible etiologic heterogeneity in NHL.

To better understand whether the one-carbon metabolism pathway influences NHL risk, we investigated 14 possibly functional single-nucleotide polymorphisms (SNPs) in 10 genes (*CBS*, *FPGS*, *FTHFD*, *MTHFR*, *MTHFS*, *MTR*, *SHMT1*, *SLC19A1*, *TCN1*, and *TYMS*) involved in one-carbon metabolism (Fig. 1) among participants of a population-based case-control study conducted in New South Wales, Australia.

Materials and methods

Subject selection and data collection

Details of the process and criteria for subject selection are described elsewhere (Hughes et al. 2004; Purdue et al. 2007). In brief, patients notified to the New South Wales (NSW) Central Cancer Registry with newly diagnosed NHL between January 1, 2000 and August 31, 2001 who were 20–74 years of age and resident in NSW or the Australian Capital Territory (ACT) were potentially eligible to be cases in this case-control study. Patients who had a history of transplantation or HIV infection were excluded. Pathology reports for consenting cases were reviewed by one anatomical pathologist to assign a cell phenotype and

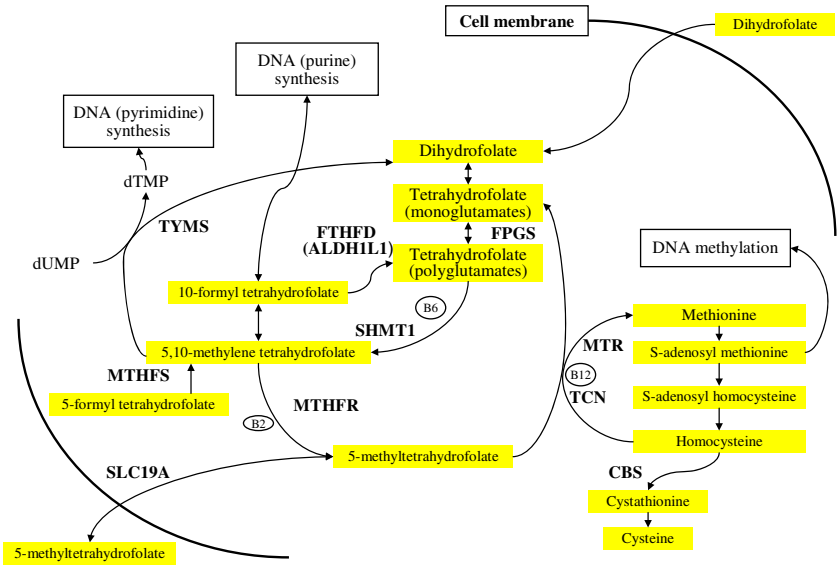

Fig. 1 The role of the proteins encoded by one-carbon metabolism genes evaluated in this study: *CBS* (cystathionine-beta-synthase), *FPGS* (folylpolyglutamate synthase), *FTHFD* (10-formyltetrahydrofolate dehydrogenase), *MTHFR* (5,10-methylenetetrahydrofolate reductase), *MTHFS* (5,10-methenyltetrahydrofolate synthetase), *MTR* (5-methyltetrahydrofolate-homocysteine methyltransferase), *SHMT1* (serine hydroxymethyltransferase 1), *SLC19A1* [solute carrier family 19 (folate transporter), member 1], *TCN1* (transcobalamin I), and *TYMS* (thymidylate synthetase)

WHO (ICD-O-3) code (Turner et al. 2004). Controls were randomly selected from the NSW and ACT electoral rolls to match approximately the expected distributions of cases with respect to age, sex and residence (NSW or ACT). Eighty five percent of cases invited to participate did so, as did 61% of controls. Of all eligible and participating subjects (687 cases and 694 controls), 597 cases and 525 controls provided blood, and genomic DNA was extracted successfully from 584 cases and 518 controls.

Genotyping

We selected 14 SNPs in 10 one-carbon metabolism genes for analysis (Fig. 1; Table 1). These SNPs were selected because they had a minor allele frequency greater than 5% (Packer et al. 2006) and laboratory evidence of functional relevance (Frosst et al. 1995; Mandola et al. 2004) or a previously reported association with human cancer (Matsuo et al. 2001; Shen et al. 2005).

