Tumor Biol. (2016) 37:3265–3275
DOI 10.1007/s13277-015-4168-6

ORIGINAL ARTICLE

Polymorphisms of 5,10-methylenetetrahydrofolate reductase and thymidylate synthase, dietary folate intake, and the risk of leukemia in adults

Ping Liu[1] · **Min Zhang**[1,2] · **Xing Xie**[3] · **Jie Jin**[4] · **C D'Arcy J Holman**[1]

Received: 20 July 2015 / Accepted: 27 September 2015 / Published online: 5 October 2015

Abstract The 5,10-methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthase (TS) are critical enzymes in folate metabolism. Previous studies have reported conflicting results on the associations between MTHFR/TS polymorphisms and adult leukemia risk, which may due to the lack of information on folate intake. We investigated the risks of adult leukemia with genetic polymorphisms of folate metabolic enzymes (MTHFR C677T, A1298C, and TS) and evaluated if the associations varied by dietary folate intake from a multicenter case-control study conducted in Chinese. This study comprised 442 incident adult leukemia cases and 442 outpatient controls, individually matched to cases by gender, birth quinquennium, and study site. Genotypes were determined by a polymerase chain reaction (PCR) or PCR-based restriction fragment length polymorphism assay. Dietary folate intake was assessed by face-to-face interviews using a validated food-frequency questionnaire. The MTHFR 677TT genotype conferred a significant higher risk of leukemia in males than in females and exhibited an increased risk of acute myeloid leukemia (AML) but a decreased risk of acute lymphoblastic leukemia (ALL). The MTHFR 1298AC genotype appeared to decrease the risks of leukemia in both genders, in AML and ALL. Stratified analysis by dietary folate intake showed the increased risks of leukemia with the MTHFR 677TT and TS 2R3R/2R2R genotypes were only significant in individuals with low folate intake. A significant interaction between TS polymorphism and dietary folate intake was observed (P= 0.03). This study suggests that dietary folate intake and gender may modify the associations between MTHFR/TS polymorphisms and adult leukemia risk.

Keywords Adult leukemia risk · MTHFR · TS · Dietary folate intake · Gene-environment interaction · Case-control study

✉ Ping Liu
ping.liu@research.uwa.edu.au

[1] School of Population Health, The University of Western Australia, M431, 35 Stirling Highway, Crawley, Perth, WA 6009, Australia

[2] Center for Healthcare Resilience and Implementation Science, Australian Institute of Health Innovation, Macquarie University, Level 6, 75 Talavera Road, Macquarie, NSW 2109, Australia

[3] Women's Hospital, School of Medicine, Zhejiang University, 1 Xueshi Road, Hangzhou, Zhejiang 310006, People's Republic of China

[4] Department of Hematology, The First Affiliated Hospital, Zhejiang University College of Medicine, 79 Qingchun Road, Hangzhou, Zhejiang 310003, People's Republic of China

Introduction

Leukemias, a group of heterogeneous malignancies, are generally characterized by acquired somatic mutations, including chromosomal translocations, deletions, and inversions [1]. Their etiology likely involves a combination of exogenous or endogenous exposures and genetic susceptibility, as well as complex gene-gene and gene-environment interactions [2, 3]. The four major leukemia subtypes are acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), and chronic lymphocytic leukemia (CLL) [4].

Folate, as a carrier of one-carbon units, is an essential nutrient for nucleotide synthesis, as well as for DNA methylation [5]. Folate deficiency increases uracil misincorporation into DNA and double-strand breaks during excision repair processes, which subsequently induces chromosomal translocations and deletions [5, 6]. Low folate status has been implicated in the development of cancers, notably of the cervix, lung, breast, brain, and colorectum [5]. Low folate status has also

been hypothesized to increase the risk of leukemia [2]. This hypothesis has received some support from our recent observation of an inverse association between dietary folate intake and adult leukemia in a Chinese population [7].

The enzymes 5,10-methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthase (TS) are critical to the metabolism of folate. MTHFR irreversibly catalyzes 5,10-methylenetetrahydrofolate (THF) to the primary form of serum folate, 5-methylTHF, which is the methyl donor for the remethylation of homocysteine to methionine. C677T (rs1801133) and A1298C (rs1801131) are two well-described genetic polymorphisms of MTHFR. The common 677 C→T (Ala→Val) and 1298 A→C (Glu→Ala) mutations of the gene could result in enzymes with reduced activity [8, 9]. Compared with the wild-type 677CC genotype, the mean activity level of MTHFR in vitro is 65 % in the heterozygous variant and approximately 30 % in the homozygous variant [8]. The 1298CC genotype has about 60 % of the activity of the wild-type 1298AA [10]. Individuals with both the 677CT and 1298AC genotypes have 50–60 % specific wild-type MTHFR activity [9, 10].

TS uses 5,10-methyleneTHF as a methyl group donor for conversion of uracil to thymidine for DNA synthesis. The TS gene contains a series of polymorphic 28-base pair tandem repeats in the 5′-terminal regulatory region (rs45445694), of which triple repeats (3R) and double repeats (2R) are the most common [11]. In vivo and in vitro studies show that the 2R allele is associated with decreased TS expression compared with the 3R [12]. TS is essential in the regulation of a balanced supply of DNA precursors for the normal replication and repair of DNA, while diminution in enzymatic activity affecting the regulation process may lead to various biological and genetic abnormalities [13].

Although several studies thus far have investigated whether variant genotypes of MTHFR/TS alter the risk of adult leukemia, the findings have generally been inconclusive [14–18]. It has been hypothesized that the risk of leukemia associated with the MTHFR polymorphisms may depend on folate intake [2]. However, none of the previous studies has assessed the postulated association. In this study, therefore, we were specifically interested to examine the associations between the genetic polymorphisms of folate metabolic enzymes (MTHFR C677T, MTHFR A1298C, and TS) and adult leukemia risk and to determine if dietary folate intake modified the associations.

Materials and methods

Study design and participants The study design has been reported in detail elsewhere [7]. Briefly, a hospital-based case-control study was conducted at three major public hospitals in southeast and northeast China between April 2008 and August 2013, namely the First and the Second Affiliated Hospitals of Zhejiang University in Hangzhou, Zhejiang Province, and the First Hospital of China Medical University in Shenyang, Liaoning Province. The project protocol was approved by the Human Research Ethics Committee of The University of Western Australia and the ethics committees of the participating hospitals in China.

