Evaluation of the Contribution of Methylenetetrahydrofolate Reductase Genotypes to Taiwan Breast Cancer

CHUNG-YU HUANG[1,2*], WEN-SHIN CHANG[3,4*], HAO-AI SHUI[1*], YUNG-HUNG HSIEH[2], CHING-HUI LOH[2], HWEI-CHUNG WANG[4], HONG-XUE JI[3,4], CHIEH-LUN HSIAO[4], CHIN-MU HSU[4], CHIA-WEN TSAI[4,5] and DA-TIAN BAU[3,4,5]

[1]*Graduate Institute of Medical Sciences, National Defense Medical Center, Taipei, Taiwan, R.O.C.;*
[2]*Taichung Armed Forces General Hospital, Taichung, Taiwan, R.O.C.;*
[3]*Graduate Institute of Clinical Medical Science, and*
[5]*Graduate Institute of Basic Medical Science, China Medical University, Taichung, Taiwan, R.O.C.;*
[4]*Terry Fox Cancer Research Laboratory, China Medical University Hospital, Taichung, Taiwan, R.O.C.*

Abstract. *The aim of the present study was to evaluate the effects of the genotypic polymorphisms in methylenetetrahydrofolate reductase (MTHFR) and its interaction with early-onset breast cancer risk in Taiwan. Two well-known polymorphic variants of MTHFR, C677T (rs1801133) and A1298C (rs1801131), were analyzed and their joint effects with individual age- and estrogen-related factors on breast cancer risk were discussed. In total, 1,232 patients with breast cancer and 1,232 healthy controls were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The MTHFR C677T genotype, but not the A1298C, was differently distributed between cancer and control groups. The T allele of MTHFR C677T was significantly more frequently found in controls than in patients with cancer. In addition, females carrying MTHFR C677T CT or TT genotypes had a higher odds ratio of 1.21 (95% confidence interval=1.03-1.42, p=1.85E-5) for breast cancer, especially before the age of 45.4 years (odds ratio=1.51 and 95% confidence interval=1.20-1.90). Our results indicate that MTHFR C677T T allele was associated with increased risk of breast cancer in Taiwan, especially in cases who were 45.4 old or younger and with earlier menarche age (<12.2 years).*

*These Authors contributed equally to this work.

Correspondence to: Da-Tian Bau, Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan, R.O.C. Tel: +886 422052121 Ext. 7534, Fax: +886 422053366, e-mail: artbau1@yahoo.com.tw; artbau2@gmail.com

Key Words: Breast cancer, carcinogenesis, genotype, *MTHFR*, polymorphism, Taiwan.

Breast cancer is now ranked first among cancer affecting women throughout the world and the latest estimates suggest that more than 1,050,000 new breast cancer cases occur worldwide annually (1, 2). Breast cancer in Asia is characterized by a lower incidence than in Western populations, but is still the leading type of cancer in Asian women, and a significantly increasing tread indicates that it is an issue of particular public health importance. In Taiwan, breast cancer is the second leading type of cancer, important for its high incidence, high mortality, and early onset (3, 4). Previous studies revealed that oriental women affected by breast cancer, such as those in Taiwan, were significantly younger than white women and had racial/ethnic difference in their survival patterns (5, 6). Recently, scientists began to explore the mechanisms underlying breast cancer formation at the molecular level. Further investigation into these racial/ethnic differences may help unravel the genomic and environmental etiology of breast cancer, and aid cancer detection, therapy and prevention.

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism and catalyzes 5,10-methylene-tetrahydrofolate to 5-methyltetrahydrofolate. The importance of MTHFR in cancer susceptibility arises from its involvement in two pathways of folate metabolism: one leads to numerous methylation processes that are dependent on S-adenosyl-methionine (SAM), while the other, *via* thymidylate synthesis, contributes to DNA replication and cell division. Reduced activity of MTHFR may decrease the methylation of homocysteine to methionine and in turn the level of SAM, resulting in DNA hypomethylation. On the other hand, a reduced level of MTHFR substrate, required for thymidylate synthesis, could lead to uracil mis-incorporation into DNA, diminished DNA repair and increased frequency of chromosomal breaks and damage. Malignancies that are derived from rapidly proliferating

Table I. *The primer sequences, polymerase chain reaction and restriction fragment length polymorphism conditions for methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms.*