Genotype analysis was performed at the National Cancer Institute Core Genotyping Facility (CGF, Advanced Technology Corporation, Gaithersburg, MD; http://cgf.nci.nih.gov). All TaqMan® assays (Applied Biosystems Inc., Foster City, CA) were optimized on the ABI 7900 HT detection system with 100% concordance with sequence analysis of 102 individuals listed on the SNP500Cancer website (http://snp500cancer.nci.nih.gov; Packer et al. 2006).

Duplicate samples from 95 study subjects were interspersed throughout each batch for all genotyping assays. The concordance rates for QC samples were 98–100% for all assays. One SNP failed a test of Hardy-Weinberg equilibrium among controls (*MTHFR Ex5+79C>T*, $P = 0.02$); however, quality control data were rechecked and the accuracy was confirmed.

Two SNPs in *SHMT1* (*Ex12+138C>T (L435F)* and *Ex12+236T>C*) were found to be in very tight linkage disequilibrium (LD; $r^2 = 0.99$). Thus, *SHMT1 Ex12+236T>C* were excluded from the global test of association and the false discovery rate test, described below.

Statistical analysis

After subjects with not well-defined ethnicity (23 cases and 12 controls) were excluded, the final analysis included 561 cases and 506 controls of European or Asian ethnicity. The chi-square test was used to identify departures from Hardy-Weinberg equilibrium among controls. To estimate the relative risk of NHL in relation to SNP genotype, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional logistic regression, adjusting for sex, age (<40, 40–49, 50–59, 60–69, 70+), state of residence (NSW vs. ACT), and ethnicity (British or Irish, Western/Northern European, Southern European, Eastern European, Mixed European, and Asian) using the most prevalent homozygous genotype as the referent group. ORs were not estimated for the main-effects of SNPs where there are fewer than five subjects in any cell.

To assess the global significance of the association between all SNPs tested and NHL risk, we used the whole-model likelihood ratio χ^2 statistic (Chapman et al. 2003), comparing a logistic regression model that included all 13 SNPs as main effects (i.e., genotypes containing one or more variant alleles vs. homozygotes of the common allele) against the null model that included none of the SNPs. For each SNP, tests for linear trend were done by assigning the ordinal values 1, 2, and 3 to homozygous wild type, heterozygous and homozygous variant genotypes, respectively, and by modeling these scores as a continuous variable. In addition to conducting analyses of overall NHL, we calculated subtype-specific ORs for follicular lymphoma (FL; $n = 211$) and diffuse large B-cell lymphoma (DLBCL; $n = 180$; see

Table 1 The 14 single nucleotide polymorphisms in 10 one-carbon metabolism genes selected for study

Gene	Name	Location	SNPs (Amino acid change)	dbSNP rs#
CBS	Cystathionine-beta-synthase	21q22.3	*Ex9+33C>T (Y233Y)* *Ex18-391A>G, 3′-UTR* *Ex13+41C>T (A360A)*	rs234706 rs12613 rs1801181
FPGS	Folylpolyglutamate synthase	9q34.1	*Ex15-263T>C, 3′-UTR*	rs10106
FTHFD (ALDH1L1)	10-Formyltetrahydrofolate dehydrogenase (aldehyde dehydrogenase 1 family, member L1)	3q21.2	*Ex10-40G>T (L395L)* *Ex21+31A>G (D793G)*	rs2305230 rs1127717
MTHFR	5,10-Methylenetetrahydrofolate reductase	1p36.3	*Ex5+79C>T (A222V: 677C>T)*	rs1801133
MTHFS	5,10-Methenyltetrahydrofolate synthetase	15q23	*IVS2-1411T>G*	rs622506
MTR	5-Methyltetrahydrofolate-homocysteine methyltransferase	1q43	*Ex26-20A>G (D919G)*	rs1805087
SHMT1	Serine hydroxymethyltransferase 1	17p11.2	*Ex12+138C>T (L435F)* *Ex12+236T>C, 3′-UTR*	rs1979277 rs1979276
SLC19A1	Solute carrier family 19 (folate transporter), member 1	21q22.3	*Ex4-254T>C (P232P)*	rs12659
TCN1	Transcobalamin I	11q11-q12	*IVS1+372T>C*	rs526934
TYMS	Thymidylate synthetase	18p11.32	*Ex8+157C>T, 3′-UTR*	rs699517

Table 2 Characteristics of NHL cases and controls (n = 1,067)

