

ORIGINAL ARTICLE

# Polymorphisms in methylenetetrahydrofolate reductase gene and risk of non-Hodgkin lymphoma in a multi-ethnic population

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An imbalance in folate metabolism can adversely affect DNA synthesis and methylation systems which can lead to susceptibility to non-Hodgkin lymphoma (NHL). Whether single nucleotide polymorphisms (SNPs) and their haplotypes in the methylenetetrahydrofolate reductase (*MTHFR*) are associated with NHL, remain inconclusive. We investigated the association between *MTHFR* C677T and A1298C SNPs and NHL risk in a population which is made up of Malay, Chinese and Indian ethnic subgroups. A total of 372 NHL patients and 722 controls were genotyped using the Sequenom MassARRAY platform. Our results of the pooled subjects failed to demonstrate significant association between the *MTHFR* C677T and A1298C SNPs with NHL and its subtypes. The results were in agreement with the previous meta-analyses. In the Indian ethnic subgroup however, single locus analysis of *MTHFR* A1298C appears to confer risk to NHL (Odds ratio (OR) 1.91, 95% confidence interval (95% CI) 1.22–3.00,  $P=0.006$ ). The risk is almost doubled in homozygous carrier of *MTHFR* 1298CC (OR 4.03, 95% CI 1.56–10.43,  $P=0.004$ ). Haplotype analysis revealed higher frequency of CC in the Indian NHL patients compared with controls (OR 1.86, 95% CI 1.18–2.93,  $P=0.007$ ). There is lack of evidence to suggest an association between *MTHFR* C677T and A1298C with the risk of NHL in the Malays and Chinese. In the Indians however, the *MTHFR* A1298C confers risk to NHL. This study suggests ethnicity modifies the relationship between polymorphisms in the folate-metabolizing gene and NHL.

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**Keywords:** folate; haplotype; *MTHFR*; non-Hodgkin's lymphoma; polymorphism

## INTRODUCTION

Non-Hodgkin's lymphoma (NHL) is a heterogeneous malignancy with a wide variety of subtypes with different incidence patterns.<sup>1</sup> It ranks among the ten most commonly diagnosed cancer and accounts for ~3–4% of all cancers worldwide, with an estimated 355 900 new cases and 191 400 deaths in 2008.<sup>2,3</sup> NHLs are derived from malignant B cells, T cells or natural killer cells, in which the first make up most of the NHLs.<sup>4,5</sup> Of these malignant B cells, diffuse large B-cell lymphoma and follicular lymphoma are the two most common subtypes.<sup>6</sup> The specific aetiologies of NHL are unknown.<sup>7</sup> What are known today, autoimmune disorders, immunodeficiency state, infectious agents (human immunodeficiency virus—HIV, Epstein-Barr virus—EBV and human T-cell lymphotropic/leukemia virus-1—HTLV-1) and exposure to chemical/pharmaceutical agents (benzene, ethylene oxide, azathioprine and cyclosporine)<sup>1,7–10</sup> are among the confirmed risk factors of NHL. Racial differences in the

incidence of NHL have given a notion that susceptibility to NHL has a genetic cause.<sup>11</sup>

Increased nutrients intake including folate has been shown to reduce the risk of NHL and its subtypes.<sup>12</sup> 5,10-Methylenetetrahydrofolate reductase (*MTHFR*) is an important folate pathway enzyme that functions in DNA synthesis and methylation systems.<sup>13</sup> Functional polymorphisms in the *MTHFR* gene encoding this enzyme have been associated with NHL.<sup>14,15</sup> Among these polymorphisms, *MTHFR* C677T and A1298C are the most studied. The C-to-T transition at the nucleotide position 677 in exon 4 (Ala222Val), causes a thermolabile enzyme of reduced activity resulting in decreased folate concentration and increased homocysteine levels in the serum. A 30% reduced enzyme activity was reported in 677CT heterozygous carriers and the percentage is doubled (60%) in homozygous TT.<sup>16</sup> The A-to-C transversion at nucleotide 1298 (Glu429Ala) may also decrease *MTHFR* activity.<sup>17</sup>

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MTHFR enzyme catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate (5,10-methylene THF) to 5-methyltetrahydrofolate (5-methyl THF), the main circulating form of folate, which acts as the methyl donor for the remethylation of homocysteine to methionine, which is further converted to S-adenosylmethionine.<sup>14</sup> The 5,10-methylene THF donates a methyl group to uracil, converting it to thymidine, by thymidylate synthase (TS) enzyme. Lower MTHFR activity can cause optimal DNA synthesis by reducing the uracil misincorporation rate, a cause of double-strand breaks during uracil excision repairing processes.<sup>18</sup> Lower availability of 5-methyl THF may decrease the synthesis of methionine and consequently of S-adenosylmethionine, which is involved in the cellular methylation processes. The decreased MTHFR activity leads to increased plasma levels of homocysteine and decreased levels of 5-methyl THF formation. The lack of 5-methyl THF may cause hypomethylation of important genes, which may then activate cell growth and promote malignant transformation.<sup>19</sup>