Polymorphism (location)	Primers sequences (5'->3')	Restriction enzyme	SNP sequence	DNA fragment size (bp)
C677T	F: TGA AGG AGA AGG TGT CTG CGG GA	*Hinf I*	C	198
(rs1801133)	R: AGG ACG GTG CGG TGA GAG TG		T	175+23
A1298C	F: GGGAGGAGCTGACCAGTGCAG	*Fnu4H I*	C	138
(rs1801131)	R: GGGGTCAGGCCAGGGGCAG		A	119+19

F and R indicate forward and reverse primers, respectively.

tissues, which have a higher requirement for DNA synthesis, should be more susceptible to folate deficiency and resultant DNA damage. The DNA variants causing reduced MTHFR activity were found to be associated with a reduced risk of leukemia, lymphoma and colorectal carcinoma. The mechanism proposed to explain these associations was the shunt of folate metabolism versus thymidine and purine synthesis, which would slow the incorporation of uracil into DNA and protect cells against carcinogenesis (7).

Previous investigations of *MTHFR* genetic variations focused on the catalytic domain and the two polymorphisms C677T and A1298C, which slightly change enzymatic activity. In the case of C677T polymorphism, the cytosine base at position number 677 changes to a thymidine base, which in turn affects the amino acid sequence at position number 222 (alanine→valine). The MTHFR enzyme resulting from the C677T polymorphism becomes thermolabile, causing a loss of its activity with increasing temperature. The modified protein also loses its co-factor flavin adenine dinucleotide (FAD) more quickly and has a lower stability. The mutation effect can be suppressed by addition of folate, which causes a higher FAD affinity and an increase in MTHFR stability. The *MTHFR* A1298C polymorphism is localized in the coding regulatory region domain (8). Studies investigating the *MTHFR* A1298C variant have found positive associations with colorectal cancer (9), breast cancer (10), acute lymphocytic leukemia (11), childhood leukemia (12).

In 2003, the association between single nucleotide polymorphisms (SNPs) of *MTHFR* and breast cancer susceptibility was firstly examined in a Taiwanese population, indicating the C677T SNP is not associated with breast cancer risk (13). However, the sample size was rather small (controls/cases=232/59), and only one SNP was investigated in that study. In 2006, the same group performed a genotype–phenotype correlation study showing that the combined genotype (677CT+TT with 1298AC+CC) conferred greater reduction of breast cancer risk among Taiwanese females with lower plasma folate levels (14), with a sample size of controls/cases=295/146. In the present work, we analyzed both *MTHFR* C677T and A1298C in a more representative population (controls/cases=1232/1232), and investigated the correlation between *MTHFR* genotypes and early onset of breast cancer in Taiwanese women.

Materials and Methods

Study population and sample collection. A total of 1232 patients diagnosed with breast cancer were recruited at our hospital. All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. An equal number of age-matched non-breast cancer healthy volunteers as controls were selected from the Health Examination Cohort of the hospital. Our study was approved by the Institutional Review Board of the China Medical University Hospital (DMR96-IRB-240).

Genotyping assays. Genomic DNA was prepared using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) as described in previous studies (15-20). The polymerase chain reaction (PCR) cycling conditions were: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 55˚C for 30 s, and 72˚C for 30 s, and a final extension at 72˚C for 10 min. Pairs of PCR primer sequences and restriction enzyme for each DNA product are listed in Table I.

Statistical analyses. Only those with both genotypic and clinical data (controls/cases=1232/1232) were selected for final analysis. Pearson's Chi-square test or Fisher's exact test was used to compare the distribution of the genotypes. The data were recognized as significant when the statistical p-value was less than 0.05.

Results

The frequency distributions of selected characteristics of 1232 breast cancer patients and 1232 non-cancer controls are shown in Table II. These characteristics of patients and controls are all well matched ($p>0.05$) (Table II). As for the individual behaviors, cigarette smoking and alcoholism were both risk factors for breast cancer in this population ($p<0.05$) (Table II).

