Breast Cancer Risk Associated with Multigenotypic Polymorphisms in Folate-metabolizing Genes: A Nested Case-control Study in Taiwan

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Abstract. *Aim:The purpose of this study was to test the hypothesis that multigenotypic polymorphisms in the folate metabolic pathway, which may result in genomic instability and inbalance of estrogen metabolism, were associated with breast cancer risk. Patients and Methods: A population-based nested case-control study of 109 cases with pathologically confirmed invasive breast cancer and 421 cancer-free controls was conducted in Taiwan between 1992 and 2001. The polymorphisms in serine hydroxymethyltransferase (SHMT1 C1420T), 5,10-methylenetetrahydrofolate reductase (MTHFR C677T), and methionine synthase (MS A2756G) genes were examined using polymerase chain reaction-restriction fragment length polymorphism. Results: There was a trend toward an increased risk of breast cancer in women harboring a greater number of putative high-risk genotypes of these genes. Furthermore, the cancer risk associated with having at least one putative high-risk genotype was more significant in women having been exposed to estrogen for a longer period before first birth. Conclusion: The present study indicates the significance of multiple low-penetrance alleles of functionally-related folate-metabolizing genes interactive with an estrogenic environment in breast tumorigenesis.*

Folate is an important nutrient required for DNA synthesis and the related methionine metabolic pathway is necessary for DNA methylation (1). Defects or polymorphisms in the genes of the folate metabolic pathway may influence cancer susceptibility (2). Several genes controlling folate metabolism are polymorphic. Serine hydroxymethyltransferase (SHMT) catalyzes the reversible conversion of tetrahydrofolate to 5,10-methylenetetrahydrofolate (methylene THF) providing one-carbon units for *S*-adenosylmethionine (SAM), purine and thymidine synthesis (3). Human SHMT genes code for two different isoforms of proteins: the cytosolic SHMT (*cSHMT* or *SHMT1*) and the mitochondrial SHMT (*mSHMT* or *SHMT2*) (4, 5). A common C1420T polymorphism of *SHMT1* has been described and results in reduced plasma and red blood cell folate levels in carriers of the 1420CC genotype (6). This polymorphism could mimic a folate deficiency by reducing the one-carbon moieties available for both remethylation of homocysteine and DNA synthesis. 5,10-methylenetetrahydrofolate reductase (*MTHFR*) plays a central role in the provision of methyl groups by converting 5,10-methylene THF to 5-methyl THF, the primary circulating form of folate and carbon donor for the remethylation of homocysteine to methionine with subsequent production of SAM, the universal donor of the methyl group, required for DNA methylation (7). A common mutation, C>T at nucleotide 677 leading to an alanine to valine conversion in the protein, has been identified in the *MTHFR* gene (8). The nucleotide 677 polymorphism results in reduced MTHFR enzyme activity and plasma folate levels, and results in hypomethylation (8). Methionine synthase (MS) catalyzes the transfer of methyl base from 5-methyl THF to homocysteine, producing methionine and tetrahydrofolate. Thus, the MS gene plays a critical role in maintaining adequate intracellular methionine concentrations and adequate intracellular SAM levels for DNA methylation. It is reported to have a polymorphism in 2756 A>G, resulting in a lower enzyme activity (9) and is thought to result in homocysteine elevation and DNA hypomethylation.

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Key Words: Breast cancer, folate metabolism, genetic susceptibility.