There are a number of conflicting results in the study of *MTHFR* C677T and A1298C polymorphisms over the past decade. Furthermore, most of the published reports are coming from the Western population. We therefore investigate the association of these functional single nucleotide polymorphisms (SNPs) in folate-metabolizing gene *MTHFR* with risk of NHL in a Malaysian population. Our study provides a multi-ethnic setting comprising Malays, Chinese and Indians, each of which are presumably of different genetic pool, hence giving an opportunity to study ethnic differences and their risk to NHL.

## MATERIALS AND METHODS

### Subjects

This is a case-control study consisting of a total of 1094 subjects, including 372 (34%) patients with NHL and 722 (66%) controls. The patients and controls were matched by gender and age. Ethnicity of the subjects was confirmed by verifications of no mixed marriages for at least three generations. This study was a part of collaboration between the University Malaya Medical Centre (UMMC) and Ampang Hospital, both of which are located in the city of Kuala Lumpur, Malaysia. Patients were recruited from hematology clinics between September 2010 and December 2012. Patients were eligible for inclusion if they were at least 18 years old, were not afflicted with other active malignancies and were not infected by HIV. The controls were unrelated healthy blood donors based on family history and following cross-checking from the patients' database. A standardized extraction template was used to collect demographic details, medical history and types of NHL from the medical records. NHL types were classified according to the World Health Organization (WHO) 2008 classification system. At the time of peripheral blood collection, written informed consent was given by all subjects. The study protocol was approved by the medical ethics committees of both centers.

### Sequenom MassARRAY genotyping

Genomic DNA was extracted from the collected blood samples using a QiAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The quality of DNA was checked consistently to confirm that 260/280 and 260/230 absorbance ratios exceed 1.8 to indicate high-quality of DNA. The genomic DNA was then diluted to 10 ng  $\mu\text{l}^{-1}$  and 20 ng  $\mu\text{l}^{-1}$ , respectively for sample and duplicate sample and then placed in the well. A volume of 1  $\mu\text{l}$  of DNA were used in every amplification reaction. The *MTHFR* C677T (rs1801133) and A1298C (rs1801131) polymorphisms were genotyped at the University of Hong Kong, Genome Research Centre using the Sequenom MassARRAY technology platform with the iPLEX GOLD chemistry (Sequenom, San Diego, CA, USA) according to the manufacturer's protocol. MassARRAY AssayDesign software package (v4.0) (Sequenom) was used to design the specific assays with proximal SNPs filtering. Quality of the PCR fragment amplification and extension primer specificity was checked before to running the reaction. Residual nucleotides were dephosphorylated before to the iPLEX Gold

reaction. Based on a single-base extension, reaction products were desalted with SpectroClean resin (Sequenom), and 10 nl was spotted onto the SpectroCHIP (Sequenom) using the MassARRAY Nanodispenser (Sequenom). MassARRAY Analyzer Compact MALDI-TOF mass spectrometer (Sequenom) was used to determine the mass. The MassARRAY Typer 4.0 software was used for proper data acquisition and analysis. Genotypes were called after cluster analysis using the default setting of Gaussian mixture model. Inspection of the clusters was done to ensure a clear cluster separation with good signal to noise cut-off. A manual review was done to further clarify uncertain genotype calls. Assay with <80% call rate within the same SpectroChip was considered failed. A blank and five duplicates were introduced as quality controls. SpectroChip with more than 25% call rate in the blank control or with <99.5% concordance in duplicate checks along with more than 10% call rate in blank check were considered to have failed and would be required to be repeated.