Eligibility criteria for cases were as follows: (i) incident patients with a hematologically confirmed diagnosis of leukemia, (ii) aged 16 years or over, (iii) residing in the selected provinces for at least 1 year, and (iv) presenting as an inpatient to the participating hospitals. Patients with other malignancies were considered ineligible for the study. A total of 442 patients aged 16–86 years were included in the analysis as the cases (response 97.8 %). All cases were interviewed within 1 year of initial diagnosis and most (86.7 %) within 3 months. Leukemia subtype information was available in 363 (82.1 %) of the 442 cases. Among these patients, 243 (66.9 %) had AML, 62 (17.1 %) had ALL, 38 (10.5 %) had CML, and 20 (5.5 %) had CLL.

Eligible outpatient controls were free of malignancy at the time of recruitment and attended the Medical Examination Center of the outpatient department at the same hospitals as their cases. Large panels of controls had been selected and interviewed for our series of case-control studies of colorectal cancer, breast cancer, and leukemia. About 4.5 % of the potential controls who were invited refused to participate. Post hoc matching was then conducted with each control for this study selected as the first attendee to individually match with each case by gender, birth quinquennium, and study site. The date of recruitment of a control never exceeded that for the matching case by more than 1 year.

Questionnaire and interview After informed consent was obtained from each participant, in-person interviews were conducted using a structured questionnaire. Each interview usually took 30–40 min. To minimize information bias, neither the participants nor the interviewers were informed about the specific hypotheses under study. Information was sought from the structured questionnaire on dietary intake, the use of cigarette, alcohol, and tea, family history of cancer, and other demographic and lifestyle characteristics.

Dietary intake was assessed by a quantitative 103-item food-frequency questionnaire (FFQ). The use of vitamin or mineral supplements was also queried on the FFQ. The FFQ used in this study has been described previously [7], and its validity and reliability have been assessed in previous studies [19–21]. The internal reliability across preliminary test, test, and retest was denoted as Cronbach's alpha being 0.81, 0.72, and 0.78, respectively [20, 21]. These high Cronbach's alpha scores suggested that the FFQ was a consistent and reliable

instrument for measuring food consumption overall. Food consumption was based on usual frequency and typical amount of intake. Participants were asked how often they had consumed each food item 1 year before their diagnosis for cases or in the previous year before interview for controls. One of nine possible responses to the frequency of each item of food consumption was assigned: never or hardly ever, once a month, two to three times a month, once a week, two to three times a week, four to six times a week, once a day, twice a day, and greater than or equal to three times a day. The amount of each food item consumed per meal was estimated using the common Chinese measure *liang* (1 *liang*=50 g).

Genotyping methods Blood samples obtained from 424 (95.9 %) cases and 420 (95.0 %) controls were used in this study for genotyping assays. Whole blood was collected in EDTA-coated tubes. Blood samples drawn from participants were stored in a −80 °C refrigerator. Then, batches of the frozen blood specimens were shipped under −20 °C during the door-to-door transportation.

Genotyping was performed at the State Key Laboratory of the Zhejiang University Women's Hospital in Hangzhou. All the samples were assayed by one technician, who was blind to the case-control status, using Biometra TG96 equipment following the manufacturer's instructions. Genotypes of MTHFR C677T and A1298C were determined using polymerase chain reaction (PCR)-restriction fragment length polymorphism methods reported by Frosst et al. [8] and Weisberg et al. [10]. Polymorphism of TS was identified by performing PCR as described by Cui et al. [22], with minor modifications of the reaction conditions for PCR. The reaction mixture was initially denatured at 94 °C for 1 min, followed by 30 cycles of 94 °C for 30 s, 66 °C for 30 s, and 72 °C for 45 s. The last extension was carried out at 72 °C for 7 min. Samples were not amplified in one case for the MTHFR C677T and in one case for the TS. A total of 300 quality control samples were assayed twice, which were randomly selected from our series of case-control studies (5 % of participants). The proportions of reproducibility were 97.7 % for MTHFR A1298C and 98.7 % for both MTHFR C677T and TS.

Data analysis In this study, we estimated the intake of folate only from food sources, as very few participants (<2 %) reported regular use of supplements. The calculation of folate intake was based on daily food consumption, taking edible portions of foods, seasonal factors, and market availability into account [20], and adjusting for energy intake using the density method [23]. Total energy intake (kcal/day) was estimated using the China Food Composition 2009 [24]. Figures for folate were only available in, and thus such figures were derived from, the previous edition of China Food Composition [25, 26]. The median value of daily folate intake (μg/1000 kcal) in the controls with blood samples was used to categorize folate intake as "low" or "high."

Other covariates were described as follows. A total lifetime consumption of 20 packs of cigarettes or more was defined as cigarette smoking. Ever consumed liquor, beer, wine, or any combination was classified as alcohol consumption using abstainers who never drank alcohol as a reference group. Drinking tea more than once per month was classified as tea consumption. Body mass index was calculated using the Quetelet's index expressed in kilograms per square meter. Metabolic equivalent task (MET) hours per week during the past year were used as a measure of physical activity.

Odds ratios (ORs) and 95 % confidence intervals (CIs) from conditional logistic regression [27] were used to evaluate the associations between genetic polymorphisms and adult leukemia risk. Multivariate analyses were conditioned on matched pairs and were further adjusted for other potential confounders [28]. Factors associated with MTHFR/TS variants and associated with the risk of adult leukemia, which were identified from univariate analyses or reported in previous studies [29], were included as covariates. These included resident locality (urban, rural), education (none, primary, secondary, tertiary), pack-years of cigarette smoking (continuous), alcohol consumption (no, yes), and tea consumption (no, yes); other polymorphisms (wild-type, heterozygous variant, and homozygous variant) were simultaneously controlled in the multivariate models. For example, we adjusted all the analyses of MTHFR C677T for the polymorphisms of MTHFR A1298C and TS and vice versa; we adjusted the TS polymorphism for the analysis of MTHFR C677T and A1298C combined. We report both univariate and multivariate adjusted ORs (95 % CIs) in the tables, but only describe the multivariate adjusted risk estimates in the text. We performed separate analyses by gender and by leukemia subtype. Gene dosage effects were tested for trend by using a quantitative variable according to the number of variant alleles (likelihood ratio tests). To evaluate possible effect modification of folate intake on adult leukemia risk associated with MTHFR/TS polymorphisms, we conducted stratified analyses in unconditional logistic regression models (because of break of matching after stratification by dietary folate intake); we compared conditional logistic regression models with and without product terms of dietary folate intake and MTHFR/TS polymorphisms using likelihood ratio tests. The data were analyzed using SAS, version 9.3 (SAS Institute Inc., Cary, NC, USA). *P* values <0.05 were considered statistically significant.