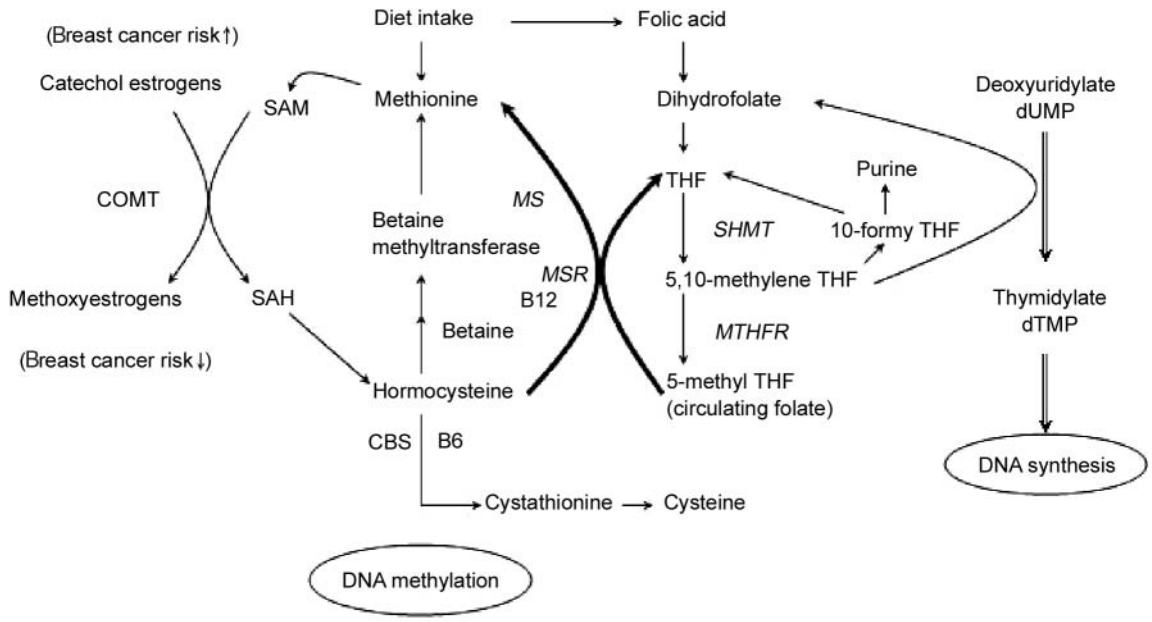

Figure 1. *Overview of the human folate metabolic pathway and its relation to hormone-dependent breast cancer risk.*

Both epidemiological and animal studies have indicated that estrogens are central in breast tumorigenesis (10). Estrogens induce proliferation, but may also initiate carcinogenesis *via* metabolic activation to potentially carcinogenic catechol estrogen metabolites (11). The principal pathway for inactivation of catechol estrogen is *O*-methylation by catechol-*O*-methyltransferase (*COMT*) (12, 13). While SAM is the necessary methyl donor for COMT-catalyzed reaction, the folate metabolic pathway largely determines the SAM level. Thus, it is possible that polymorphisms in folate-metabolizing genes might be interactive with estrogen metabolism in determining breast cancer risk (Figure 1). In fact, we previously demonstrated that genetic polymorphisms in *MTHFR* were associated with elevated risk of breast cancer in women with prolonged exposure to estrogens prior to first full-term pregnancy (FFTP) (14).

Given the importance of the folate metabolic pathway in estrogen metabolism, we carried out a multigenic nested case-control study to examine whether polymorphisms in the folate-metabolizing genes *SHMT1, MTHFR* and *MS* influence breast cancer risk.

Patients and Methods

Study cohort. This nested case-control study was conducted within a cancer screening cohort of individuals who were between 30- and 64-years-old and lived in seven townships in Taiwan. The cohort characteristics, methods of screening and follow-up, and implementation of nested case-control studies have been described elsewhere (15, 16). Briefly, from January 1991 to December 1992, a community-based cancer screening project was carried out in seven townships. There were 47,079 eligible males and 42,214 eligible females who were invited by letter to participate. A total of 12,020 male and 11,923 female adults enrolled; approximately 25% agreed to participate. Non-smokers, the elderly and those with a higher level of education showed higher rates of response (15). For molecular epidemiological studies of breast cancer, only female participants were considered in the present study.

Study participants. Cases of female breast cancer were ascertained by computerized linkage of data with information from the National Cancer Registry in Taiwan. The registry data are evaluated on an annual basis for completeness and accuracy, and case ascertainment by the registry through the hospital system is estimated to be 94% complete (17). With respect to the diagnosis of breast cancer, microscopic confirmation has been accomplished in 98% of reported breast cancer cases (17). In the original cohort study, we have defined prevalent breast cancer cases as tumors occurring prior to the date of recruitment or those detected within the first year of enrollment. Accordingly, a total of 109 pathologically confirmed primary breast carcinoma patients were identified among female cohort members between July 1992 and December 2001. Four female control participants were matched to each case by age (±2 years), residence and date of blood sample collection (±3 months). Controls were free of cancer when their matched cases were diagnosed. Among control individuals, 15 with missing data on date of birth and/or all other breast cancer risk factors considered in this study were excluded from the analysis. As a consequence, there were 98 case-control sets with 1 case matched to 4 controls, 8 sets with 1 case and 3 controls, 2 sets with 1 case and 2 controls, and 1 set with 1 case and 1 control. Therefore, the final study participants included 109 cases and 421 controls.