### Statistical analysis

All values are presented as mean  $\pm$  s.d. for continuous data and as percentages for categorical data. A goodness-of-fit  $\chi^2$ -test was used to assess whether each individual variant was in equilibrium at each locus in the population (Hardy-Weinberg equilibrium). Deviation from Hardy-Weinberg equilibrium was set at default  $P < 0.05$ . Although gender and age were matched, a multivariate analysis reconfirmed no significant contribution of gender and age in the analysis. Association of allele was performed using logistic regression. In order to avoid false discoveries owing to population stratification, the association analysis was performed for each marker (SNP/haplotype) separately, for each ethnic group. The overall estimate was evaluated using multiple logistic regression with ethnicity as a cofactor. Correction for multiple testing was performed using Bonferroni method. The calibration and fit of the model were assessed using Hosmer-Lemeshow goodness-of-fit and receiver-operating characteristic curves. All statistical tests were two-sided and results were considered significant if  $P < 0.05$ . All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 21.0 (SPSS, IBM Corp., Chicago, IL, USA). Linkage disequilibrium and haplotype analyses for the *MTHFR* C677T and A1298C were performed using Haploview 4.2 program (<http://www.broad.mit.edu/mpg/haploview>). The P values were generated using 100 000 permutations. The OR of the haplotypes was calculated using R software version 2.11.1 (<http://www.R-project.org>).

## RESULTS

The patients' demographic information is summarized in Table 1. The 372 patients consisted of 199 Malays, 121 Chinese and 52 Indians. Out of the 722 controls, 307 were Malays, 265 Chinese and 150 Indians. Male patients (59%) were more than female (41%) and the mean age of the subjects was 48 years. Majority of the patients had diffuse large B-cell lymphoma (51%) and followed by follicular lymphoma (13%). Of the 372 NHL patients, 332 (89%) were B-cell NHL and 40 (11%) were T-cell NHL. Table 2 lists the distribution of NHL histological subtypes among patients.

Table 3 shows the frequencies and association between *MTHFR* and SNPs with overall NHL. All genotypes were in Hardy-Weinberg equilibrium for both patients and controls, as well as when stratified according to ethnicity. No significant association was found between the SNPs and overall NHL for the pooled subjects. Similar trend was observed for diffuse large B-cell lymphoma (Table 4) and follicular lymphoma (Table 5). The association was however observed for the *MTHFR* A1298C in the Indians after ethnic stratification (OR 1.91, 95% CI 1.22–3.00,  $P = 0.006$ ). Patients bearing single risk allele C of the *MTHFR* A1298C have 1.91 risk of NHL. The risk is doubled when they have a homozygous CC genotype (OR 4.03, 95% CI 1.56–10.43,  $P = 0.004$ ). The  $P$ -value remained significant after correction of multiple testing (0.05/2). No association was found in all the three ethnic subgroups for *MTHFR* C677T.

Tables 3–5 also demonstrated the distribution of the haplotype frequency. There are four possible combination of haplotypes where

**Table 1 Demographic characteristics of the subjects**

Characteristics	Malay (n = 506)			Chinese (n = 386)			Indian (n = 202)			Total (n = 1094)			
	Case (%)	Control (%)	P-value	Case (%)	Control (%)	P-value	Case (%)	Control (%)	P-value	Case (%)	Control (%)	P-value <sup>a</sup>	OR <sup>b</sup> (95% CI)
Age (years) <sup>F</sup>													
Mean (s.d.)	45 (16)	45 (15)	0.92	0.99 (0.99–1.01)	52 (16)	51 (15)	0.83	1.00 (0.99–1.01)	49 (16)	50 (16)	0.92	1.00 (0.98–1.02)	48 (16)
Sex, n (%)													
Males	118 (59)	171 (56)	—	1	67 (55)	143 (54)	—	1	35 (67)	97 (65)	—	—	411 (57)
Females	81 (41)	136 (44)	0.43	1.16 (0.81–1.66)	54 (45)	122 (46)	0.80	1.06 (0.69–1.63)	17 (33)	53 (35)	0.73	1.13 (0.58–2.20)	152 (41)
NHL, n (%)													
DLBCL	109 (55)				65 (54)				17 (33)				191 (51)
FL	25 (12)				15 (12)				10 (19)				50 (13)
Others <sup>d</sup>	65 (33)				41 (34)				25 (48)				131 (35)
Total	199 (100)	307 (100)			121 (100)	265 (100)			52 (100)	150 (100)			372 (100)