The frequencies of the genotypes for the *MTHFR* C677T and A1298C in controls and patients with breast cancer are shown in Table III. The genotype distribution of the *MTHFR* C677T was significantly different between breast cancer and control groups ($p=1.85\times10^{-5}$), while that for A1298C

Table II. *Distributions of demographic and life-style of breast cancer patients and the matched controls.*

Characteristic	Controls (n=1232)			Patients (n=1232)			*p*-Value
	n	%	Mean (SD)	n	%	Mean (SD)	
Age at onset (years)							
<40	359	29.1%		362	29.4%		0.89[a]
40-55	558	45.3%		547	44.4%		
>55	315	25.6%		323	26.2%		
Age at menarche (years)			12.4 (0.7)			12.1 (0.6)	0.79[b]
Age at first birth of child (years)			29.4 (1.2)			29.8 (1.4)	0.63[b]
Age at menopause (years)			48.8 (1.8)			49.3 (2.0)	0.59[b]
Site							
Unilateral				1198	97.2%		
Bilateral				34	2.8%		
Family history of breast cancer							
First degree (Mother, sister and daughter)				55	4.5%		
Second degree				6	0.5%		
No history				1171	95%		
Personal habits							
Cigarette smokers	86	7.0%		170	13.8%		<0.0001[a]
Alcohol drinkers	91	7.4%		162	13.1%		<0.0001[a]

Statistical results based on [a]Chi-square or [b]unpaired Student's *t*-test.

Table III. *Distribution of methylenetetrahydrofolate reductase (MTHFR) genotypes among breast cancer and control groups.*

Genotype	Controls	%	Cases	%	*p*-Value[a]	OR (95% CI)
C677T rs1801133					1.85E-5	
CC	596	48.4%	538	43.7%		Reference
CT	533	43.3%	519	42.1%	0.3917	1.08 (0.91-1.28)
TT	103	8.3%	175	14.2%	0.0001	1.88 (1.44-2.47)
CT+TT	636	51.6%	694	56.3%	0.0212	1.21 (1.03-1.42)
A1298C rs1801131					0.3738	
AA	796	64.6%	787	63.9%		Reference
AC	391	31.7%	386	31.3%	1.0000	1.00 (0.84-1.19)
CC	45	3.7%	59	4.8%	0.1882	1.33 (0.89-1.98)
AC+CC	436	35.4%	445	36.1%	0.7367	1.03 (0.88-1.22)

[a]Based on Chi-square test; OR: odds ratio, CI: confidence interval.

polymorphisms was not ($p>0.05$) (Table III). Those who carried the TT genotype were found to have a 1.88-fold odds of breast cancer compared with those with CC genotype (95% CI=1.44-2.47).

Since age and estrogen exposure are the predominant risk factors for breast cancer, and Taiwan is well-known for early-onset breast cancer, the interactions between *MTHFR* genotype and age- and estrogen-related factors were also analyzed and presented in Tables IV-VII. The average age of all participants for age at onset, age at menarche, age at first birth of child, and age at menopause were 45.4, 12.2, 29.6 and 49.0 years, respectively, and were set as the cutting point for stratification. We noticed that those with CT or TT genotypes for *MTHFR* C677T had higher risk of breast cancer than those with CC genotype in the younger group (<45 years), but not in the case of the elder group (≥45 years) (Table IV). Those with CT or TT genotypes for *MTHFR* C677T also had higher risk of breast cancer than those with CC genotype in the group with earlier menarche (<12.2 years) but not in the case of later menarche (Table V). To sum up, there was an interaction between age at early menarche and onset with *MTHFR* C677T genotype for breast cancer susceptibility.

Discussion

In order to determine the role of *MTHFR* and to find potential biomarkers of breast cancer, in the present study, we selected two SNPs of *MTHFR* and investigated their

Table IV. *Distribution of methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C genotypes and age-related demographic characteristics (onset age).*