Data collection. At baseline recruitment, well-trained research assistants administered a structured questionnaire to participants. The information collected from female participants included sociodemographic characteristics, history of cigarette and alcohol consumption, personal and family history of cancer, age at menarche and/or menopause, parity and age at FFTP. Blood specimens, including samples of serum, plasma and white blood cells, were also obtained from participants and were frozen at –70˚C until subsequent analysis. All participants gave informed consent for both interview and blood collection; in addition, their anonymity was maintained through numerical coding of questionnaires and blood samples. This community-based cancer screening program was supported by the Department of Health, Executive Yuan and approved by National Defense Medical Center institutional review board.

Genotype analyses. Genomic DNA of each participant was isolated from peripheral blood lymphocytes, coded and subjected to polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Genotyping was performed according to previously described methods for *SHMT1* 1420 C>T (18), *MTHFR* 677 C>T (8) and *MS* 2756 A>G (19) polymorphisms. For *SHMT1* C1420T, restriction enzyme digestion was carried out using *EAR1* (New England Biolab® Inc, UK) which cuts the wild-type sequence into 113 bp and 179 bp fragments. For *MTHFR* C677T, based on the *HinfI* (Promega, USA) RFLP analysis, a single undigested band at 198 bp represents a homozygous wild-type genotype (677CC), two bands at 198 and 175 bps represent the heterozygous genotype (677CT), and a single band at 175 bp represents a homozygous mutant genotype (677TT). For MS A2756G, this A-to-G base pair substitution in the MS gene creates a *Hae III* (Promega, USA) restriction site. Wild-types (2756AA) produced a single band at 211 bp. Heterozygotes (2756AG) produced 211 bp, 131 bp, and 80 bp fragments. Homozygous mutants (2756GG) produced two fragments of 80 bp and 131 bp. To ensure that the observed polymorphisms were specific and not the results of experimental variation, the results were confirmed by repeating 10% of the assays. In addition, laboratory personnel were blinded as to case and control status.

Statistical analysis. The genotype frequencies of studied genes were calculated and tested for Hardy-Weinberg equilibrium (HWE), stratified by case-control status. Because the data were matched, analyses were carried out using conditional logistic regression to obtain odds ratios (ORs) and 95% confidence intervals (CIs) to measure the strength of association between hormonal risk factors and genetic polymorphisms of studied genes and the risk of developing breast cancer. To explore a possible association between breast cancer risk and individual polymorphisms in *SHMT1, MTHFR* and *MS*, the homozygous wild-type genotype of the studied genes (*SHMT1* 1420CC, *MTHFR* 677CC and *MS* 2756AA) was treated as the reference group. Given that an increased cancer risk due to a joint effect of genes belonging to a common metabolic pathway has been demonstrated in a mouse model (20) and epidemiological studies (13, 21), a joint effect of folate-metabolizing genes was analyzed by determining the breast cancer risk associated with harboring different numbers of putative high-risk genotypes. The putative high-risk genotype was defined as *SHMT1* 1420CC, *MTHFR* 677TT and *MS* 2756GG on the basis of analytical results of cancer risk associated with individual polymorphisms (as shown in Table I). Using a dummy variable coding scheme, breast cancer risk in women harboring different numbers of putative high-risk genotypes was estimated. Of particular interest was the relationship between polymorphic folate-metabolizing genotypes and breast cancer risk in women with different duration of estrogen exposure, which was examined using the stratified analysis. In this study, we defined the time period between age at menarche and age at FFTP as the duration of critical estrogen exposure period, as this interval is the critical time period when the breast tissue was most vulnerable to mutagenesis (22) and a significant elevation in breast cancer risk with increasing duration of this time period was observed in our study population (23). In the stratified method, possible effect modification of risk associated with folate-metabolizing genotypes by estrogen exposure was evaluated by calculating the OR of breast cancer in relation to the number of high-risk genotypes within different levels of estrogen exposure, with a test of homogeneity of ORs across strata. All analyses were performed using either Statistical Analysis System v.8.0 (SAS Institute Inc., NC, USA) or STATA (STATA Cooperation, College Station, TX, USA) statistical software.