Characteristics	Cases (n = 561)		Controls (n = 506)	
	N	%	*N*	%
Sex/age group				
Males	323	57.6	288	56.9
20–39	21	6.5	20	6.9
40–49	48	14.9	46	16.0
50–59	106	32.8	86	29.9
60–69	96	29.7	85	29.5
70–74	52	16.1	51	17.7
Females	238	42.4	218	43.1
20–39	20	8.4	19	8.7
40–49	43	18.1	28	12.8
50–59	74	31.1	61	28.0
60–69	74	31.1	81	37.2
70–74	27	11.3	29	13.3
State				
NSW	536	95.5	483	95.5
ACT	25	4.5	23	4.5
Ethnicity				
British or Irish	429	76.5	419	82.8
Western or Northern European	29	5.2	20	4.0
Southern European	30	5.4	13	2.6
Eastern European	12	2.1	6	1.2
Mixed European	45	8.0	40	7.9
Asian	16	2.8	8	1.5
Case pathology				
B-cell	542	96.6		
Follicular	211	37.6		
Diffuse large	180	32.1		
Marginal zone	53	9.4		
SLL/CLL	18	3.2		
Other	80	14.3		
T-cell	16	2.9		
NOS	3	0.5		

NSW New South Wales, *ACT* Australian Capital Territory, *SLL* small lymphocytic lymphoma, *CLL* chronic lymphocytic leukemia

Table 2). Other subgroups [marginal zone and small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL)] were too few to conduct subtype-specific analysis.

To evaluate the probability of false positive associations, we computed the false discovery rate (FDR) (Benjamini and Hochberg 1995; Curran-Everett 2000), which estimates the proportion of false positivity for statistically significant findings in a specified set. Main-effect results for the 13 SNPs assuming a dominant model (i.e., variant allele containing genotypes vs. homozygotes of the common allele) were assessed as one set by the FDR method as the resulting ORs were less susceptible to problems of instability due to small numbers. We considered FDR <0.2 as noteworthy. The results from subgroup analysis by NHL subtypes were assessed as a separate set. We also computed the false positive report probabilities (FPRP) (Wacholder et al. 2004) using prior probabilities ranging from 0.1 to 0.01 based on gene selection criteria described above and considered values below a criterion of 0.2 noteworthy as recommended in the initial description of the method (Wacholder et al. 2004).

Haplotype analyses were conducted for genes with two or more SNPs (*CBS*, *FTHFD* and *SHMT1*) using the haplo.stat statistical package (http://mayoresearch.mayo.edu/mayo/research/schaid_lab/software.cfm) in the R program (v. 2.2.1: http://www.r-project.org), which uses an expectation-maximization algorithm to estimate haplotypes from genotype data (Schaid et al. 2002).

A meta-analysis was conducted to summarize the associations between *MTHFR Ex5+79C>T* genotype and NHL risk in populations of largely Caucasian origin. Published reports were identified through electronic searches in MEDLINE, Biological Abstracts and Web of Science using the terms of "Methylenetetrahydrofolate Reductase", "MTHFR", "Polymorphism", "Genotype", and "Lymphoma". Among the 18 studies published as articles (n = 14) or abstracts (n = 4), we excluded non-Caucasian studies conducted in Japan, China, Korea, Egypt and Saudi Arabia. Thus our analysis included 13 studies (4,245 cases and 5,594 controls) that were conducted in predominantly Caucasian populations (Gonzalez Ordonez et al. 2000; Lincz et al. 2003; Toffoli et al. 2003; Linnebank et al. 2004; Gemmati et al. 2004; Skibola et al. 2004; Lightfoot et al. 2005; Deligezer et al. 2006; Niclot et al. 2006; Timuragaoglu et al. 2006; Lim et al. 2007), including our study (Fig. 2). Case and control frequencies by genotype for NHL were obtained for all 13 studies and these frequency data were used to estimate summary ORs and 95% CIs using a random-effect model. Some of the studies were on specific subtypes as the outcome; Toffoli (2003) on DLBCL, Niclot (2006) on FL, Linnebank (2004) on primary CNS lymphoma, mostly DLBCL, and Deligezer (2006) on B-cell neoplasms including myeloma. Although almost all studies appeared to include small proportions of non-Caucasian subjects [less than 5% except for Lim et al. 2007 (17%)], the data for all subjects were used because we could not extract ethnicity-specific data from the studies. Less than 3% of our study subjects were non-Caucasian.