Results

Table 1 reports the selected demographic characteristics, lifestyle, and dietary factors in cases and controls by gender, AML, and ALL. Cases were comparable to controls in terms

Table 1 Selected characteristics of adult leukemia cases and controls by gender, AML, and ALL

Characteristics	Male (*n*=512)		Female (*n*=372)		AML (*n*=486)		ALL (*n*=124)	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Age at interview (years)	46.2±14.9	46.2±14.6	44.1±14.0	44.4±14.1	46.5±13.3	46.6±13.4	37.7±13.8	38.3±13.7
16–29	42 (16.4)	42 (16.4)	28 (15.1)	28 (15.1)	29 (11.9)	29 (11.9)	22 (35.5)	22 (35.5)
30–39	42 (16.4)	42 (16.4)	43 (23.1)	43 (23.1)	46 (18.9)	46 (18.9)	13 (21.0)	13 (21.0)
40–49	60 (23.4)	60 (23.4)	53 (28.5)	53 (28.5)	65 (26.7)	65 (26.7)	14 (22.6)	14 (22.6)
50–59	60 (23.4)	60 (23.4)	33 (17.7)	33 (17.7)	60 (24.7)	60 (24.7)	7 (11.3)	7 (11.3)
60–69	40 (15.6)	40 (15.6)	23 (12.4)	23 (12.4)	37 (15.2)	37 (15.2)	5 (8.1)	5 (8.1)
70–86	12 (4.7)	12 (4.7)	6 (3.2)	6 (3.2)	6 (2.5)	6 (2.5)	1 (1.6)	1 (1.6)
Male	256 (100.0)	256 (100.0)	0 (0.0)	0 (0.0)	136 (56.0)	136 (56.0)	35 (56.5)	35 (56.5)
Resident locality								
Urban	202 (78.9)	219 (85.5)	149 (80.1)	156 (83.9)	191 (78.6)	208 (85.6)	49 (79.0)	54 (87.1)
Rural	54 (21.1)	37 (14.5)*	37 (19.9)	30 (16.1)	52 (21.4)	35 (14.4)*	13 (21.0)	8 (12.9)
Education								
None	4 (1.6)	4 (1.6)	11 (5.9)	9 (4.8)	9 (3.7)	6 (2.5)	1 (1.6)	2 (3.2)
Primary	32 (12.5)	27 (10.5)	28 (15.1)	21 (11.3)	30 (12.3)	22 (9.1)	4 (6.5)	3 (4.8)
Secondary	169 (66.0)	112 (43.8)	117 (62.9)	87 (46.8)	164 (67.5)	99 (40.7)	42 (67.7)	23 (37.1)
Tertiary	51 (19.9)	113 (44.1)*	30 (16.1)	69 (37.1)*	40 (16.5)	116 (47.7)*	15 (24.2)	34 (54.8)*
Cigarette smoking	157 (61.3)	126 (49.2)*	5 (2.7)	4 (2.2)	83 (34.2)	76 (31.3)	21 (33.9)	9 (14.5)*
Alcohol consumption	186 (72.7)	174 (68.0)	70 (37.6)	57 (30.6)	135 (55.6)	136 (56.0)	36 (58.1)	28 (45.2)
Tea consumption	161 (62.9)	183 (71.5)*	61 (32.8)	93 (50.0)*	118 (48.6)	167 (68.7)*	27 (43.5)	38 (61.3)*
Body mass index (kg/m^2)								
<25	214 (83.6)	190 (74.2)	153 (82.3)	165 (88.7)	191 (78.6)	198 (81.5)	55 (88.7)	53 (85.5)
≥25	42 (16.4)	66 (25.8)*	33 (17.7)	21 (11.3)	52 (21.4)	45 (18.5)	7 (11.3)	9 (14.5)
Physical activity (weekly MET hour)	84.4±96.4	78.3±83.6	86.4±80.2	87.4±75.0	80.9±83.9	83.6±82.0	84.8±74.5	79.7±78.6
Any cancer in first-degree relatives	32 (12.5)	23 (9.0)	14 (7.5)	21 (11.3)	29 (11.9)	26 (10.7)	5 (8.1)	7 (11.3)
Daily folate intake (μg/1000 kcal)	109.6±27.4	119.7±26.2*	120.2±31.1	126.6±27.3*	113.5±29.8	126.4±27.1*	113.7±31.4	124.0±25.7
Daily energy intake (kcal)	3081.6±1235.8	2846.0±768.4*	2215.6±650.2	2195.7±632.0	2740.6±1219.9	2429.9±687.7*	2799.9±1265.5	2511.1±788.7*

Values expressed as mean±SD or number (percent)

*$P<0.05$ derived from univariate conditional logistic regression with one degree of freedom

of age, gender, alcohol consumption, physical activity, and cancer history in first-degree relatives. The cases were less educated and were less likely to consume tea than the controls. The male cases and the AML cases were more likely to live in rural areas than their controls. Compared with their controls, the male cases and the ALL cases were more likely to smoke. In males, the cases had a lower proportion of body mass index ≥25 than the controls. The cases in both genders and the AML cases had a lower average daily intake of folate per 1000 kcal than their controls. The male cases and the AML and ALL cases had a higher mean daily intake of energy than their controls.

Table 2 shows the distributions of the MTHFR/TS genotypes between the cases and controls by gender. The MTHFR C677T and A1298C genotypes were found to deviate slightly from Hardy-Weinberg equilibrium (HWE) in the controls (χ^2=4.61, P=0.03 for MTHFR C677T; χ^2=4.75, P=0.03 for MTHFR A1298C). Among the controls, the distribution of TS genotypes was in accordance with HWE (χ^2=2.26, P=0.13).