Characteristic	*MTHFR* C677T				*MTHFR* A1298C			
	Controls n (%)	Cases n (%)	*p*-Value[a]	Crude OR (95% CI)[b]	Controls n (%)	Cases n (%)	*p*-Value[a]	Crude OR (95% CI)[b]
Age at onset								
<45.4 years			3.60E-6*				0.6482	
CC	299 (50.68)	242 (40.47)		1.00 (Ref.)	390 (63.73)	393 (64.11)		1.00 (Ref.)
CT	245 (41.52)	258 (43.14)		1.30 (1.02-1.66)*	199 (32.51)	191 (31.16)		0.95 (0.75-1.21)
TT	46 (7.80)	98 (16.39)		2.63 (1.78-3.88)*	23 (3.76)	29 (4.73)		1.25 (0.71-2.20)
CT+TT	291 (49.32)	356 (59.53)		1.51 (1.20-1.90)*	222 (36.27)	220 (35.89)		0.98 (0.78-1.24)
≥45.4 years			0.1186				0.4884	
CC	297 (46.26)	296 (46.69)		1.00 (Ref.)	406 (65.48)	394 (63.65)		1.00 (Ref.)
CT	288 (44.86)	261 (41.17)		0.91 (0.72-1.15)	192 (30.97)	195 (31.50)		1.05 (0.82-1.33)
TT	57 (8.88)	77 (12.14)		1.36 (0.93-1.98)	22 (3.55)	30 (4.85)		1.41 (0.80-2.48)
CT+TT	345 (53.74)	338 (53.31)		0.98 (0.79-1.22)	214 (34.52)	225 (36.35)		1.08 (0.86-1.37)

[a]Based on Chi-square. [b]No difference in the trend in statistical significance before and after adjustments for individual habits such as smoking (pack-years). OR, Odds ratio; CI, confidence interval; Ref., reference. *Statistically significant.

Table V. *Distribution of methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C genotypes and age-related demographic characteristics (age at menarche).*

Characteristics	*MTHFR* C677T				*MTHFR* A1298C			
	Controls n (%)	Cases n (%)	*p*-Value[a]	Crude OR (95% CI)[b]	Controls n (%)	Cases n (%)	*p*-Value[a]	Crude OR (95% CI)[b]
Age at menarche								
<12.2 years			7.42E-6*				0.6383	
CC	304 (49.67)	260 (41.67)		1.00 (Ref.[d])	397 (64.55)	397 (63.93)		1.00 (Ref.[d])
CT	261 (42.65)	262 (41.99)		1.17 (0.92-1.49)	195 (31.71)	194 (31.24)		0.99 (0.78-1.27)
TT	47 (7.68)	102 (16.35)		2.54 (1.73-3.72)*	23 (3.74)	30 (4.83)		1.30 (0.74-2.29)
CT+TT	308 (50.33)	364 (58.33)		1.38 (1.10-1.73)*	218 (35.45)	224 (36.07)		1.03 (0.81-1.30)
≥12.2 years			0.2355				0.5841	
CC	292 (47.10)	278 (45.72)		1.00 (Ref.[d])	399 (64.67)	390 (63.83)		1.00 (Ref.[d])
CT	272 (43.87)	257 (42.27)		0.99 (0.78-1.26)	196 (31.77)	192 (31.42)		1.00 (0.79-1.28)
TT	56 (9.03)	73 (12.01)		1.37 (0.93-2.01)	22 (3.56)	29 (4.75)		1.35 (0.76-2.39)
CT+TT	328 (52.90)	330 (54.28)		1.06 (0.84-1.32)	218 (35.33)	221 (36.17)		1.04 (0.82-1.31)

[a]Based on Chi-square. [b]No difference in the trend in statistical significance before and after adjustments for individual habits such as smoking (pack-years). OR, Odds ratio; CI, confidence interval; Ref., reference. *Statistically significant.

associations with breast cancer risk in Taiwan. We found CT and TT genotypes of *MTHFR* C677T were significantly associated with a lower susceptibility for breast cancer (Tables III). These data are consistent with those finding the T allele to confer a higher risk (21-23), but not those reporting no association (13, 24-28). This may be caused by differences in ethnicity; moreover, our sample size was much larger than that of Jeng *et al*. and more representative of Taiwanese patients (13). Thus, the effects of MTHFR C677T polymorphism on carcinogenesis are complex, exerting either an adverse effect on DNA methylation or an advantageous influence on nucleotide synthesis in determining cancer risk.