Abbreviations: CI, confidence interval; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; NHL, non-Hodgkin's lymphoma; OR, odds ratio.  
<sup>a</sup>P-values based on combining results across ethnicities.  
<sup>b</sup>ORs and 95% CIs were estimated using logistic regression analysis and adjusted for age, gender and ethnicity.  
<sup>c</sup>Age at study for patients and controls.  
<sup>d</sup>Others: Chronic lymphocytic leukemia (CLL), Adult T-cell leukemia (ATLL), Anaplastic large cell lymphoma, T-cell (ALCL), Burkitt lymphoma (BL), Mantle cell lymphoma (MCL), Lymphoplasmacytic lymphoma (LPL), Hairy cell leukemia-variant (HCL-V), Peripheral T-cell lymphoma, NOS (PTCL, NOS), Marginal zone lymphoma (MZL), Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) (MAL), Extranodal NK/T-cell lymphoma, nasal type (ENNKTL), Splenic marginal zone lymphoma (SMZL), Small lymphocytic lymphoma (SLL), Primary cutaneous follicle center lymphoma (PCFCL), Angioimmunoblastic T-cell lymphoma (AITL), Aggressive NK-cell leukemia (ANKL), Mycosis fungoides (MF), Primary mediastinal (thymic) large B-cell lymphoma (MLBCL), Primary cutaneous T-cell lymphoma, NOS (PCTCL, NOS) and Subcutaneous panniculitis-like T-cell lymphoma (SPTCL).

**Table 2 Distribution of histological subtypes among cases**

Histological subtype	Patient (%), n = 372
<i>Non-Hodgkin's lymphoma (NHL)</i>	
<i>B-cell NHL</i>	
Diffuse large B-cell lymphoma (DLBCL)	191 (51)
Follicular lymphoma (FL)	50 (13)
Other B-cell NHL subtype	
Chronic lymphocytic leukemia (CLL)	36 (10)
Burkitt lymphoma (BL)	9 (3)
Mantle cell lymphoma (MCL)	9 (3)
Lymphoplasmacytic lymphoma (LPL)	10 (3)
Hairy cell leukemia-variant (HCL-V)	6 (2)
Others <sup>a</sup>	21 (6)
Total	332 (89)
<i>T-cell NHL</i>	
Adult T-cell leukemia (ATLL)	15 (4)
Anaplastic large cell lymphoma, T-cell (ALCL)	10 (3)
Peripheral T-cell lymphoma, NOS (PTCL, NOS)	6 (2)
Extranodal NK/T-cell lymphoma, nasal type (ENNKTL)	4 (1)
Others <sup>b</sup>	5 (1)
Total	40 (11)

<sup>a</sup>Others: Marginal zone lymphoma (MZL), Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) (MAL), Splenic marginal zone lymphoma (SMZL), Small lymphocytic lymphoma (SLL), Primary cutaneous follicle center lymphoma (PCFCL) and Primary mediastinal (thymic) large B-cell lymphoma (MLBCL).

<sup>b</sup>Others: Angioimmunoblastic T-cell lymphoma (AITL), Aggressive NK-cell leukemia (ANKL), Mycosis fungoides (MF), Primary cutaneous T-cell lymphoma, NOS (PCTCL, NOS) and Subcutaneous panniculitis-like T-cell lymphoma (SPTCL).

only three (CA, CC and TA) were presented with frequencies of above 5%. Haplotype TC (< 5%) was not taken into account in the analysis as result generated will not be meaningful. The highest frequency was observed for haplotype CA, followed by CC and TA, and this was seen in all the three ethnic subgroups. Haplotype analysis revealed a significantly higher frequency of the haplotype CC in the Indian patients compared with controls ( $P = 0.007$ ). Although we did not see significant finding for single marker of *MTHFR* C677T, combination of markers (haplotype) showed increased susceptibility to NHL (OR 1.86, 95% CI 1.18–2.93,  $P = 0.007$ ). Interestingly, both marker SNPs were found to be in strong linkage disequilibrium ( $D' = 0.83$ ).

**DISCUSSION**

In the present study, we investigated the associations between the *MTHFR* C677T and A1298C polymorphisms and susceptibility to overall NHL and its subtypes in the Malaysian tri-ethnic population. Our results showed no significant association for both SNPs in the pooled subjects, however significant difference was shown for *MTHFR* A1298C in the Indians.

Single marker SNP analysis of *MTHFR* C677T failed to demonstrate any significant association with susceptibility to NHL in the pooled population. This finding is in contrast with reports from the Koreans<sup>20</sup> and Italians,<sup>13</sup> but is in the direction with many other published reports; West Siberians,<sup>21</sup> Jordanians,<sup>22</sup> Swedish,<sup>23</sup> Russians,<sup>24</sup> Saudi-Arabians,<sup>25</sup> Turkish,<sup>26,27</sup> French,<sup>28</sup> Germans,<sup>29,30</sup> Egyptians,<sup>17</sup> British,<sup>14</sup> Italians<sup>31</sup> and Spanish.<sup>32</sup> The difference in the findings probably reflects the differences in genetic pools between various populations studied.