We further excluded two Turkish studies (Deligezer et al. 2006; Timuragaoglu et al. 2006) to estimate summary ORs and 95% CIs for Caucasians of European origin (N = 11, 4,121 cases and 5,358 controls). All meta-analytic procedures were performed using STATA version 9 (College Station, TX) (Sterne et al. 2001). The effects of *CT* and *TT* genotype compared to *CC* genotype were evaluated

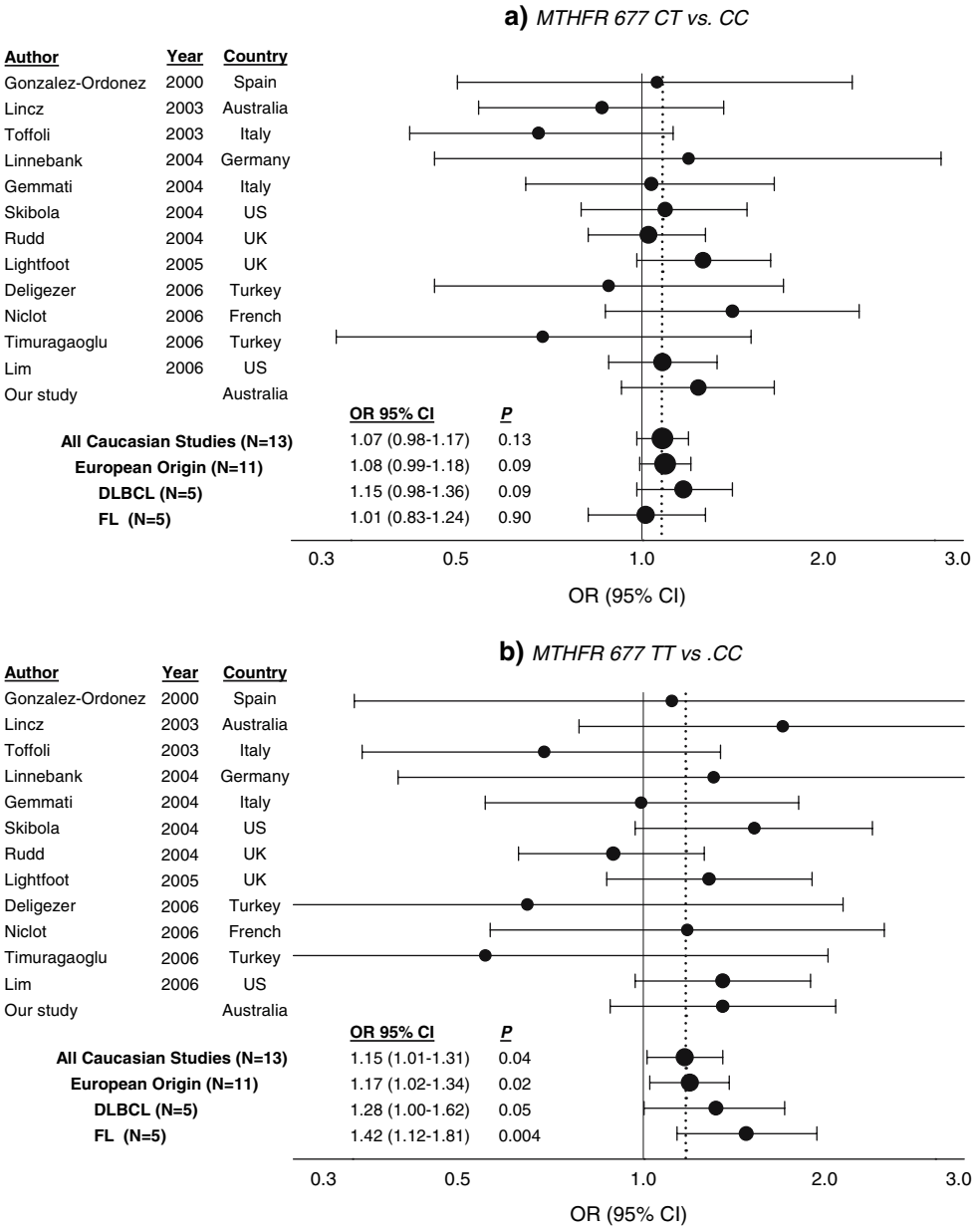

Fig. 2 Meta-analysis of the association between *MTHFR Ex5+79* (a.k.a., *677*) *CT* (**a**) or *TT* (**b**) and NHL among the studies conducted for Caucasian populations (4,245 cases and 5,594 controls): The *T* allele was associated with elevation of NHL risk in Caucasians (*CT* vs. *CC*: OR = 1.07, 95% CI = 0.98–1.17, *TT* vs. *CC*: OR = 1.15, 95% CI = 1.01–1.31, $P_{trend} = 0.03$). The association became stronger when the analysis was restricted to Caucasians of European origin (4,121 cases and 5,358 controls) (*CT* vs. *CC*: OR = 1.08, 95% CI = 0.99–1.18, *TT* vs. *CC*: OR = 1.17, 95% CI = 1.02–1.34, $P_{trend} = 0.01$). Subgroup analysis by NHL subtype showed similar increasing trend in the risk of both DLBCL ($n = 971$) and FL ($n = 849$) ($P_{trend} = 0.02$ and 0.03, respectively); tests of heterogeneity between these subtypes were not statistically significant (*CT*, $P = 0.44$; *TT*, $P = 0.44$). Note: Case-control frequencies by genotype from each study are shown in Supplementary Table II

separately and the trend in the risk of NHL was evaluated by fitting the additive model using case-control frequencies by genotype from each studies. Heterogeneity among the studies was evaluated by means of the Cochrane Q test and publication bias was assessed by Begg's (Begg and Mazumdar 1994) and Egger's test (Egger et al. 1997). Meta-analysis results for two common subtypes were also compared in studies which reported both data ($N = 5$) (DLBCL: 971 cases and 3,204 cases; FL: 849 cases and 3,204 controls) (Lincz et al. 2003; Skibola et al. 2004; Lightfoot et al. 2005; Lim et al. 2007; our study); a meta-analysis of case-case comparisons between these two subtypes was performed to evaluate the possibility of etiologic heterogeneity.