We further analyzed the association between C677T genotype and breast cancer risk in those aged younger to investigate the role of *MTHFR* in early onset of breast cancer in Taiwan. Interestingly, the interaction between *MTHFR* C677T and age is clear, younger females (diagnosed with breast cancer at an age earlier than 45.4 years) with the CT or TT genotype have a 1.51-fold greater odds of breast cancer than those younger females with the CC genotype,

Table VI. *Distribution of methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C genotypes and age-related demographic characteristics (age at birth of first child).*

Characteristics	*MTHFR* C677T				*MTHFR* A1298C			
	Controls n (%)	Cases n (%)	*p*-Value[a]	Crude OR (95% CI)[b]	Controls n (%)	Cases n (%)	*p*-Value[a]	Crude[b] OR (95% CI)[c]
Age at birth of first child								
<29.6 years			0.0176*				0.5362	
CC	287 (46.44)	280 (45.68)		1.00 (Ref.[d])	390 (64.14)	386 (63.18)		1.00 (Ref.[d])
CT	277 (44.82)	249 (40.62)		0.92 (0.73-1.17)	196 (32.24)	195 (31.91)		1.01 (0.79-1.28)
TT	54 (8.74)	84 (13.70)		1.59 (1.09-2.33)*	22 (3.62)	30 (4.91)		1.38 (0.78-2.43)
CT+TT	331 (53.56)	333 (54.32)		1.03 (0.82-1.29)	218 (35.86)	225 (36.82)		1.04 (0.83-1.32)
≥29.6 years			0.1186				0.6847	
CC	309 (50.33)	258 (41.68)		1.00 (Ref.[d])	406 (65.06)	401 (64.57)		1.00 (Ref.[d])
CT	256 (41.69)	270 (43.62)		1.26 (1.00-1.60)*	195 (31.25)	191 (30.76)		0.99 (0.78-1.26)
TT	49 (7.98)	91 (14.70)		2.22 (1.51-3.27)*	23 (3.69)	29 (4.67)		1.28 (0.73-2.24)
CT+TT	315 (49.67)	358 (58.32)		1.36 (1.09-1.70)*	218 (34.94)	220 (35.43)		1.02 (0.81-1.29)

[a]Based on Chi-square. [b]No difference in the trend in statistical significance before and after adjustments for individual habits such as smoking (pack-years). OR, Odds ratio; CI, confidence interval; Ref., reference. *Statistically significant.

Table VII. *Distribution of methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C genotypes and age-related demographic characteristics (age at menopause).*

Characteristics	*MTHFR* C677T				*MTHFR* A1298C			
	Controls n (%)	Cases n (%)	*p*-Value[a]	Crude OR (95% CI)[b]	Controls n (%)	Cases n (%)	*p*-Value[a]	Crude OR (95% CI)[b]
Age at menopause								
<49.0 years			0.0082*				0.6899	
CC	292 (47.33)	280 (44.59)		1.00 (Ref.[d])	386 (63.59)	392 (64.37)		1.00 (Ref.[d])
CT	272 (44.08)	259 (41.24)		0.99 (0.78-1.26)	198 (32.62)	189 (31.03)		0.94 (0.72-1.20)
TT	53 (8.59)	89 (14.17)		1.75 (1.20-2.55)*	23 (3.79)	28 (4.60)		1.20 (0.68-2.12)
CT+TT	325 (52.67)	348 (55.41)		1.11 (0.89-1.40)	221 (36.41)	217 (35.63)		0.97 (0.77-1.22)
≥49.0 years			0.0014*				0.3974	
CC	304 (49.43)	258 (42.72)		1.00 (Ref.[d])	410 (65.60)	395 (63.40)		1.00 (Ref.[d])
CT	261 (42.44)	260 (43.05)		1.17 (0.92-1.49)	193 (30.88)	197 (31.62)		1.06 (0.83-1.35)
TT	50 (8.13)	86 (14.24)		2.03 (1.38-2.98)*	22 (3.52)	31 (4.98)		1.46 (0.83-2.57)
CT+TT	311 (50.57)	346 (57.28)		1.31 (1.05-1.64)*	215 (34.40)	228 (36.60)		1.10 (0.87-1.39)

[a]Based on Chi-square. [b]No difference in the trend in statistical significance before and after adjustments for individual habits such as smoking (pack-years). OR, Odds ratio; CI, confidence interval; Ref., reference. *Statistically significant.