Table I. *Genotype and allele frequencies of folate-metabolizing genes among breast cancer cases and controls and their contributions to the risk of breast cancer.*

Genotype /allele	Cases No. (%)	Controls No. (%)	OR[a] (95% CI[a])
SHMT1[a] C1420T[b]			
CC	79 (75.2)	283 (70.2)	1.0 (Reference)
CT	20 (19.1)	89 (22.1)	0.8 (0.4-1.3)
TT	6 (5.7)	31 (7.7)	0.7 (0.2-1.7)
C allele	178 (84.8)	655 (81.3)	
T allele	32 (15.2)	151 (18.7)	
HWE test[a]	$p=0.01$	$p<0.0001$	
MTHFR[a] C677T[b]			
CC	56 (51.4)	225 (53.6)	1.0 (Reference)
CT	44 (40.4)	170 (40.5)	1.0 (0.7-1.6)
TT	9 (8.3)	25 (6.0)	1.5 (0.6-3.3)
C allele	156 (71.6)	620 (73.8)	
T allele	62 (28.4)	220 (26.2)	
HWE test	$p=0.9312$	$p=0.3363$	
MS[a] A2756G[b]			
AA	85 (78.7)	324 (77.5)	1.0 (Reference)
AG	22 (20.4)	92 (22.0)	0.9 (0.5-1.5)
GG	1 (0.9)	2 (0.5)	1.9 (0.2-21.3)
A allele	192 (88.9)	740 (88.5)	
G allele	24 (11.1)	96 (11.5)	
HWE test	$p=0.7454$	$p=0.0910$	

[a]*SHMT1*: serine hydroxymethyltransferase 1; *MTHFR*: methylenetetrahydrofolate reductase; *MS*: methionine synthase; HWE test: Hardy-Weinberg equilibrium test; OR: odds ratio; CI: confidence interval; [b]Four cases and 18 controls had missing data on the *SHMT1* C1420T genotype, one control had missing data on the *MTHFR* C677T genotype, and one case and three controls had missing data on the MS A2756G genotype.

Results

The distributions of ethnicity (67.9% of cases and 63.0% of controls were Fukienese), proportion of parous women

Table II. *Risk of breast cancer associated with the number of putative high-risk genotypes of folate-metabolizing genes.*

No. of high-risk genotypes[a]	Cases No. (%)	Controls No. (%)	OR[b,c] (95% CI[b])
0	24 (22.9)	113 (28.0)	1.0 (Reference)
1	73 (69.5)	271 (67.3)	1.2 (0.7-2.1)
≥2	8 (7.6)	19 (4.7)	2.0 (0.9-4.9)

[a]The high-risk genotypes included in the analysis were serine hydroxymethyltransferase 1 (*SHMT1*) 1420 CC, methylenetetrahydrofolate reductase (*MTHFR*) 677 TT, and methionine synthase (MS) 2756 GG; [b]OR: odds ratio; CI: confidence interval; [c]ORs are adjusted for age and ethnicity.

Table III. *Joint effect of the folate-metabolizing genes[a] compound genotypes and duration of critical estrogen exposure on breast cancer risk.*

Duration of critical estrogen exposure (years)[b]	No. of high-risk genotypes[c]	Cases No. (%)	Controls No. (%)	OR[d,e] (95% CI[d])
≤9	0	17 (16.2)	79 (19.7)	1.0 (Reference)
	≥1	43 (41.0)	221 (55.1)	0.9 (0.5-1.6)
>9	0	7 (6.7)	33 (8.2)	1.0 (Reference)
	≥1	38 (36.2)	68 (17.0)	2.6 (1.1-6.4)

[a]The folate-metabolizing genes included in the analysis were serine hydroxymethyltransferase 1 (*SHMT1*), methylenetetrahydrofolate reductase (*MTHFR*), and methionine synthase (*MS*); [b]Duration of critical estrogen exposure was defined as the interval between age at menarche and age at first full-term pregnancy and was categorized based on the third tertile value in controls; [c]The high-risk genotypes of the folate-metabolizing genes included *SHMT1* 1420CC, *MTHFR* 677TT, and *MS* 2756GG; [d]OR: odds ratio; CI: confidence interval. [e]ORs are adjusted for age and ethnicity.

(99.0% and 98.6%), average number of parity (5.1±2.1 and 5.1±2.0), and menopausal status (39.4% and 42.9% were post-menopausal women) were similar in cases and controls, as were the matching variables age at enrollment (47.8 years ±9.2 years) and residential area.