Our result of the *MTHFR* A1298C in the pooled subjects was supported by most of the studies which found no significant

**Table 3 The relationship of C677T and A1298C polymorphisms with susceptibility to NHL**

Allele/genotype/ haplotype	Malay (n = 506)				Chinese (n = 386)				Indian (n = 202)				Total (n = 1094)			
	Case (%)	Control (%)	P-value	OR (95% CI)	Case (%)	Control (%)	P-value	OR (95% CI)	Case (%)	Control (%)	P-value	OR (95% CI)	Case (%)	Control (%)	P-value <sup>a</sup>	OR <sup>b</sup> (95% CI)
<b>C667T</b>																
C	337 (85)	538 (88)	—	1	182 (75)	408 (77)	—	1	97 (93)	276 (92)	—	1	616 (83)	1222 (85)	—	1
T	61 (15)	76 (12)	0.21	1.28 (0.89–1.84)	60 (25)	122 (23)	0.66	1.10 (0.77–1.57)	7 (7)	24 (8)	0.84	0.83 (0.35–1.99)	128 (17)	222 (15)	0.29	1.14 (0.90–1.45)
CC	144 (72)	236 (77)	—	1	67 (55)	155 (59)	—	1	45 (87)	128 (85)	—	1	256 (69)	519 (72)	—	1
CT	49 (25)	66 (21)	0.36	1.22 (0.80–1.86)	48 (40)	98 (37)	0.59	1.13 (0.72–1.78)	7 (13)	20 (14)	0.99	1.00 (0.40–2.51)	104 (28)	184 (25)	0.37	1.14 (0.86–1.51)
TT	6 (3)	5 (2)	0.27	1.97 (0.59–6.56)	6 (5)	12 (4)	0.78	1.16 (0.42–3.21)	0 (0)	2 (1)	1.00	0 (0)	12 (3)	19 (3)	0.51	1.28 (0.61–2.69)
<b>A1298C</b>																
A	290 (73)	421 (69)	—	1	188 (78)	405 (76)	—	1	49 (47)	189 (63)	—	1	527 (71)	1015 (70)	—	1
C	108 (27)	193 (31)	0.16	0.81 (0.62–1.07)	54 (22)	125 (24)	0.77	0.93 (0.65–1.34)	55 (53)	111 (37)	0.006*	1.91 (1.22–3.00)	217 (29)	429 (30)	0.98	1.00 (0.82–1.22)
AA	104 (52)	137 (45)	—	1	74 (61)	160 (60)	—	1	11 (21)	57 (38)	—	1	189 (51)	354 (49)	—	1
CA	82 (41)	147 (48)	0.10	0.74 (0.51–1.07)	40 (33)	85 (32)	0.94	1.02 (0.64–1.62)	27 (52)	75 (50)	0.12	1.87 (0.85–4.07)	149 (40)	307 (43)	0.52	0.92 (0.70–1.20)
CC	13 (7)	23 (7)	0.43	0.75 (0.36–1.54)	7 (6)	20 (8)	0.55	0.76 (0.31–1.87)	14 (27)	18 (12)	0.004*	4.03 (1.56–10.43)	34 (9)	61 (8)	0.58	1.14 (0.72–1.81)
<b>C667T-A1298C</b>																
CA	232 (58)	353 (58)	0.89	1	128 (53)	285 (54)	0.76	1	44 (43)	167 (56)	0.06	1	405 (55)	804 (55)	0.58	1
CC	105 (26)	185 (30)	0.18	0.82 (0.62–1.09)	54 (22)	123 (23)	0.77	0.95 (0.66–1.36)	52 (50)	109 (36)	0.007*	1.86 (1.18–2.93)	211 (28)	418 (29)	0.78	0.97 (0.80–1.18)
TA	58 (15)	68 (11)	0.11	1.36 (0.94–1.98)	60 (25)	120 (23)	0.52	1.12 (0.79–1.60)	4 (4)	22 (7)	0.28	0.56 (0.20–1.62)	122 (16)	211 (15)	0.26	1.15 (0.90–1.46)
TC	3 (1)	8 (1)	—	—	0 (0)	2 (0)	—	—	2 (3)	2 (1)	—	—	6 (1)	11 (1)	—	—

Abbreviations: CI, confidence interval; OR, odds ratio.

\*Significant value.

<sup>a</sup>P-values based on combining results across ethnicities.<sup>b</sup>ORs and 95% CIs were estimated using logistic regression analysis and adjusted for ethnicity.