In the analyses of MTHFR/TS polymorphisms with the susceptibility of leukemia by gender (Table 2), we found significant differences in risks between males and females. Among the males, the homozygous variants were significantly associated with elevated risks of leukemia, with adjusted ORs (95 % CIs) of 2.88 (1.51–5.49) for MTHFR 677TT versus CC, 10.43 (1.71–63.44) for MTHFR 1298CC versus AA, and 5.70 (1.72–18.86) for TS 2R2R versus 3R3R. Positive trends were found with the increasing variant alleles of MTHFR C677T (P=0.001) and TS (P=0.03), with an exception of the MTHFR A1298C. Using the MTHFR 677CC and 1298AA as a referent group, the combined 677CT and

Table 2 Associations between MTHFR/TS polymorphisms and adult leukemia risk by gender

Genotype	Males (n = 491)			Females (n = 353)			$P_{interaction}$[e]
	Cases/controls	Univariate OR (95% CI)[a]	Adjusted OR (95% CI)[b]	Cases/controls	Univariate OR (95% CI)[a]	Adjusted OR (95% CI)[b]	
MTHFR C677T							0.02
CC	75/107	1.00[c]	1.00[c]	65/53	1.00[c]	1.00[c]	
CT	112/103	1.61 (1.09–2.39)	1.63 (1.02–2.61)	71/78	0.69 (0.42–1.14)	0.69 (0.38–1.26)	
TT	58/36	2.06 (1.24–3.41)	2.88 (1.51–5.49)	42/43	0.71 (0.37–1.38)	0.64 (0.28–1.48)	
P_{trend}[d]		0.002	0.001		0.20	0.22	
MTHFR A1298C							0.82
AA	180/168	1.00[c]	1.00[c]	132/114	1.00[c]	1.00[c]	
AC	53/75	0.67 (0.44–1.03)	0.63 (0.37–1.05)	34/57	0.54 (0.33–0.90)	0.46 (0.25–0.86)	
CC	12/3	3.94 (0.84–18.42)	10.43 (1.71–63.44)	13/3	3.80 (1.07–13.45)	3.80 (0.95–15.22)	
P_{trend}[d]		0.58	0.96		0.85	0.74	
TS							0.42
3R3R	148/160	1.00[c]	1.00[c]	114/108	1.00[c]	1.00[c]	
2R3R	76/80	1.03 (0.68–1.55)	1.13 (0.70–1.84)	51/61	0.83 (0.53–1.30)	0.98 (0.57–1.68)	
2R2R	21/6	3.55 (1.40–8.98)	5.70 (1.72–18.86)	13/5	2.49 (0.78–8.02)	2.65 (0.74–9.52)	
P_{trend}[d]		0.05	0.03		0.78	0.41	
MTHFR C677T and A1298C							0.14
CC/AA	42/65	1.00[c]	1.00[c]	37/25	1.00[c]	1.00[c]	
CT/AA	82/69	1.86 (1.11–3.09)	1.88 (1.05–3.34)	53/49	0.69 (0.36–1.33)	0.74 (0.36–1.54)	
TT/AA	56/34	2.30 (1.30–4.08)	3.22 (1.61–6.44)	41/40	0.67 (0.31–1.42)	0.82 (0.34–1.97)	
CC/AC	23/40	0.88 (0.45–1.71)	0.75 (0.36–1.58)	19/26	0.52 (0.24–1.14)	0.58 (0.23–1.45)	
CT/AC	28/33	1.24 (0.66–2.34)	1.05 (0.48–2.29)	15/28	0.35 (0.15–0.82)	0.34 (0.13–0.88)	
TT/AC	2/2	4.15 (1.06–16.16)[f]	7.25 (1.46–35.96)[f]	0/3	1.44 (0.49–4.28)[f]	1.76 (0.53–5.90)[f]	
CC/CC	10/2			9/2			
CT/CC	2/1			3/1			
TT/CC	0/0			1/0			
MTHFR C677T and A1298C variant alleles							0.03
0	42/65	1.00[c]	1.00[c]	37/25	1.00[c]	1.00[c]	
1	105/109	1.52 (0.95–2.45)	1.52 (0.90–2.59)	72/75	0.63 (0.34–1.15)	0.68 (0.35–1.32)	
2	94/69	1.89 (1.17–3.03)[f]	2.21 (1.26–3.88)[f]	65/70	0.60 (0.32–1.12)[f]	0.68 (0.34–1.38)[f]	
≥3	4/3			4/4			
P_{trend}[d]		0.01	0.006		0.20	0.41	
Sum of MTHFR/TS variant alleles							0.02
0	26/50	1.00[c]	1.00[c]	21/19	1.00[c]	1.00[c]	
1	74/85	1.88 (1.05–3.37)	1.92 (1.02–3.59)	67/49	1.25 (0.56–2.76)	1.41 (0.59–3.39)	
2	96/74	2.53 (1.42–4.52)	3.11 (1.61–5.99)	53/72	0.63 (0.30–1.33)	0.76 (0.33–1.77)	
≥3	49/37	2.43 (1.26–4.69)	3.07 (1.48–6.36)	36/34	0.89 (0.41–1.94)	1.29 (0.54–3.12)	
P_{trend}[d]		0.002	<0.001		0.39	0.94	

[a] Estimates were derived from univariate conditional logistic regression model, matched-pair conditioned on gender, birth quinquennium, and study site

[b] Estimates were derived from multivariate conditional logistic regression model adjusted for resident locality, education, pack-years of cigarette smoking, alcohol consumption, tea consumption, and other polymorphisms (as described in "Data analysis")

[c] Reference category

[d] Tests for trend were performed by using quantitative variable according to the number of variant alleles

[e] P for interaction was based on Wald chi-square value derived from multivariate conditional logistic regression model by including main effect variables (gender and MTHFR/TS polymorphisms) and their product terms and plausible confounding factors listed above

[f] Risk estimates were for combined groups

1298AA genotypes (adjusted OR=1.88; 95 % CI 1.05–3.34) and the combined 677TT and 1298AA genotypes (adjusted OR=3.54; 95 % CI 1.41–8.91) were associated with increased risks; the risk further increased for the combination of 677TT and 1298AC and the combination of harboring 1298CC regardless of the C677T genotypes (adjusted OR=7.25; 95 % CI 1.46–35.96). Finally, we observed a significant and positive dose-response relationship between the sum of MTHFR/TS variant alleles and leukemia risk in males (*P*, trend=0.002).

Among the females, the risks of leukemia with the MTHFR/TS polymorphisms were consistently lower than those in males. Compared with the 677CC genotype, the adjusted ORs (95 % CIs) were 0.69 (0.38–1.26) for 677CT and 0.64 (0.28–1.48) for 677TT in females. A significant interaction between the MTHFR C677T polymorphism and gender was seen (*P*, interaction=0.02). There was no significant difference in susceptibility between males and females with MTHFR A1298C and TS polymorphisms. It was noteworthy that the heterozygous variant MTHFR 1298AC was associated with a lower risk of leukemia relative to the wild-type 1298AA in both genders, with adjusted ORs (95 % CIs) being 0.63 (0.37–1.05) in males and 0.46 (0.25–0.86) in females.