More interestingly, there was a significant elevation in breast cancer risk with increasing duration of estrogen exposure prior to FFTP (p-value for trend test=0.0023). Compared to women with a duration of ≤6 years, women with a duration between 7 and 8 years had a 1.5-fold increased risk of breast cancer (95% CI 0.8-2.8), and those who had been exposed to estrogen for >8 years had a 2.6-fold increased risk of breast cancer (95% CI 1.4-4.8).

The allelic frequencies and genotype distributions of *SHMT1* 1420 C>T, *MTHFR* 677 C>T, and *MS* 2756 A>G among cases and controls are shown in Table I. Genotype distributions among controls were in conformity with HWE, with the exception of the *SHMT1* 1420 C>T polymorphism. For *SHMT1* C1420T, the frequency of the T allele was 15.2% in the cases and 18.7% in the controls. Considering the *SHMT1* 1420CC genotype as the reference, individuals with the 1420CT genotype showed a 1.2-fold reduction in breast cancer risk (OR 0.8; 95% CI 0.4-1.3), while individuals with the 1420TT genotype showed a 1.4-fold decrease in breast cancer risk (OR 0.7; 95% CI 0.2-1.7). The variant allele frequencies for *MTHFR* 677 C>T in cases and controls were 28.4% and 26.2%, respectively. In the main effect model using the *MTHFR* 677CC genotype as the reference, there was no association between the *MTHFR* 677CT genotype and breast cancer risk (OR 1.0; 95% CI 0.7-1.6), while a 1.5-fold increased risk estimate (95% CI 0.6-3.3) for breast cancer risk was observed among those with the *MTHFR* 677TT genotype. For *MS* A2756G, the frequency of the 2756G polymorphic allele was 11.1% in the cases and 11.5% in the controls. In a main effect model, an increased risk estimate for breast cancer risk was associated with the *MS* 2756GG genotype when the *MS* 2756AA genotype was the reference group (OR 1.9; 95% CI 0.2-21.3), while the OR of breast cancer associated with the *MS* 2756AG genotype was 0.9 (95% CI 0.5-1.5).

Following this univariate analysis, we next investigated whether a joint effect of folate-metabolizing genes was associated with breast cancer development by determining the breast cancer risk associated with harboring different numbers of putative high-risk genotypes. The results showed an elevation in breast cancer risk with increasing numbers of putative high-risk genotypes (Table II), albeit the trend was not statistically significant (p=0.1838). This finding suggested a joint effect of genes involved in folate metabolic pathway.

We further examined the potential importance of estrogen exposure in conjunction with these susceptibility genotypes using the stratified analysis. Our hypothesis was supported by the finding that a significant association between an increased cancer risk and harboring at least one putative high-risk genotype was only observed in those women who had been exposed to estrogen for >9 years before FFTP (OR 2.6; 95% CI 1.1-6.4). In contrast, among women with a short menarche to FFTP interval (≤9 years), there was no significant association (OR 0.9; 95% CI 0.5-1.6) (Table III). The test for heterogeneity of ORs was statistically significant (p=0.0453).

Discussion

On the basis of a multigenic model, this study investigated the tumorigenic contribution to breast cancer development of critical genes participating in folate metabolic pathway. In our entire sample of breast cancer cases and their matched controls, we observed no significant associations for each individual polymorphism in studied genes including *SHMT1, MTHFR* and *MS*. However, there was a trend toward an

increased risk of breast cancer in women harboring a greater number of high-risk genotypes of folate-metabolizing genes. Our data suggest that functional polymorphisms within these genes like *SHMT1* 1420 and *MS* 2756 might act in concert with *MTHFR* polymorphism to shift greater folate for nucleotide synthesis and cause DNA hypomethylation *via* reduction levels of SAM, the major cellular methyl donor for methylation reactions, including the *O*-methylation catalyzed by *COMT* for detoxification of carcinogenic estrogen metabolites (1, 11, 24). As a consequence, multigenic mutations in the folate metabolic pathway may contribute to breast tumorigenesis through the influence of estrogen metabolism, as illustrated in Figure 1. This finding may suggest the potential importance of nutrient-hormone interactions in breast tumorigenesis. Our data futher support the notion that a single polymorphism may only contribute a modest effect, if any, and that a compound analysis of genotypic polymorphisms of multigenes that were active in related metabolic pathways may provide a more complete picture of genetic susceptility associated with candidate genes with low penetrance (13, 25).