Results

Selected demographic characteristics of 561 cases and 506 controls are shown in Table 1. The distributions of participating cases and controls were similar with respect to sex, age and ethnicity. Most pathological types among cases were of B-cell origin (96.6%). Among B-cell malignancies, FL was the most prevalent (37.6%), followed by DLBCL (32.1%).

Genotype distributions for cases and controls and main effects of each SNP are shown in Table 2. A global test of the 13 one-carbon metabolism gene SNPs was statistically significant for DLBCL ($P = 0.008$), but not for FL ($P = 0.27$) or all NHL ($P = 0.17$).

Three SNPs out of the 13 SNPs in 10 genes were associated with all NHL or at least one subtype of NHL (Table 3 and Supplementary Table I). The *T* allele at *MTHFR Ex5+79* was marginally significantly associated with an elevated risk of all NHL (OR = 1.25, 95% CI = 0.98–1.59) and DLBCL (OR = 1.36, 95% CI = 0.96–1.93). The *T* allele at *TYMS Ex8+157* was less prevalent among FL cases than controls (OR = 0.64, 95% CI = 0.46–0.91) with a statistically significant trend ($P = 0.01$). The *G* allele at *FTHFD Ex21+31* was associated with all NHL (OR = 1.31, 95% CI = 1.02–1.69), and the association was stronger for DLBCL (OR = 1.50, 95% CI = 1.05–2.14). Additionally, the *A* allele at *CBS Ex18-391* and the C allele at *FPGS Ex15-263* were moderately associated with risk of DLBCL (OR = 1.50, 95% CI = 0.96-2.33 and OR = 0.71, 95% CI = 0.49–1.02, respectively).

The FDR value for the strongest dominant-model association in this study (*TYMS Ex8+157 CT/TT* in relation to risk of FL) was 0.15 when taking into account all SNPs tested for association with risk of FL. Other FDR values and all FPRP values were above the cut-point value 0.2.