Table 3 presents the risks of AML and ALL with MTHFR/TS polymorphisms. A discrepancy in risks was observed between AML and ALL. We found elevated risks of AML for the MTHFR 677TT genotype and for a greater number of MTHFR/TS variant alleles, whereas we observed inverse associations for ALL with these variants, though these associations were not statistically significant. Notably, 1298AC individuals were at lower risk of AML (adjusted OR=0.60; 95 % CI 0.35−1.03) and ALL (adjusted OR=0.14; 95 % CI 0.03−0.69), compared with those with the MTHFR 1298AA genotype. It was impractical to calculate risk estimates for CML and CLL due to small numbers.

The associations between MTHFR/TS polymorphisms and adult leukemia risk after stratification by dietary folate intake are presented in Table 4. Overall, the risks of adult leukemia with variant alleles of MTHFR C677T and TS were higher in the subgroup whose daily dietary intake of folate was <119.5 μg/1000 kcal. There was a significant multiplicative interaction between TS polymorphism and folate intake (*P*=0.03). The presence of TS 2R3R/2R2R significantly elevated the risk in individuals with a low dietary folate intake (adjusted OR=1.53; 95 % CI 1.01–2.31) compared with those with a high dietary folate intake (adjusted OR=0.66; 95 % CI 0.41–1.06). Although dietary folate intake did not significantly modulate the risks of adult leukemia with polymorphisms of MTHFR C677T and A1298C, the risk associated with the 677TT genotype was 2.26-fold increased (95 % CI 1.24–4.10) in individuals with a low dietary folate intake; in contrast, the risk did not significantly vary by C677T genotype in individuals with a high dietary folate intake.

Discussion

We report a multicenter case-control study conducted in China to investigate the associations between three polymorphisms of MTHFR C677T, A1298C, and TS and leukemia susceptibility in adults. We conducted separate analyses by gender, by leukemia subtype, and by dietary folate intake.

A slight departure from HWE was observed for the MTHFR C677T and A1298C polymorphisms among the controls. Such a departure was unlikely to have resulted from genotyping error because of the high concordance of quality control samples (98.7 % for C677T and 97.7 % for A1298C). Moreover, the frequencies of 677T (40 %) and 1298C (17 %) alleles in our controls were very similar to those in other studies conducted in the Chinese and Korean populations [30, 31]. No controls and only one case had the combined MTHFR 677TT and 1298CC genotypes, which was also consistent with literature described that both homozygous variants of C677T and A1298C are rare in general populations [2, 9, 10]. The frequency of the TS 2R allele in our controls (19 %) was consistent with that in Koreans (18 %) [31].

MTHFR/TS polymorphisms and adult leukemia risk by gender Results from this investigation suggest that adult males with the MTHFR 677TT, 1298CC, and TS 2R2R genotypes are more likely to develop leukemia. MTHFR is a key regulatory enzyme that can direct available folate toward the methylation of homocysteine at the expense of nucleotide synthesis. The MTHFR 677TT and 1298CC genotypes result in lower enzyme activity. The reduced MTHFR enzyme activity is less efficient in converting 5,10-methyleneTHF to 5-methylTHF as a consequence, leading to an increased level of cytosolic 5,10-methyleneTHF available for thymidylate, an essential precursor of de novo synthesis of DNA. On the other hand, less MTHFR enzyme activity also affects de novo methionine synthesis and causes imbalanced DNA methylation. The current study found that the risk of leukemia in males was considerably elevated for 677TT (2.88-fold) and for 1298CC (10.43-fold) compared with their wild-type homozygotes. The observed positive associations may suggest that the effects on genomic instability are mediated more by influences on insufficient DNA methylation than by influences on nucleotide synthesis [32] and thus aberrant DNA methylation may contribute to tumorigenesis.

The study found that the MTHFR 1298AC genotype was inversely associated with leukemia risk in both genders. Significant lower risks of leukemia in females were noted in the analyses of the single polymorphism of A1298C, and the joint associations of A1298C and C677T. Non-statistically significant decreased risks were observed in males for the 1298AC genotype or combined genotypes of 1298AC with 677CC. The protective effect of the MTHFR 1298AC genotype may

Table 3 Associations between MTHFR/TS polymorphisms and the risk of AML and ALL

Genotype	AML (n=462)			ALL (n=119)		
	Cases/controls	Univariate OR (95 % CI)[a]	Adjusted OR (95 % CI)[b]	Cases/controls	Univariate OR (95 % CI)[a]	Adjusted OR (95 % CI)[b]
MTHFR C677T						
CC	72/77	1.00[c]	1.00[c]	23/23	1.00[c]	1.00[c]
CT	105/107	1.12 (0.75–1.69)	1.35 (0.79–2.29)	24/25	0.96 (0.42–2.19)	0.70 (0.16–3.20)
TT	51/49	1.04 (0.61–1.77)	1.51 (0.73–3.11)	12/12	0.86 (0.29–2.54)	0.32 (0.05–1.97)
P_{trend}[d]		0.79	0.24		0.79	0.22
MTHFR A1298C						
AA	166/157	1.00[c]	1.00[c]	44/32	1.00[c]	1.00[c]
AC	49/75	0.66 (0.43–1.01)	0.60 (0.35–1.03)	12/27	0.29 (0.12–0.73)	0.14 (0.03–0.69)
CC	14/1	NP	NP	3/1	2.37 (0.24–23.81)	NP
P_{trend}[d]		0.93	0.96		0.07	0.04
TS						
3R3R	139/138	1.00[c]	1.00[c]	45/40	1.00[c]	1.00[c]
2R3R	69/86	0.84 (0.56–1.26)	0.99 (0.60–1.63)	13/19	0.53 (0.22–1.25)	0.82 (0.20–3.34)
2R2R	20/9	2.17 (0.93–5.08)	1.85 (0.66–5.20)	1/1	0.73 (0.04–12.42)	NP
P_{trend}[d]		0.52	0.35		0.17	0.81
Sum of MTHFR/TS variant alleles						
0	25/30	1.00[c]	1.00[c]	10/6	1.00[c]	1.00[c]
1	76/70	1.33 (0.72–2.45)	1.29 (0.63–2.61)	24/19	0.86 (0.21–3.52)	0.58 (0.07–5.06)
2	75/87	1.10 (0.60–2.01)	1.37 (0.68–2.80)	19/25	0.40 (0.12–1.40)	0.18 (0.02–1.60)
≥3	51/46	1.26 (0.65–2.43)	1.60 (0.73–3.51)	6/10	0.35 (0.08–1.55)	0.38 (0.04–3.18)
P_{trend}[d]		0.57	0.19		0.04	0.15