**Table 4 The relationship of C677T and A1298C polymorphisms with susceptibility to DLBCL**

Allele/genotype/ haplotype	Malay (n = 416)				Chinese (n = 330)				Indian (n = 167)				Total (n = 913)			
	Case (%)	Control (%)	P-value	OR (95% CI)	Case (%)	Control (%)	P-value	OR (95% CI)	Case (%)	Control (%)	P-value	OR (95% CI)	Case (%)	Control (%)	P-value <sup>a</sup>	OR <sup>b</sup> (95% CI)
<b>C667T</b>																
C	180 (83)	538 (88)	—	1	100 (77)	408 (77)	—	1	31 (91)	276 (92)	—	1	311 (81)	1222 (85)	—	1
T	38 (17)	76 (12)	0.08	1.49 (0.98–2.29)	30 (23)	122 (23)	0.92	1.00 (0.64–1.58)	3 (9)	24 (8)	0.87	1.11 (0.32–3.91)	71 (19)	222 (15)	0.13	1.26 (0.94–1.70)
CC	75 (69)	236 (77)	—	1	37 (57)	155 (59)	—	1	14 (82)	128 (86)	—	1	126 (66)	519 (72)	—	1
CT	30 (27)	66 (21)	0.16	1.43 (0.86–2.37)	26 (40)	98 (37)	0.71	1.11 (0.63–1.95)	3 (18)	20 (13)	0.64	1.37 (0.36–5.20)	59 (31)	184 (25)	0.12	1.32 (0.93–1.89)
TT	4 (4)	5 (2)	0.18	2.52 (0.66–9.62)	2 (3)	12 (4)	0.65	0.70 (0.15–3.26)	0 (0)	2 (1)	1.00	0 (0)	6 (3)	19 (3)	0.56	1.32 (0.51–3.41)
<b>A1298C</b>																
A	155 (71)	421 (69)	—	1	101 (78)	405 (76)	—	1	17 (50)	189 (63)	—	1	273 (71)	1015 (70)	—	1
C	63 (29)	193 (31)	0.54	0.89 (0.63–1.24)	29 (22)	125 (24)	0.85	0.93 (0.59–1.47)	17 (50)	111 (37)	0.20	1.70 (0.84–3.47)	109 (29)	429 (30)	0.74	0.96 (0.75–1.23)
AA	53 (49)	137 (45)	—	1	40 (62)	160 (60)	—	1	5 (29)	57 (38)	—	1	98 (51)	354 (49)	—	1
CA	49 (45)	147 (48)	0.52	0.86 (0.55–1.36)	21 (32)	85 (32)	0.97	0.99 (0.55–1.78)	7 (42)	75 (50)	0.92	1.06 (0.32–3.53)	77 (40)	307 (43)	0.49	0.89 (0.63–1.25)
CC	7 (6)	23 (7)	0.60	0.79 (0.32–1.94)	4 (6)	20 (8)	0.70	0.80 (0.26–2.47)	5 (29)	18 (12)	0.09	3.17 (0.82–12.19)	16 (9)	61 (8)	0.93	1.03 (0.56–1.88)
<b>C667T-A1298C</b>																
CA	119 (55)	352 (58)	0.47	1	71 (55)	285 (54)	0.91	1	14 (41)	167 (56)	0.10	1	204 (53)	804 (55)	0.41	1
CC	61 (28)	185 (30)	0.50	0.89 (0.63–1.25)	29 (22)	122 (23)	0.82	0.95 (0.60–1.50)	17 (51)	109 (36)	0.13	1.74 (0.85–3.54)	107 (28)	418 (29)	0.69	0.95 (0.74–1.22)
TA	36 (16)	68 (11)	0.08	1.56 (1.01–2.42)	30 (23)	120 (23)	0.92	1.02 (0.65–1.61)	3 (8)	22 (7)	0.75	1.23 (0.35–4.34)	69 (18)	211 (15)	0.10	1.28 (0.95–1.73)
TC	2 (1)	8 (1)	—	—	0 (0)	2 (0)	—	—	0 (0)	2 (1)	—	—	2 (1)	11 (1)	—	—

Abbreviations: CI, confidence interval; DLBCL, diffuse large B-cell lymphoma; OR, odds ratio.  
<sup>a</sup>P-values based on combining results across ethnicities.  
<sup>b</sup>ORs and 95% CIs were estimated using logistic regression analysis and adjusted for ethnicity.