Analyses stratified across categories of age, gender and ethnicity provided similar findings (data not shown). Haplotype analysis did not reveal associations beyond those that

Table 3 Main effect of SNPs in one-carbon metabolism genes on NHL risk in Australia: SNPs for which there was some evidence of a statistically significant association with NHL

SNPs	Controls ($n = 506$)	All NHL			Follicular			DLBCL		
		Cases ($n = 561$)	OR (95% CI) [a]	*P*	Cases ($n = 211$)	OR (95% CI) [a]	*P*	Cases ($n = 180$)	OR (95% CI) [a]	*P*
MTHFR Ex5+79C>T (677C>T)										
CC	256 (50.9)	253 (45.7)	Ref.		107 (51.2)	Ref.		77 (43.0)	Ref.	
CT	190 (37.8)	227 (40.9)	1.22 (0.94–1.59)	0.13	69 (33.0)	0.91 (0.63–1.31)	0.62	85 (47.5)	**1.46 (1.01–2.12)**	**0.04**
TT	57 (11.3)	74 (13.4)	1.32 (0.89–1.95)	0.16	33 (15.8)	1.35 (0.82–2.22)	0.23	17 (9.5)	1.00 (0.55–1.85)	0.99
TC/TT	247 (49.1)	301 (54.3)	1.25 (0.98–1.59)	0.08	102 (48.8)	1.02 (0.73–1.42)	0.92	102 (57.0)	1.36 (0.96–1.93)	**0.08**
Trend			1.17 (0.98–1.40)	0.08		1.09 (0.87–1.38)	0.46		1.14 (0.88–1.47)	0.31
TYMS Ex8+157C>T										
CC	237 (48.8)	268 (48.9)	Ref.		120 (58.0)	Ref.		71 (39.9)	Ref.	
CT	197 (40.5)	225 (41.1)	0.99 (0.76–1.28)	0.91	72 (34.8)	**0.68 (0.48–0.98)**	**0.04**	83 (46.6)	1.40 (0.96–2.04)	0.08
TT	52 (10.7)	55 (10.0)	0.84 (0.54–1.30)	0.43	15 (7.2)	**0.49 (0.25–0.94)**	**0.03**	24 (13.5)	1.29 (0.72–2.34)	0.39
CT/TT	249 (51.2)	280 (51.1)	0.96 (0.75–1.23)	0.72	87 (42.0)	**0.64 (0.46–0.91)**	**0.01**	107 (60.1)	1.38 (0.96–1.98)	0.08
Trend			0.94 (0.78–1.14)	0.53		**0.69 (0.53–0.90)**	**0.01**		1.22 (0.93–1.58)	0.15
FTHFD Ex21+31A>G (D793G)										
AA	329 (65.5)	332 (59.5)	Ref.		131 (62.0)	Ref.		102 (56.7)	Ref.	
AG	146 (29.1)	195 (35.0)	**1.34 (1.03–1.75)**	**0.03**	71 (33.7)	1.26 (0.88–1.80)	0.21	69 (38.3)	**1.57 (1.09–2.28)**	**0.02**
GG	27 (5.4)	31 (5.5)	1.16 (0.67–2.00)	0.59	9 (4.3)	0.84 (0.37–1.86)	0.66	9 (5.0)	1.09 (0.49–2.43)	0.83
AG +GG	173 (34.5)	226 (40.5)	**1.31 (1.02–1.69)**	**0.04**	80 (38.0)	1.19 (0.84–1.68)	0.32	78 (43.3)	**1.50 (1.05–2.14)**	**0.03**
Trend			**1.21 (0.98–1.48)**	**0.08**		1.09 (0.82–1.44)	0.57		**1.29 (0.97–1.72)**	**0.08**
CBS Ex18–391A>G										
GG	423 (84.3)	464 (83.3)	Ref.		179 (85.2)	Ref.		143 (79.4)	Ref.	
GA	73 (14.5)	91 (16.3)	1.17 (0.84–1.65)	0.35	29 (13.8)	0.99 (0.62–1.60)	0.97	37 (20.6)	**1.62 (1.03–2.53)**	**0.04**
AA	6 (1.2)	2 (0.4)	NC		2 (1.0)	NC		–	NC	
AG/AA	79 (15.7)	93 (16.7)	1.11 (0.80–1.54)	0.54	31 (14.8)	0.97 (0.61–1.55)	0.91	37 (20.6)	**1.50 (0.96–2.33)**	**0.07**
Trend			1.04 (0.76–1.41)	0.81		0.96 (0.63–1.46)	0.85		1.33 (0.88–2.01)	0.17
FPGS Ex15–263T>C										
TT	170 (34.3)	197 (35.8)	Ref.		61 (29.2)	Ref.		73 (41.2)	Ref.	
TC	232 (46.8)	248 (45.0)	0.93 (0.71–1.23)	0.62	104 (49.8)	1.34 (0.91–1.97)	0.14	66 (37.3)	**0.64 (0.43–0.95)**	**0.03**
CC	94 (18.9)	106 (19.2)	0.98 (0.69–1.39)	0.91	44 (21.0)	1.34 (0.83–2.16)	0.23	38 (21.5)	0.87 (0.54–1.41)	0.58
TC/CC	326 (65.7)	354 (64.2)	0.95 (0.73–1.23)	0.67	148 (70.8)	1.34 (0.93–1.93)	0.11	104 (58.8)	**0.71 (0.49–1.02)**	**0.06**
Trend			0.98 (0.83–1.17)	0.83		1.17 (0.93–1.48)	0.19		0.89 (0.70–1.13)	0.33