NP risk estimates did not provide due to small numbers

[a] Estimates were derived from univariate conditional logistic regression model, matched-pair conditioned on gender, birth quinquennium, and study site

[b] Estimates were derived from multivariate conditional logistic regression model adjusted for resident locality, education, pack-years of cigarette smoking, alcohol consumption, tea consumption, and other polymorphisms (as described in "Data analysis")

[c] Reference category

[d] Tests for trend were performed by using quantitative variable according to the number of variant alleles

be related to a more efficient DNA synthesis [2]. The potential biological mechanism of the protection could involve increased levels of the methyl group donor 5,10-methyleneTHF being available for DNA synthesis, thereby reducing levels of incorporation of the abnormal base uracil instead of thymidine into DNA [33], and thus generating a more desirable balance between DNA methylation and DNA synthesis.

TS binds 5,10-methyleneTHF, which serves as a methyl group donor in the conversion of dUMP to dTMP in DNA synthesis. This study found that individuals with the TS 2R2R genotype experienced a significant 5.70-fold increased risk of leukemia in males and a non-statistically significant increase in females relative to those with the 3R3R genotype. The findings support the underlying effect of the decreased TS enzyme activity, which may affect the balanced supply of deoxynucleotides required for normal DNA synthesis, particularly in rapidly proliferating cells such as hematopoietic cells [33].

A gender difference in risks of adult leukemia with MTHFR polymorphisms was first reported in a case-control study in a Chinese population (no. of B-ALL cases 127, no. of controls 182), from which a preferential protection of females against B-ALL was reported with an OR of 0.06 (95 % CI 0.00–0.53) and an OR of 0.71 (95 % CI 0.20–2.53) in males for 677CC and 1298AC in contrast to 677CC and 1298AA [30]. Thus, that study also found that the associations between MTHFR polymorphisms and leukemia risk were modulated by gender. In the present study, we consistently observed higher risks of adult leukemia associated with the MTHFR 677TT, 1298CC, and TS 2R2R variants in males than in females. The presence of the MTHFR C677T variant genotypes significantly increased the risk of leukemia only in males, but no such effect was manifested in females. The findings on the modification of effects of MTHFR/TS polymorphisms by gender could partly explain the male predominance in adult leukemia incidence in Chinese [34]. Interestingly, a previous

Table 4 Associations between MTHFR/TS polymorphisms and adult leukemia risk by dietary folate intake

Genotype	Folate low[a] (n=471)		Folate high[a] (n=373)		$P_{interaction}$[e]
	Cases/controls	OR (95 % CI)[b]	Cases/controls	OR (95 % CI)[b]	
MTHFR C677T					0.08
CC	82/81	1.00[c]	58/79	1.00[c]	
CT	115/98	1.26 (0.80–1.98)	68/83	1.20 (0.71–2.02)	
TT	64/31	2.26 (1.24–4.10)	36/48	1.29 (0.67–2.46)	
P_{trend}[d]		0.01		0.42	
MTHFR A1298C					0.16
AA	208/149	1.00[c]	104/133	1.00[c]	
AC/CC	53/61	0.69 (0.43–1.13)	59/77	0.91 (0.55–1.49)	
TS					0.03
3R3R	148/135	1.00[c]	114/133	1.00[c]	
2R3R/2R2R	112/75	1.53 (1.01–2.31)	49/77	0.66 (0.41–1.06)	
MTHFR/TS variant alleles					0.19
0	30/40	1.00[c]	17/29	1.00[c]	
1	84/71	1.70 (0.92–3.12)	57/63	1.53 (0.73–3.19)	
2	90/67	2.20 (1.19–4.06)	59/79	1.32 (0.64–2.73)	
≥3	56/32	3.21 (1.60–6.43)	29/39	1.28 (0.57–2.88)	
P_{trend}[d]		<0.001		0.75	

[a] Cutoff value for folate was 119.5 μg/1000 kcal per day using the nutrient density method, based on the medium value among the controls with blood samples (n=420)

[b] Estimates were derived from multivariate unconditional logistic regression model adjusted for years of age, gender, study site, resident locality, education, pack-years of cigarette smoking, alcohol consumption, tea consumption, other polymorphisms, and kilocalorie of daily energy intake (as described in "Data analysis")

[c] Reference category

[d] Tests for trend were performed by using quantitative variable according to the number of variant alleles

[e] P for interaction was based on the likelihood ratio tests to compare multivariate conditional logistic regression models with and without product terms. The full model included main effect variables (dietary folate intake and MTHFR/TS polymorphisms) and their product terms and plausible confounding factors listed above (excluding matching variables)

study has shown that the influence of one-carbon metabolism relevant genetic polymorphism on plasma S-adenosylmethionine (a primary methyl donor for the methylation) concentrations varied with gender and one-carbon metabolism nutrients [35]. Further studies are warranted to investigate the gender difference in risks of adult leukemia with MTHFR polymorphisms.

It should be noted that upon stratification according to gender, the associations between MTHFR A1298C and TS genetic variations and the risks of adult leukemia were based on a very small sample size of the homozygous variant genotypes, as shown by their wide 95 % CIs in Table 2. Thus, interpretation of these associations should be cautious.

MTHFR/TS polymorphisms and adult leukemia risk by subtype This study observed that the risks with MTHFR/TS polymorphisms differed between AML and ALL. Specifically, the MTHFR C677T variant genotypes as well as a greater number of MTHFR/TS variant alleles, appeared to increase the risk of AML, but decrease the risk of ALL. Although previous case-control studies on the relationships between MTHFR C677T polymorphism and risks of ALL and AML in adults have produced inconsistent results, the discrepancy observed in this study is somewhat supported by previous observational studies.