Previously, we had reported that there was a significant elevation in breast cancer risk with increasing duration of the interval between menarche and FFTP among women in Taiwan (23). In the present study, we further reported that a significant association between an increased cancer risk and harboring at least one putative high-risk genotype of folate-metabolizing genes was only seen in women who had been exposed to estrogen before FFTP for a longer time period. In contrast, among women with a short menarche to FFTP interval, there was no significant association. Thus, multigenotypic polymorphism in the folate metabolic pathway could pose enhanced risk of breast cancer in the presence of a relevant environmental exposure, as most low-penetrance genes are expected to act through gene-environment interactions (13, 14, 26-28).

Certain limitations of this study should be noted. The primary shortcoming of this study is a lack of data on dietary intake of folate for further examination of the gene-nutrient interaction, because the effect of genetic variations on cancer risk in folate-metabolizing genes depends on the level of folate intake (27-29). In addition, the results from this study were based on a small number of participants leading to risk estimates with wide confidence intervals. Because the genotype distribution of the *SHMT1* C1420T polymorphism departed from the Hardy-Weinberg equilibrium, possible selection bias in the present study may exist due to the inclusion of a small sample size of control participants.

Conclusion

A strong birth cohort effect among women in Taiwan, as reflected by increasing prevalence of central obesity, later age at FFTP, and the decrease of parity, could correspond to a continued shift in the incidence of breast cancer among women in Taiwan toward that of Caucasian Americans (23, 30). Given this background of a shift toward a high-risk profile of estrogen exposure among women in Taiwan, the findings of the present study indicate the increasing significance of genetic susceptibility due to multiple low-penetrance alleles of functionally related folate-metabolizing genes interactive with an estrogenic environment on breast tumorigenesis in this area.

Acknowledgements

We are grateful to all the women who participated in this study, in particular those who took the effort to supply this study with a blood sample. This study was supported by grants from National Science Council (NSC93-2320-B-016-016) and Department of Health (DOH93-TD-F-113-029), Executive Yuan, Taiwan, Republic of China.

References

1 Stover PJ: Physiology of folate and vitamin B12 in health and disease. Nutr Rev *62*: S3-S12, 2004.

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

3 Herbig K, Chiang EP, Lee LR, Hills J, Shane B and Stover PJ: Cytoplasmic serine hydroxymethyltransferase mediates competition between folate-dependent deoxyribonucleotide and *S*-adenosylmethionine biosynthesis. J Biol Chem *277*: 38381-38389, 2002.

4 Stover PJ, Chen LH, Suh JR, Stover DM, Keyomarsi K and Shane B: Molecular cloning, characterization, and regulation of the human mitochondrial serine hydroxymethyltransferase gene. J Biol Chem *272*: 1842-1848, 1997.

5 Girgis S, Nasrallah IM, Suh JR, Stover DM, Keyomarsi K and Shane B: Molecular cloning, characterization and alternative splicing of the human cytoplasmic serine hydroxymethyltransferase gene. Gene *210*: 315-324, 1998.

6 Heil SG, Van der Put NM, Waas ET, den Heijer M, Trijbels FJ and Blom HJ: Is mutated serine hydroxymethyltransferase (SHMT) involved in the etiology of neural tube defects? Mol Genet Metab *73*: 164-172, 2001.

7 Bailey LB and Gregory JF: Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: metabolic significance, risks and impact on folate requirement. J Nutr *129*: 919-922, 1999.

8 Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP and Rozen R: A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nature Genet *10*: 111-113, 1995.

9 Chen LH, Liu ML, Hwang HY, Chen LS, Korenberg J and Shane B: Human methionine synthase: cDNA cloning, gene localization, and expression. J Biol Chem *272*: 3628-3634, 1997.