Statistically significant results were represented in bold style

NC not calculated for sparse data

[a] Adjusted for age, sex, state of residence and ethnicity

were apparent in the single SNP analysis; the omnibus tests for haplotypes of *CBS* and *FTHFD* were not statistically significant for all NHL or either major subtype (data not shown).

The meta-analysis of all reported findings for *MTHFR Ex5+79* and NHL showed that the T allele was associated with elevated risk in Caucasian populations (*CT* vs. *CC*: OR = 1.07, 95% CI = 0.98–1.17, *TT* vs. *CC*: OR = 1.15, 95% CI = 1.01–1.31, $P_{trend} = 0.03$) (Fig. 2). The association became stronger when the analysis was restricted to Caucasians of European origin (*CT* vs. *CC*: OR = 1.08, 95% CI = 0.99–1.18, *TT* vs. *CC*: OR = 1.17, 95% CI = 1.02–1.34, $P_{trend} = 0.01$). Subgroup analysis by NHL subtype showed similar increasing trends in risk for both DLBCL and FL ($P_{trend} = 0.02$ and 0.03, respectively); tests of heterogeneity between these subtypes were not statistically significant (*CT*, $P = 0.44$; *TT*, $P = 0.44$). There was no evidence of significant heterogeneity among studies or of publication bias.

Discussion

We investigated the relationship between NHL risk and polymorphisms in genes involved in the one-carbon metabolism pathway (i.e., *CBS, FPGS, FTHFD, MTHFR, MTHFS, MTR, SHMT1, SHMT1, SLC19A1, TCN1*, and *TYMS*). Our results provide moderately strong evidence that *TYMS*, and our meta-analysis that *MTHFR* may influence lymphomagenesis.

The association with the *MTHFR Ex5+79 CT/TT* genotype was marginally significant for all NHL and DLBCL in this study, but no dose-response relationship with allele copy number was present. These findings are similar to those of Skibola et al. (2004) who reported a moderate association between the *TT* genotype and all NHL and DLBCL. The findings of other studies, however, have offered equivocal or no evidence of such an association with increased risk of NHL (Gonzalez Ordonez et al. 2000; Lincz et al. 2003; Toffoli et al. 2003; Linnebank et al. 2004; Gemmati et al. 2004; Matsuo et al. 2004; Lightfoot et al. 2005; Deligezer et al. 2006; Niclot et al. 2006; Timuragaoglu et al. 2006; Lim et al. 2007). This inconsistency could be due to small sample sizes and ethnic diversity, and our meta-analysis of studies of Caucasian populations provides quite strong support for an increased risk of NHL in carriers of the *MTHFR T* allele.

Methylenetetrahydrofolate reductase (MTHFR) directs 5,10-methylenetetrahydrofolate toward methionine synthesis at the expense of DNA synthesis (Fig. 1). The *MTHFR Ex5+79C>T* variant codes for a thermolabile enzyme with reduced activity (Frosst et al. 1995). As a consequence, the DNA methylation pathway is decreased while the thymidylate pool is increased, thereby reducing uracil misincorporation in DNA synthesis (Blount et al. 1997). As Skibola et al. (2004) discussed, the biological mechanism underlying an increased risk of NHL with the T allele could be global DNA hypomethylation, which might cause proto-oncogene activation and transcription, and malignant transformation (Das and Singal 2004). It is noteworthy that a recent meta-analysis of the *MTHFR 677T* variant on acute lymphoblastic leukemia (ALL) showed a significant protective effect in adults (Pereira et al. 2006). This protective effect has often been explained by reduction of uracil misincorporation in DNA synthesis suggesting the possible existence of different 677T effects for NHL and ALL. However, more study is needed to elucidate the discrepant results between NHL and ALL.