With regard to the influence of MTHFR C677T polymorphism on ALL susceptibility in adults, a recent meta-analysis based on nine studies found that 677TT individuals were at an insignificantly decreased risk of ALL (OR=0.82; 95 % CI 0.47–1.42), when compared with their counterparts with the 677CC genotype [14]. A similar result (OR=0.75; 95 % CI 0.36–1.57) has been reported in another meta-analysis conducted only in Chinese populations [15]. In assessing the risk of AML with the C677T polymorphism, a non-statistically significant positive association was observed in Asian populations with the 677TT genotype (OR=1.12; 95 % CI 0.88–1.42) compared with 677CC [16]. More recently, a case-control study conducted in a Chinese population comprising 98 AML cases (both genders, aged 14–76 years) and 2016

controls (male only, aged 20–69 years) yielded an OR of 2.25 (95 % CI 0.95–5.33) for the MTHFR 677TT versus 677CC after adjusting for age, smoking status, and alcohol consumption [36].

Caution should also be exercised when interpreting the variation in risks between AML and ALL. First, the statistical power to calculate the subtype-specific risk estimates was reduced in our study, since subtype information was unavailable for 18 % of the cases. Second, the variation may be ascribed to differences in the genetic backgrounds of study populations, statistical power, dietary folate and other nutrients intake, and other potential confounding factors. For example, alcohol and green tea may influence the associations because of their antifolate effect [37, 38]. In our study, the multivariate analyses not only controlled demographic characteristics and other polymorphisms but also adjusted for important lifestyle and dietary factors, such as cigarette smoking and alcohol and tea consumption. However, most previous studies performed either univariate analyses [39–41] or adjusted for relatively few confounders such as age and gender [30, 31, 42]. Despite these concerns, it is possible that gene-disease associations may vary by leukemia subtype, since it has been suggested that a difference in folate requirements or susceptibility to chromosomal damage may exist between myeloid and lymphoid cells [2, 43].

Interaction between MTHFR/TS polymorphisms and dietary folate intake on leukemia susceptibility We previously reported that higher dietary folate intake was inversely associated with adult leukemia risk [7]. This study is the first report so far to examine the risks of adult leukemia with polymorphisms of folate pathway genes in conjunction with dietary folate intake. Most notably, we found that low dietary folate intake appeared to exacerbate the deleterious effect of TS 2R3R/2R2R on leukemia susceptibility. Our observation is biologically plausible, since folate deficiency has been shown to depress thymidylate synthesis, which increases the misincorporation of uracil and increases the frequency of chromosome breaks in human leukocytes [6] and bone marrow cells [44]. Simultaneous suppression of thymidylate synthesis due to impaired TS enzyme activity may further increase these lesions.

This study found a trend toward the 677TT genotype that significantly elevated the risk of adult leukemia only in individuals with low dietary folate intake. Although the test of interaction was not statistically significant in our analysis, the MTHFR C677T polymorphism is likely to modulate leukemia susceptibility depending on folate intake. Existing evidence has shown that only the 677TT individuals with low plasma folate levels account for the diminished genomic DNA methylation in peripheral blood mononuclear cells [32]. The pattern of interaction between dietary folate intake and MTHFR C677T on leukemia susceptibility, as observed in the present study, is similar to breast carcinogenesis and other solid tumors [45–47], i.e., the highest risk was found among 677TT individuals with low dietary folate intake. No indication of effect modification by the A1298C polymorphism was apparent in the study.

In summary, this study suggests that the MTHFR C677T, A1298C, and TS homozygous variants significantly increase the risk of leukemia in male adults. The MTHFR 1298AC genotype was associated with lower risks of adult leukemia in both genders and in subtypes of AML and ALL. Our data add support to the literature that disparities in risks with MTHFR/TS polymorphisms may exist between males and females, between AML and ALL. This study provides the first evidence that the associations between MTHFR/TS polymorphisms and adult leukemia risk may depend on dietary folate intake. The study of gene-diet interaction may be of importance in pointing to the etiology of adult leukemia, targeting susceptible populations, as well as guiding an effective dietary modification strategy against the disease.

Acknowledgments The authors are grateful to the participants in this study for their cooperation. The authors would also like to thank the staff from the three participating hospitals and Zhejiang University Women's Hospital for their kind assistance with fieldwork. The first author was supported by the Scholarship for International Research Fees and the University Postgraduate Award of The University of Western Australia.

Compliance with ethical standards

Funding This study was funded by the National Health and Medical Research Council (Australia) Project Grant (grant number 572542).

Conflicts of interest None

References

1. Gilliland DG. Molecular genetics of human leukemia. Leukemia. 1998;12:S7–S12.
2. Robien K, Ulrich CM. 5,10-Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: a HuGE minireview. Am J Epidemiol. 2003;157:571–82.
3. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet. 2013;381:1943–55.
4. Cole P, Rodu B. Descriptive epidemiology: cancer statistics. In: DeVita VT, Hellman S, Rosenberg SA, editors. Cancer: principles and practice of oncology. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 228–41.
5. Duthie SJ. Folic acid deficiency and cancer: mechanisms of DNA instability. Br Med Bull. 1999;55:578–92.
6. Blount BC, Mack MM, Wehr CM, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A. 1997;94:3290–5.