10 Clemons M and Goss P: Estrogen and the risk of breast cancer. N Engl J Med *344*: 276-285, 2001.

11 Yager JD: Endogenous estrogens as carcinogens through metabolic activation. J Natl Cancer Inst Monogr 27: 67-73, 2000.
12 Yager JD and Liehr JG: Molecular mechanisms of estrogen carcinogenesis. Annu Rev Pharmacol Toxicol *36*: 203-232, 1996.
13 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.
14 Lin WY, Chou YC, Wu MH, Huang HB, Jeng YL, Wu CC, Yu CP, Yu JC, You SL, Chu TY, Chen CJ and Sun CA: The MTHFR C677T polymorphism, estrogen exposure and breast cancer risk: a nested case-control study in Taiwan. Anticancer Res *24*: 3863-3868, 2004.
15 Chen CJ, Lu SN, You SL, Wu MH, Wang LY, Lee LT, Huang GT, Yang PM and Lee HS: Community-based hepatocellular carcinoma screening in seven townships in Taiwan. J Formos Med Assoc *94(Suppl.2)*: S94-S102, 1995.
16 Chen CJ, You SL, Pwu RF, Wang LY, Lin YP, His CC, His MS, Ho HC, Lee CT, Lin CC, Ou YC, Yuan ML, Hsing AW, Huang TW and Hsieh CY: Community-based cervical cancer screening in seven townships in Taiwan. J Formos Med Assoc *94(Suppl.2)*: S103-S111, 1995.
17 Department of Health. Cancer Registry: Annual Report, 2000. Department of Health. Executive Yuan, Republic of China, 2003.
18 Skibola CF, Smith MT, Hubbard A, Shane B, Roberts AC, Law GR, Rollinson S, Roman E, Cartwright RA and Morgan GJ: Polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and risk of adult acute lymphocytic leukemia. Blood *99*: 3786-3791, 2002.
19 Ma J, Stampfer MJ, Christensen B, Giovannucci E, Hunter DJ, Chen J, Willett WC, Selhub J, Hennekens CH, Gravel R and Rozen R: A polymorphism of the methionine synthase gene: association with plasma folate, vitamin B12, homocysteine, and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev *8*: 825-829, 1999.
20 Kim MJ, Cardiff RD, Desai N Banach-Petrosky WA, Parsons R, Shen MM and Abate-Shen C: Cooperativity of *Nkx3.1* and *Pten* loss of function in a mouse model of prostate carcinogenesis. Proc Natl Acad Sci USA *99*: 2884-2889, 2002.
21 Perera FP, Mooney LA, Stampfer M, Phillips DH, Bell DA, Rundle A, Cho S, Tsai WY, Ma J, Blackwood A and Tang D: Associations between carcinogen-DNA damage, glutathione *S*-transferase genotypes, and risk of lung cancer in the prospective Physicians' Health Cohort Study. Carcinogenesis *23*: 1641-1646, 2002.
22 Colditz GA and Frazier AL: Models of breast cancer show that risk is set by events of early life: prevention efforts must shift focus. Cancer Epidemiol Biomarkers Prev *4*: 567-571, 1995.
23 Wu MH, Chou YC, Yu JC, Yu CP, Wu CC, Chu CM, Yang T, Lai CH, Hsieh CY, You SL, Chen CJ and Sun CA: Hormonal and body-size factors in relation to breast cancer risk: A prospective study of 11,889 women in a low-incidence area. Ann Epidemiol *16*: 223-229, 2006.
24 Wagner C: Biochemical role of folate in cellular metabolism. *In*: Folate in Health and Disease. Bailey LB (ed.). New York, Marcel Dekker, pp23-42, 1995.
25 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.
26 Nakachi K, Imai K, Hayashi S and Kawajiri K: Polymorphisms of the *CYP1A1* and glutathione *S*-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. Cancer Res *53*: 2994-2999, 1993.
27 Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, Willett WC, Selhub J, Hennekens CH and Rozen R: Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res *57*: 1098-1102, 1997.
28 Vairaktaris E, Yapijakis C, Kessler P, Vylliotis A, Ries J, Wiltfang J, Vassiliou S, Derka S and Neukam EW: Methylene-tetrahydrofolate reductase polymorphism and minor increase of risk for oral cancer. J Cancer Res Clin Oncol *132*: 219-222, 2006.
29 Shrubsole MJ, Gao YT, Cai Q, Shu XO, Dai Q, Hebert JR, Jin F and Zheng W: *MTHFR* polymorphisms, dietary folate intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. Cancer Epidemiol Biomarkers Prev *13*: 190-196, 2004.
30 Shen YC, Chang CJ, Hsu C, Cheng CC, Chiu CF and Cheng AL: Significant difference in the trends of female breast cancer incidence between Taiwanese and Caucasian Americans: implications from age-period-cohort analysis. Cancer Epidemiol Biomarkers Prev *14*: 1986-1990, 2005.

Received January 10, 2007
Revised March 14, 2007
Accepted March 27, 2007