which is not the case in the elder group (Table IV). This trend also fits well with the history of earlier menarche in the same population, those with the TT and CT+TT genotypes have a 2.54- and 1.38-fold greater odds for breast cancer than those with younger age at first menarche with CC genotype, which is not the case in the elder group (Table V). Since early first full-term pregnancy and late menopause were considered to be protective (29-31) and risky (32-34) factors of breast cancer in Taiwan, respectively, we also analyzed the interactions of *MTHFR* genotype with these two age- and estrogen-related factors. The data showed that TT genotype of *MTHFR* C677T has a 1.59 -fold greater odds for breast cancer than those with CC at early (<29.6 years) birth of first child, while CT, TT and CT+TT genotypes have a 1.26-, 2.22- and 1.36 -fold greater odds for breast cancer respectively than those with CC at late (>29.6 years) birth of first child (Table VI). As for age of menopause, TT genotype of *MTHFR* C677T has a 1.75-fold greater odds for breast

cancer than those with CC at early (<49 years) menopause, while TT and CT+TT genotypes have a 2.03- and 1.31-fold greater odds for breast cancer respectively than those with CC at late (>49 years) menopause (Table VII).

We propose that T allele at C677T may affect MTHFR activity, influencing the normal function of MTHFR. In literature, it has been shown that *MTHFR* 677T variants result in 70% lower functional activity (35). Those with T allele(s) not only have an imbalance in their folate pool available for DNA synthesis and cell proliferation, but may not be able to remove the DNA adducts caused by estrogen-induced insults (36) as soon as those with C allele, or cannot regulate the methylation status of other genes normally. All the above mechanisms may lead to earlier onset of breast cancer. To sum up, the alterations toward early breast carcinogenesis may be caused by decreased functions of MTHFR and cascading effects, which may finally lead to early onset of breast cancer.

In conclusion, as far as we are aware of, this is the first study focusing on *MTHFR* genotype and joint effects with age- and estrogen-related risk factors for early-onset breast cancer. The presence of T allele at C677T was not only associated with a higher cancer risk, but involved in early breast carcinogenesis. The T allele of *MTHFR* C677T may be a useful marker in breast oncology and early cancer detection.

Acknowledgements

We thank Tzu-Chia Wang, Yun-Ru Syu, Lin-Lin Hou and Chia-En Miao for their technical assistance. This study was supported by research grants from Terry Fox Cancer Research Foundation, and Taichung Armed Forces General Hospital (grant number: 103A24 and 103A25).

References

1 Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB and Ames BN: Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci USA *94*: 3290-3295, 1997.

2 Kim YI: Folate and carcinogenesis: evidence, mechanisms, and implications. J Nutr Biochem *10*: 66-88, 1999.

3 Cheng SH, Tsou MH, Liu MC, Jian JJ, Cheng JC, Leu SY, Hsieh CY and Huang AT: Unique features of breast cancer in Taiwan. Breast Cancer Res Treat *63*: 213-223, 2000.

4 Kuo WH, Yen AM, Lee PH, Chen KM, Wang J, Chang KJ, Chen TH and Tsau HS: Cumulative survival in early-onset unilateral and bilateral breast cancer: an analysis of 1907 Taiwanese women. Br J Cancer *100*: 563-570, 2009.

5 Hsu JL, Glaser SL and West DW: Racial/ethnic differences in breast cancer survival among San Francisco Bay Area women. J Natl Cancer Inst *89*: 1311-1312, 1997.

6 Natarajan N, Nemoto D, Nemoto T and Mettlin C: Breast cancer survival among Orientals and whites living in the United States. J Surg Oncol *39*: 206-209, 1988.

7 Krajinovic M, Lamothe S, Labuda D, Lemieux-Blanchard E, Theoret Y, Moghrabi A and Sinnett D: Role of MTHFR genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia. Blood *103*: 252-257, 2004.

8 Homberger A, Linnebank M, Winter C, Willenbring H, Marquardt T, Harms E and Koch HG: Genomic structure and transcript variants of the human methylenetetrahydrofolate reductase gene. Eur J Hum Genet *8*: 725-729, 2000.

9 Chen J, Giovannucci E, Hankinson SE, Ma J, Willett WC, Spiegelman D, Kelsey KT and Hunter DJ: A prospective study of methylenetetrahydrofolate reductase and methionine synthase gene polymorphisms, and risk of colorectal adenoma. Carcinogenesis *19*: 2129-2132, 1998.