7. Liu P, Holman CD, Jin J, Zhang M. Diet and risk of adult leukemia: a multicenter case-control study in China. Cancer Causes Control. 2015;26:1141–51.
8. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995;10:111–3.
9. van der Put NM, Gabreels F, Stevens EM, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet. 1998;62:1044–51.
10. Weisberg I, Tran P, Christensen B, et al. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab. 1998;64:169–72.
11. Kaneda S, Takeishi K, Ayusawa D, et al. Role in translation of a triple tandemly repeated sequence in the 5′-untranslated region of human thymidylate synthase mRNA. Nucleic Acids Res. 1987;15:1259–70.
12. Horie N, Aiba H, Oguro K, et al. Functional analysis and DNA polymorphism of the tandemly repeated sequences in the 5′-terminal regulatory region of the human gene for thymidylate synthase. Cell Struct Funct. 1995;20:191–7.
13. Lehman NL. Future potential of thymidylate synthase inhibitors in cancer therapy. Expert Opin Investig Drugs. 2002;11:1775–87.
14. Li SY, Ye JY, Liang EY, et al. Association between MTHFR C677T polymorphism and risk of acute lymphoblastic leukemia: a meta-analysis based on 51 case-control studies. Med Sci Monit. 2015;21:740–8.
15. Xiao Y, Deng TR, Su CL, Shang Z. Methylenetetrahydrofolate reductase polymorphisms and susceptibility to acute lymphoblastic leukemia in a Chinese population: a meta-analysis. Oncol Res Treat. 2014;37:576–82.
16. Qin YT, Zhang Y, Wu F, et al. Association between MTHFR polymorphisms and acute myeloid leukemia risk: a meta-analysis. PLoS One. 2014;9, e88823.
17. Li B, Zhang J, Wang L, et al. MTHFR genetic polymorphisms may contribute to the risk of chronic myelogenous leukemia in adults: a meta-analysis of 12 genetic association studies. Tumour Biol. 2014;35:4233–45.
18. Weng Y, Zhang J, Tang X, et al. Thymidylate synthase polymorphisms and hematological cancer risk: a meta-analysis. Leuk Lymphoma. 2012;53:1345–51.
19. Shu XO, Yang G, Jin F, et al. Validity and reproducibility of the food frequency questionnaire used in the Shanghai Women's Health Study. Eur J Clin Nutr. 2004;58:17–23.
20. Zhang M, Binns CW, Lee AH. A quantitative food frequency questionnaire for women in southeast China: development and reproducibility. Asia Pac J Public Health. 2005;17:29–35.
21. Zhang M (2002) Dietary factors and lifestyle characteristics in the aetiology of ovarian cancer: a case-control study in China. Dissertation, Curtin University of Technology
22. Cui YH, Liu TS, Zhuang RY, et al. Polymorphism of thymidylate synthase gene and chemosensitivity of 5-fluorouracil regimen in metastatic gastrointestinal cancer. J Dig Dis. 2009;10:118–23.
23. Willett W, Stampfer M. Implications of total energy intake for epidemiologic analysis. In: Willett W, editor. Nutritional epidemiology. 2nd ed. New York: Oxford University Press; 1998. p. 273–301.
24. National Institute of Nutrition and Food Safety, China CDC. China food composition book 1. 2nd ed. Beijing: Peking University Medical Press; 2009. p. 3–191.
25. National Institute of Nutrition and Food Safety, China CDC. China food composition book 1. 1st ed. Beijing: Peking University Medical Press; 2002. p. 326–8.
26. National Institute of Nutrition and Food Safety, China CDC. China food composition book 2. Beijing: Peking University Medical Press; 2005. p. 77–211.
27. Breslow NE, Day NE. Statistical methods in cancer research. Vol. 1: The analysis of case-control studies. Lyon: IARC Scientific Publications; 1980. p. 162–91.
28. Rothman KJ, Greenland S, Lash TL. Validity in epidemiologic studies. In: Rothman KJ, Greenland S, Lash TL, editors. Modern epidemiology. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2008. p. 129–34.
29. Rota M, Porta L, Pelucchi C, et al. Alcohol drinking and risk of leukemia—a systematic review and meta-analysis of the dose-risk relation. Cancer Epidemiol. 2014;38:339–45.
30. Lv L, Wu C, Sun H, et al. Combined 677CC/1298AC genotypes of methylenetetrahydrofolate reductase (MTHFR) reduce susceptibility to precursor B lymphoblastic leukemia in a Chinese population. Eur J Haematol. 2010;84:506–12.
31. Kim HN, Kim YK, Lee IK, et al. Association between polymorphisms of folate-metabolizing enzymes and hematological malignancies. Leuk Res. 2009;33:82–7.
32. Friso S, Choi SW, Girelli D, et al. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci U S A. 2002;99:5606–11.
33. Skibola CF, Smith MT, Hubbard A, et al. Polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and risk of adult acute lymphocytic leukemia. Blood. 2002;99:3786–91.
34. Ferlay J, Soerjomataram I, Ervik M, et al (2013) GLOBOCAN 2012 v1.0, cancer incidence and mortality worldwide: IARC CancerBase No. 11 [Internet]. International Agency for Research on Cancer, Lyon. Available from: http://globocan.iarc.fr. Accessed 26 April 2015
35. Inoue-Choi M, Nelson HH, Robien K, et al. One-carbon metabolism nutrient status and plasma S-adenosylmethionine concentrations in middle-aged and older Chinese in Singapore. Int J Mol Epidemiol Genet. 2012;3:160–73.
36. Huang L, Deng D, Peng Z, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene (MTHFR) are associated with susceptibility to adult acute myeloid leukemia in a Chinese population. Cancer Epidemiol. 2015;39:328–33.
37. Mason JB, Choi SW. Effects of alcohol on folate metabolism: implications for carcinogenesis. Alcohol. 2005;35:235–41.
38. Navarro-Peran E, Cabezas-Herrera J, Garcia-Canovas F, et al. The antifolate activity of tea catechins. Cancer Res. 2005;65:2059–64.
39. Gemmati D, De Mattei M, Catozzi L, et al. DHFR 19-bp insertion/deletion polymorphism and MTHFR C677T in adult acute lymphoblastic leukaemia: is the risk reduction due to intracellular folate unbalancing? Am J Hematol. 2009;84:526–9.
40. Yang L, Liu L, Wang J, et al. Polymorphisms in folate-related genes: impact on risk of adult acute lymphoblastic leukemia rather than pediatric in Han Chinese. Leuk Lymphoma. 2011;52:1770–6.
41. Khorshied MM, Shaheen IA, Abu Khalil RE, Sheir RE. Methylene tetrahydrofolate reductase (MTHFR) gene polymorphisms in chronic myeloid leukemia: an Egyptian study. Med Oncol. 2014;31:794.
42. Oh D, Kim NK, Jang MJ, et al. Association of the 5,10-methylenetetrahydrofolate reductase (MTHFR C677T and A1298C) polymorphisms in Korean patients with adult acute lymphoblastic leukemia. Anticancer Res. 2007;27:3419–24.
43. Skibola CF, Smith MT, Kane E, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc Natl Acad Sci U S A. 1999;96:12810–5.
44. Wickramasinghe SN, Fida S. Bone marrow cells from vitamin B12- and folate-deficient patients misincorporate uracil into DNA. Blood. 1994;83:1656–61.

45. Chen J, Gammon MD, Chan W, et al. One-carbon metabolism, MTHFR polymorphisms, and risk of breast cancer. Cancer Res. 2005;65:1606–14.
46. Shrubsole MJ, Gao YT, Cai Q, et al. MTHFR polymorphisms, dietary folate intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. Cancer Epidemiol Biomarkers Prev. 2004;13:190–6.
47. Heijmans BT, Boer JM, Suchiman HE, et al. A common variant of the methylenetetrahydrofolate reductase gene (1p36) is associated with an increased risk of cancer. Cancer Res. 2003;63:1249–53.