We also observed a statistically significant association between the *TYMS Ex8+157T* allele and decreased risk of FL, for which the FDR was 0.15. The *Ex8+157T* variant is in complete LD with a 6-bp deletion in the 3′-untranslated region (rs16430) and *IVS7-68T>C* (rs1059394) in Caucasian populations (Skibola et al. 2004), which have been associated with decreased NHL risk in one previous study (Skibola et al. 2004). In a recent experiment, the 6-bp deletion polymorphism significantly lowered mRNA stability in vitro and intratumoral TYMS expression in vivo (Mandola et al. 2004). TYMS is a key enzyme in the nucleotide biosynthetic pathway that methylates deoxyuridine monophosphate (dUMP) using 5,10-methylene tetrahydrofolate to produce deoxythymine monophosphate (dTMP) (Fig. 1). Accumulation of 5,10-methylene tetrahydrofolate, as might be expected to occur with reduced TYMS activity, favors DNA methylation reactions, which could lead to a decreased risk of lymphoma. On the other hand, reduced TYMS activity would also be expected to lead to an increase in uracil misincorporation into DNA, causing potentially lymphomagenic strand breaks initiated by uracil-DNA-glycosylase (Blount et al. 1997; Ladner 2001). The significant association between the *MTHFR Ex5+79T* allele and an increased risk of NHL in our meta-analysis might suggest that an effect of *TYMS Ex8+157T* through DNA methylation could be the dominant effect. However, further investigation is warranted to test this hypothesis considering that a previous study found a significantly increased DLBCL risk associated with the homozygous 6-bp deletion polymorphism (Lightfoot et al. 2005).

The *FTHFD Ex21+31A>G (D793G)* variant, which was associated with all NHL and DLBCL risk, is located in the catalytic carboxyl-terminal domain (Reuland et al. 2006) and might affect enzyme activity through the amino acid change from aspartic acid to glycine. FTHFD catalyzes the conversion of 10-formayl-tetrahydrofolate (THF) to THF (Fig. 1). It has been suggested that down-regulation of FTHFD in tumors may increase proliferation of tumor cells (Krupenko and Oleinik 2002). Low FTHFD expression facilitates the incorporation of one-carbon units into purine

while increased FTHFD expression may impair cell growth by depleting the supply of 10-formyl-THF for purine biosynthesis (Anguera et al. 2006). However, the FDR and FPRP values for the association of *FTHFD Ex21+31A>G (D793G)* suggest that this finding might be due to chance ($P > 0.2$).

The associations between *CBS Ex18-391A* allele or *FPGS Ex15-263C* allele and DLBCL observed in this study are not consistent with the findings from a recently published case-control study from the United States (Lim et al. 2007). In it, the *CBS Ex18-391A* allele was associated with decreased risk of DLBCL, whereas the same allele was associated with increased risk of DLBCL in our study. Additionally, the *FPGS Ex15-263C* allele marginally significantly increased the risk of FL, whereas the same allele had little effect on overall risk in our study but moderately decreased the risk of DLBCL. Because these associations are of borderline statistical significance and were identified through subgroup analysis, we cannot rule out chances as an explanation for these findings.

Our meta-analysis provides strong support for a true positive association between *MTHFR Ex5+79C>T* and NHL risk. Our literature search included abstracts or letters, and the test results for publication bias was not statistically significant in our meta-analysis. While there is also moderate evidence for a negative association between *TYMS Ex8+157C>T* and FL, its lack of effect on risk of NHL as a whole weakens any conclusions we might draw from this association. Other significant associations found in this study did not remain noteworthy after applying the FDR or FPRP method ($P > 0.2$). However, the significant global association between all genotypes we studied and DLBCL ($P = 0.008$) suggests that variants in one-carbon metabolism genes may influence the development of this subgroup of NHL.

Further studies, preferably involving the use of tagging SNPs summarizing LD patterns within each gene, should be done of the association between variants in one-carbon metabolism genes including *TYMS* and NHL. Ideally, such studies would explore the gene-nutrient interaction of polymorphisms with intake of nutritional factors in one-carbon metabolism, given the reasonably strong evidence found by Lim et al. (2007) of interaction of vitamin B6 or methionine intake and SNPs in some one-carbon metabolism genes.

In conclusion, the results of this study suggest that genetic polymorphisms of one-carbon metabolism genes such as *MTHFR* and *TYMS* may influence susceptibility to NHL.

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