Table 5 The relationship of C677T and A1298C polymorphisms with susceptibility to FL

Allele/genotype/ haplotype	Malay (n = 332)			Chinese (n = 280)			Indian (n = 160)			Total (n = 772)						
	Case (%)	Control (%)	P-value	Case (%)	Control (%)	P-value	Case (%)	Control (%)	P-value	Case (%)	Control (%)	P-value <sup>a</sup>	OR <sup>b</sup> (95% CI)			
<b>C667T</b>																
C	43 (86)	538 (88)	—	1	23 (77)	408 (77)	—	1	19 (95)	276 (92)	—	1	85 (85)	1222 (85)	—	1
T	7 (14)	76 (12)	0.91	1.15 (0.50–2.65)	7 (23)	121 (23)	0.87	1.03 (0.43–2.45)	1 (5)	24 (8)	0.96	0.61 (0.08–4.72)	15 (15)	222 (15)	0.92	0.97 (0.55–1.71)
CC	20 (80)	236 (77)	—	1	10 (67)	155 (59)	—	1	9 (90)	128 (86)	—	1	39 (78)	519 (72)	—	1
CT	4 (16)	66 (21)	0.33	0.54 (0.16–1.86)	3 (20)	98 (37)	0.27	0.47 (0.13–1.77)	1 (10)	20 (13)	0.75	0.71 (0.09–5.92)	9 (18)	184 (25)	0.10	0.50 (0.22–1.15)
TT	1 (4)	5 (2)	0.07	4.72 (0.86–25.89)	2 (13)	12 (4)	0.25	2.58 (0.51–13.16)	0 (0)	2 (1)	1.00	0 (0)	2 (4)	19 (3)	0.07	2.81 (0.91–8.67)
<b>A1298C</b>																
A	39 (78)	421 (69)	—	1	21 (70)	405 (76)	—	1	10 (50)	189 (63)	—	1	70 (70)	1015 (70)	—	1
C	11 (22)	193 (31)	0.22	0.62 (0.31–1.23)	9 (30)	125 (24)	0.56	1.39 (0.62–3.11)	10 (50)	111 (37)	0.36	1.70 (0.69–4.22)	30 (30)	429 (30)	0.93	1.02 (0.66–1.59)
AA	16 (64)	137 (45)	—	1	7 (47)	160 (60)	—	1	1 (10)	57 (38)	—	1	24 (48)	354 (49)	—	1
CA	7 (28)	147 (48)	0.06	0.41 (0.16–1.02)	7 (47)	85 (32)	0.25	1.88 (0.64–5.54)	8 (80)	75 (50)	0.09	6.08 (0.74–50.00)	22 (44)	307 (43)	0.85	1.06 (0.58–1.93)
CC	2 (8)	23 (7)	0.71	0.75 (0.16–3.46)	1 (6)	20 (8)	0.90	1.14 (0.13–9.77)	1 (10)	18 (12)	0.42	3.17 (0.19–53.23)	4 (8)	61 (8)	0.98	0.99 (0.33–2.95)
<b>C667T-A1298C</b>																
CA	32 (64)	353 (58)	0.43	1	14 (47)	285 (54)	0.43	1	10 (47)	167 (56)	0.55	1	55 (55)	804 (55)	0.83	1
CC	11 (22)	185 (30)	0.21	0.64 (0.32–1.28)	9 (30)	122 (23)	0.40	1.41 (0.63–3.17)	10 (47)	109 (36)	0.30	1.62 (0.65–4.07)	30 (30)	418 (29)	0.86	1.04 (0.67–1.62)
TA	7 (14)	68 (11)	0.56	1.29 (0.56–2.97)	7 (23)	120 (23)	0.93	1.04 (0.43–2.48)	1 (3)	22 (7)	0.66	0.33 (0.04–2.58)	15 (15)	211 (15)	0.94	1.02 (0.58–1.80)
TC	0 (0)	8 (1)	—	—	0 (0)	2 (0)	—	—	1 (3)	2 (1)	—	—	0 (0)	11 (1)	—	—

Abbreviations: CI, confidence interval; OR, odds ratio.

<sup>a</sup>P-values based on combining results across ethnicities.<sup>b</sup>ORs and 95% CIs were estimated using logistic regression analysis and adjusted for ethnicity.

association to NHL; West Siberians,<sup>21</sup> Swedish,<sup>23</sup> Russians,<sup>24</sup> French,<sup>28</sup> British,<sup>14</sup> Italians<sup>13</sup> and Germans.<sup>33</sup> Studies by the Jordanians,<sup>22</sup> Koreans<sup>20</sup> and Egyptians<sup>17</sup> however demonstrated positive significant findings. Surprisingly, we were able to replicate these Middle East and Eastern reports in our Indian ethnic subgroup. The risk of NHL in our Indians is however greater than the others (1.91, 1.63, 1.20 and 0.365, for the Malaysian Indians, Jordanians, Koreans and Egyptians, respectively). The significant association in the Malaysian Indians is in contrast to the absence of such association in the Malays and Chinese. In the 1800s, the Malaysian Indians migrated to Malaysia from Southern India.<sup>34</sup> Studies have suggested that the Indians from India were proto-Asian origin with West Eurasian admixture, therefore giving them the genetic affinity towards both Asian and European.<sup>11,35</sup> It is known that the highest incidence rates of NHL is in the USA, New Zealand and Australia, and Europe, and the lowest in the Eastern and South Central Asia.<sup>36</sup> Furthermore, the *MTHFR* A1298C C-allele and CC genotype frequencies were shown to be significantly higher among South Indians.<sup>37</sup>