10 Sharp L, Little J, Schofield AC, Pavlidou E, Cotton SC, Miedzybrodzka Z, Baird JO, Haites NE, Heys SD and Grubb DA: Folate and breast cancer: the role of polymorphisms in methylenetetrahydrofolate reductase (*MTHFR*). Cancer Lett *181*: 65-71, 2002.

11 Skibola CF, Smith MT, Kane E, Roman E, Rollinson S, Cartwright RA and Morgan G: Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc Natl Acad Sci USA *96*: 12810-12815, 1999.

12 Wiemels JL, Smith RN, Taylor GM, Eden OB, Alexander FE and Greaves MF: Methylenetetrahydrofolate reductase (*MTHFR*) polymorphisms and risk of molecularly defined subtypes of childhood acute leukemia. Proc Natl Acad Sci USA *98*: 4004-4009, 2001.

13 Jeng YL, Wu MH, Huang HB, Lin WY, You SL, Chu TY, Chen CJ and Sun CA: The methylenetetrahydrofolate reductase 677C->T polymorphism and lung cancer risk in a Chinese population. Anticancer Res *23*: 5149-5152, 2003.

14 Chou YC, Wu MH, Yu JC, Lee MS, Yang T, Shih HL, Wu TY and Sun CA: Genetic polymorphisms of the methylenetetrahydrofolate reductase gene, plasma folate levels and breast cancer susceptibility: a case-control study in Taiwan. Carcinogenesis *27*: 2295-2300, 2006.

15 Tsai CW, Tsai MH, Shih LC, Chang WS, Lin CC, and Bau DT: Associations of interleukin-10 (*IL10*) promoter genotypes with nasopharyngeal carcinoma risk in Taiwan. Anticancer Res *33*: 3391-3396, 2013.

16 Hsia TC, Tsai CW, Liang SJ, Chang WS, Lin LY, Chen WC, Tu CY, Tsai CH and Bau DT: Effects of ataxia telangiectasia mutated (*ATM*) genotypes and smoking habits on lung cancer risk in Taiwan. Anticancer Res *33*: 4067-4071, 2013.

17 Chang WS, Tsai CW, Ji HX, Wu HC, Chang YT, Lien CS, Liao WL, Shen WC, Tsai CH and Bau DT: Associations of cyclooxygenase 2 polymorphic genotypes with bladder cancer risk in Taiwan. Anticancer Res *33*: 5401-5405, 2013.

18 Lin CH, Lin CC, Tsai CW, Chang WS, Yang CW and Bau DT: Association of caveolin-1 genotypes with gastric cancer in Taiwan. Anticancer Res *34*: 2263-2267, 2014

19 Hsu CM, Yang MD, Tsai CW, Ho CY, Chang WS, Chang SC, Jeng LB, Tsai Y, Tsai FJ and Bau DT: The contribution of caveolin-1 genotype and phenotype to hepatocellular carcinoma. Anticancer Res *33*: 671-677, 2013.

20 Pei JS, Lee YM, Lo HH, Hsu YN, Lin SS and Bau DT: Association of X-ray repair cross-complementing-6 genotypes with childhood leukemia. Anticancer Res *33*: 5395-5399, 2013.