Despite showing no single marker association between *MTHFR* C677T and NHL, association was found for the haplotype (677C–1298C) in the Indians. This finding is anticipated given that the R-squared value is not that high between C677T and A1298C (HapMap 0.184 and 0.096), for JPT+CHB and South Indian population, respectively. Both SNPs were in strong linkage disequilibrium, but were nevertheless lower than that reported in the HapMap. This could be owing to the relatively low sample size in this study and population-specific differences compared with the HapMap data. Another possible explanation could be that the frequency of the *MTHFR* A1298C minor allele was similar between Malaysian Indians (0.37) and Gujarati Indians (0.39) from the HapMap database ([www.hapmap.org](http://www.hapmap.org)). The *MTHFR* C677T minor allele on the other hand, was lower in the Malaysian Indians (0.08) as compared with the Gujarati Indians (0.16).

The genetic effect alone is insufficient to explain the NHL risk. The differences in NHL susceptibility in various populations have implied the significant contribution of genetics. However, most of the Western (Europeans/Caucasians) studies failed to demonstrate positive associations,<sup>14,23,24,28–30,32,38</sup> despite being the population with the highest incidence rate (double or triple than the Eastern).<sup>3</sup> This indicates possible gene-environment interplay, such as diet, or nutritional intake of folate and related vitamins. The *MTHFR* 677TT and 1298CC genotype leads to decreased levels of 5-methyl THF for DNA methylation and increased 5,10-methylene THF availability for DNA synthesis by thymidylate synthase (TS), which protect cells from DNA damage.<sup>16,39</sup> The decreased *MTHFR* activity modifies the normal intracellular supply of folate substrates in favor of precursors for nucleotide synthesis.<sup>40</sup> This may provide a possible mechanism by which, if sufficient levels of folate are available, although *MTHFR* activity is low, there is adequate conversion of 5-methyl THF for DNA methylation, whereas still shifting 5,10-methylene THF toward the synthesis of deoxyuridine monophosphate to deoxythymidine monophosphate and preventing uracil incorporation as well as causing chromosomal damage. This suggests that differences in folate availability may influence functional effects of *MTHFR* polymorphisms.<sup>41</sup> The addition of folate to foods in Western countries may cover the impact of differential enzyme actions along the folate metabolic pathway.<sup>12</sup>

There are several limitations and strengths of this study. The low incidence of the disease in our study population has caused difficulty in sampling, hence the relatively small study sample size. The limitation in sample size is shared by most of the studies including

the European population which were found to be of high risk for NHL. In spite of that, our study is presented with the total number of NHL patients that are greater than 76% and 69% of the studies, for *MTHFR* C677T and A1298C respectively. Our findings in the pooled subjects did not show significant association between the SNPs and NHL, and this was supported by the latest meta-analysis.<sup>21</sup> Although positive genetic association studies between *MTHFR* SNPs and NHL has been shown for various populations, when further investigated into different NHL subtypes, the results have not always been in concordance for the different populations. This could be owing to the differences in pathogenesis of different NHL subtypes. Limitation is also imposed by the relatively low sample size while trying to preserve the genetic effect.<sup>19</sup> In this study, gene effect alone seems to provide a moderate effect on the association. A study that measures both gene and environmental effects would provide a better result and increase the confidence of the finding. However, none of the studies to date has measured both gene and environmental effects. A major strength of this study was the ability to compare the association between *MTHFR* polymorphisms and NHL among the three major ethnic groups in Malaysia, which are also the main ethnic groups in Asia.<sup>42</sup> This study provides data on the low-risk population that is among the least studied.

In conclusion, this study suggests that ethnicity modifies the relationship between *MTHFR* gene polymorphisms and NHL risk. Future studies that incorporate both genetic and environmental factors will be able to provide a better understanding on the outcome of NHL. Since most of the studies are limited by sample size, a genome-wide association study from multi-centers is necessary. A meta-analysis that analyzes both the Western and Eastern populations separately will give a better picture on disease association with regard to population difference.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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