21 Hung RJ, Hashibe M, McKay J, Gaborieau V, Szeszenia-Dabrowska N, Zaridze D, Lissowska J, Rudnai P, Fabianova E, Mates I, Foretova L, Janout V, Bencko V, Chabrier A, Moullan N, Canzian F, Hall J, Boffetta P and Brennan P: Folate-related genes and the risk of tobacco-related cancers in Central Europe. Carcinogenesis *28*: 1334-1340, 2007.
22 Siemianowicz K, Gminski J, Garczorz W, Slabiak N, Goss M, Machalski M and Magiera-Molendowska H: Methylene-tetrahydrofolate reductase gene C677T and A1298C polymorphisms in patients with small cell and non-small cell lung cancer. Oncol Rep *10*: 1341-1344, 2003.
23 Zhang XM, Miao XP, Tan W, Qu SN, Sun T, Zhou YF and Lin DX: Association between genetic polymorphisms in methylentetrahydrofolate reductase and risk of lung cancer. Zhongguo Yi Xue Ke Xue Yuan Xue Bao *27*: 700-703, 2005.
24 Shen H, Spitz MR, Wang LE, Hong WK and Wei Q: Polymorphisms of methylene-tetrahydrofolate reductase and risk of lung cancer: a case-control study. Cancer Epidemiol Biomarkers Prev *10*: 397-401, 2001.
25 Shen M, Rothman N, Berndt SI, He X, Yeager M, Welch R, Chanock S, Caporaso N and Lan Q: Polymorphisms in folate metabolic genes and lung cancer risk in Xuan Wei, China. Lung Cancer *49*: 299-309, 2005.
26 Shi Q, Zhang Z, Li G, Pillow PC, Hernandez LM, Spitz MR and Wei Q: Sex differences in risk of lung cancer associated with methylene-tetrahydrofolate reductase polymorphisms. Cancer Epidemiol Biomarkers Prev *14*: 1477-1484, 2005.
27 Suzuki T, Matsuo K, Hiraki A, Saito T, Sato S, Yatabe Y, Mitsudomi T, Hida T, Ueda R and Tajima K: Impact of one-carbon metabolism-related gene polymorphisms on risk of lung cancer in Japan: a case control study. Carcinogenesis *28*: 1718-1725, 2007.
28 Vineis P, Veglia F, Garte S, Malaveille C, Matullo G, Dunning A, Peluso M, Airoldi L, Overvad K, Raaschou-Nielsen O, Clavel-Chapelon F, Linseisen JP, Kaaks R, Boeing H, Trichopoulou A, Palli D, Crosignani P, Tumino R, Panico S, Bueno-De-Mesquita HB, Peeters PH, Lund E, Gonzalez CA, Martinez C, Dorronsoro M, Barricarte A, Navarro C, Quiros JR, Berglund G, Jarvholm B, Day NE, Key TJ, Saracci R, Riboli E and Autrup H: Genetic susceptibility according to three metabolic pathways in cancers of the lung and bladder and in myeloid leukemias in nonsmokers. Ann Oncol *18*: 1230-1242, 2007.
29 Ding SL, Yu JC, Chen ST, Hsu GC and Shen CY: Genetic variation in the premature aging gene WRN: a case-control study on breast cancer susceptibility. Cancer Epidemiol Biomarkers Prev *16*: 263-269, 2007.
30 Fu YP, Yu JC, Cheng TC, Lou MA, Hsu GC, Wu CY, Chen ST, Wu HS, Wu PE and Shen CY: Breast cancer risk associated with genotypic polymorphism of the nonhomologous end-joining genes: a multigenic study on cancer susceptibility. Cancer Res *63*: 2440-2446, 2003.
31 Hsu HM, Wang HC, Chen ST, Hsu GC, Shen CY and Yu JC: Breast cancer risk is associated with the genes encoding the DNA double-strand break repair Mre11/Rad50/Nbs1 complex. Cancer Epidemiol Biomarkers Prev *16*: 2024-2032, 2007.
32 Huang KE, Xu L, I NN and Jaisamrarn U: The Asian Menopause Survey: knowledge, perceptions, hormone treatment and sexual function. Maturitas *65*: 276-283, 2010.
33 Kuo WH, Yen AM, Lee PH, Hou MF, Chen SC, Chen KM, Chen TH and Chang KJ: Incidence and risk factors associated with bilateral breast cancer in area with early age diagnosis but low incidence of primary breast cancer: analysis of 10-year longitudinal cohort in Taiwan. Breast Cancer Res Treat *99*: 221-228, 2006.
34 Shin HR, Joubert C, Boniol M, Hery C, Ahn SH, Won YJ, Nishino Y, Sobue T, Chen CJ, You SL, Mirasol-Lumague MR, Law SC, Mang O, Xiang YB, Chia KS, Rattanamongkolgul S, Chen JG, Curado MP and Autier P: Recent trends and patterns in breast cancer incidence among Eastern and Southeastern Asian women. Cancer Causes Control *21*: 1777-1785, 2010.
35 Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP *et al*: A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet *10*: 111-113, 1995.
36 Cheng TC, Chen ST, Huang CS, Fu YP, Yu JC, Cheng CW, Wu PE and Shen CY: Breast cancer risk associated with genotype polymorphism of the catechol estrogen-metabolizing genes: a multigenic study on cancer susceptibility. Int J Cancer *113*: 345-353, 2005.

Received May 7, 2014
Revised June 19, 2014
Accepted June 20, 2014